ADDIS ABABA SCIENCE AND TECHNOLOGY UNIVERSITY

REDUCING SUGAR RECOVERY FROM WATER HYACINTH FOR BIOETHANOL PRODUCTION AND PARAMETRIC OPTIMIZATION

A MASTER'S THESIS

By

RAKEB KIFLE ALEMU

DEPARTMENT OF CHEMICAL ENGINEERING

COLLEGE OF BIOLOGICAL AND CHEMICAL ENGINEERING

July, 2021

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A thesis submitted as a partial fulfillment to the requirements for the award of the degree of Master of Science in bioenergy engineering

To

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Approval Page

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Declaration

I hereby declare that this thesis entitled "**Reducing Sugar Recovery From Water Hyacinth For Bioethanol Production And Parametric Optimization"** was prepared by me, with the guidance of my advisor. The work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted, in whole or in part, for any other degree professional qualification.

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Abstract

The wide and fast invasion of water hyacinth on water bodies is a threat worldwide. The invasion, however, has not been possible to halt due to the fast growth of the weed. This call for a solution that could benefit the country and help preserve the water body. Even though water hyacinth has unmerciful ways of ruining aesthetic and physicochemical values of water bodies, it has a solution that is undeniably beneficial, especially in a developing country such as Ethiopia where fuel is very expensive. Water hyacinth has a good potential for biofuel production. During the present work, the water hyacinth was converted into bioethanol through two-step pretreatments, enzymatic hydrolysis and fermentation. The two-step pretreatments included liquid hot water pretreatment and dilute acid hydrolysis. The liquid hot water pretreatment was mainly for delignification but some amount of hemicellulose gets degraded as well. The dilute acid hydrolysis was optimized to get the best process condition that could break down the hemicellulose leaving the cellulose vulnerable for enzymatic hydrolysis. The parameters optimized were: dilute acid concentration, hydrolysis temperature and hydrolysis time. The optimum condition for dilute acid concentration was 1.5% (v/v), for hydrolysis temperature was 140^0C and for hydrolysis time was 85 min in order to produce an optimum reducing sugar amount of 11.884 g/L. The enzymatic hydrolysis step was where the cellulose was broken down into glucose and other hexose sugar. This step was carried out using a cellulase enzyme (activity: 10 U/mg) that was extracted from *trichoderma virdie.* The reducing sugar yield was obtained to be 588 mg of reducing sugar per gram of water hyacinth. The theoretical yield of enzymatic hydrolysis was 886 mg of reducing sugar per gram of water hyacinth and in turn the efficiency was obtained to be 66.36%. Finally, during the fermentation process, *Saccharomyces cerevisae* was used for the production of ethanol from the fermentable sugars. The bioethanol obtained in the fermentation broth was 7.18 %(v/v). After distillation, 72 %(v/v) was recovered and used for further characterization by FTIR and other physic-chemical characteristics such as density, API gravity, specific gravity, refractive index and pH. It was concluded that 0.168 g of bioethanol can be produced from 1 g of water hyacinth if *Saccharomyces cerevisae* is used and a total reducing sugar of 0.759 g is produced per gram of water hyacinth.

Keywords: Acid hydrolysis, Enzymatic hydrolysis, Reducing sugar, Water hyacinth,

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Abberaviations and Acronyms

CO_2	carbon dioxide
IEA	international energy agency
UNDP	united nation development programme
FDRE	Federal Democratic Republic of Ethiopia
Gt	giga tonnes
Mt. CO_2 e	million metric tonnes of carbon dioxide equivalents
ASM	American society for microbiology
SANAS	South African national accreditation system
API	American petroleum institute
ASTM	American society for testing and materials
Wt%	weight percent
g/g	gram per gram
wb	wet basis
db	dry basis
C	carbon
N	nitrogen
P	phosphorous
K	potassium
CV	calorific value
Cal/g	calories per gram
Kgh^{-1}	kilogram per hour
gL^{-1}	gram per liter
^oC	Degree Celsius
Rpm	revolution per minute
mL	milliliter

mm	millimeter
KH_2PO_4	potassium hypophosphate
$(NH_4)_2SO_4$	ammonium sulfate
w/v	weight by volume
%(V/V)	percent volume by volume
M	molarity
$MgSO_4$	magnesium sulfate
FTIR	Fourier transform infrared spectroscopy
SC	Saccharomyces Cerevisae
XRD	X-ray diffraction
Nm	Nanometer
SEM	scanning electron microscope
CV	coefficient of variation
RSM	response surface methodology
CCD	central composite design
WH	water hyacinth

1 Introduction

1.1 Background

The development of fast industrialization, global population growth followed by modernization of life style usher for increasing the rate of global energy consumption. It was reported that global energy demand rose by 2.3% in 2018(Dale, 2019). This was the fastest pace in decades. In order to meet this rising global energy, the demand for all fuel sources has increased significantly. Fossil fuel supplies the majority of world energy and has increased to its highest peak than before. This increased use of fossil fuel has also counter impact as it increased the release of CO_2 to the environment. In 2018 alone, global energy-related CO_2 emissions rose by 1.7% amounting to 33 Giga tonnes (Gt) as compared to 1.6% the previous year (IEA report, 2019; Industrial Development Report, 2018). Carbon dioxide emitted from fossil fuel sources is a major component of greenhouse gas sources. The rising level of CO_2 in the environment causes an overabundance of greenhouse gases that trap additional heat and rapid increase in surface temperature with especial pronounced warming trend in recent times. This additional heat result in melting of ice caps, glaciers, rising of sea and ocean levels that cause flooding, devastating and irrecoverable weather pattern.

Since fossil fuel is the major sources of CO_2 emission to the environment, use of alternative renewable energy sources with net zero or limited carbon dioxide emission is necessary to reduce the effect of greenhouse gases. Presently, there is a promising interest in the utilization of renewable energy sources both in developing and developed nations; however, its global energy share is still the lowest. According to International Energy Agency (IEA) report, renewable energy sources cover only about 11% of global energy demand. Among renewable energy sources, biofuel covers the majority of global renewable energy supply which is about 77%. Biomass, obtained by photosynthesis, is a versatile feedstock available in different part of the world. Biomass as biofuel can be used for heating and cooking purposes without further processing in most rural areas of developing nations or it can be converted into solid, liquid or gaseous biofuel to increase its fuel efficiency and diversity. It includes bio-ethanol, bio-methanol, biodiesel and bio-hydrogen. In 2016, about 4% of world road transport fuel is covered by biofuels particularly bioethanol.

Among biofuels, bioethanol (CH_3CH_2OH) is the most widely used commercial fuel. It is used for complementing petrol as a liquid biofuel either in pure form or blended with petrol at different proportions. In US and Brazil more than 70% of petrol vehicles, including nearly all new passenger cars, can use a petrol blend containing up to 10% of ethanol (E10). There are also automobiles in the market that are able to use blends containing up to 85% of ethanol (E85). In 2016, the use of ethanol in gasoline reduced the emission of CO_2 from transportation by 43.5 million metric tonnes which is equivalent of removing 9.3 million cars from the road for an entire year (Robak and Balcerek, 2018). Use of bioethanol further decreases dependence on imported crude petroleum, increasing energy security and diversifying energy supplies as well as increasing employment and stimulating the economy in rural areas.

Currently, the use of bioethanol is widespread, and more than 98% of gasoline in United States contains ethanol at different proportion. Next to US, Brazil is the largest producer and consumer of bioenergy. Brazil has planned to increase its renewable energy mix to 18% by 2030 by expanding biofuel consumption, increasing ethanol supply, including by increasing the share of advanced biofuels production such as second and third generation bioethanol.

Conventionally bioethanol is produced by fermenting any sucrose rich biomass resources in the presence of enzymes or it can be produced from fossil based carbon chemical through chemical conversion. As the demand for bioethanol as a renewable energy source is increasing at a faster rate, the production of ethanol from any biomass sources using different technique is also diversifying. Bioethanol is referred as first, second, third generation bioethanol based on its feedstock sources and technology employed during production (Robak, 2018).

First generation bioethanol is produced from crops with high levels of starch and sugar such as corn, maize, wheat, barley, cassava, potato and sucrose-containing feedstock of sugarcane, sugar beet, sweet sorghum. These energy sources compete with food production since in most developing countries they are used as food source. This raised a serious concern of choosing between food and energy as it pushes up both the price of food and energy. This leads to the search for alternative non-edible biomass resources that can be used to produce bioethanol. The ethanol obtained from non-edible sources is referred to as second generation and third generation bioethanol.

Bioethanol produced from these sources do not compete against food supplies as they are based on non-food raw material (Devarapalli and Atiyeh, 2015).

Lignocellulose biomass is used as a feedstock source of second generation bioethanol. It is relatively cheap as well as readily and locally available. Its conversion into reducing sugars and then to ethanol is more complex than the conventional way of producing bioethanol. Various agricultural wastes, such as cereal straw of Stover, wheat straw, corn cob, rice husk and bagasse from processing sugar cane have also been examined as potential sources of lignocellulose biomass. Industrial wastes, such as brewer's spent grains and spent grains from distilleries, and municipal solid wastes such as food waste, Kraft paper and paper sludge containing cellulose have also been considered (Robak and Balcerek, 2018).

Lignocellulose biomass is a promising substrate as a future bioethanol energy sources as its supply may not be depleted and susceptible to permanent damage. Its composition depends on its species, variety, growth conditions and maturity. Consequently, ethanol yield and conversion rate relies on the type of biomass that needs a high cellulose and hemicellulose content as well as low content of lignin. Ethanol production from lignocellulosic biomass is also affected by the development of efficient technologies and the selection of appropriate microorganisms. It was reported that the degradation of lignocellulosic structure is difficult due to its compositional heterogeneity; consisting of cellulose, hemicellulose and lignin. Processing of lignocellulose biomass to bioethanol, therefore, encompasses four major processes of lignocellulose pretreatment, hydrolysis, fermentation and product separation and purification (Rocha–Meneses et al., 2017).

In Ethiopia, ethanol is produced from sugar cane by product molasses for different purposes such as for the production of liquors, as inputs for pharmaceuticals and cosmetic industries. However, in response to the increasing oil prices and environmental concern, the government has decided to diversify its fuel sources and include ethanol in the national liquid fuel mix by blending up to 10% volume of Ethanol content in gasoline. In response to this government direction, the country has started providing a 5% ethanol and 95% benzene blended (technically known as E5) for the market as a motor fuel since 2009, and has also planned to increase the ethanol blend to petrol oil up to 25% (E25) based on the supply of ethanol. On the other hand, ethanol is produced from molasses, a byproduct of sugar factories. The capacity of

ethanol produced from such sources is limited to satisfy the current ambition of the government; blending up to 10% ethanol gasoline let alone expanding the composition of ethanol in the blend up to 25%. This calls for assessing all sources of local biomass that can be converted into bioethanol and developing appropriate technology for the production of these alternative sources. One such option is the use of lignocellulosic biomass as second generation feedstock. Currently, among different biomass sources available locally, water hyacinth appeared to be an appropriate option for various reasons presented here under (Bhattacharya et. al., 2010):

- It is naturally grown vegetation and a perennial.
- It has low lignin and high cellulose content per unit volume on a dry basis.
- It does not compete with land grown crops since it grows on fresh water bodies.
- It is pest and disease resistant
- It is not polluted easily by cross breeding with cultivated food crops.

Water hyacinth has an attractive blue, lilac to purplish flowers and round to oval leaves. They are native to Brazil and Ecuador region (Bhattacharya et. al., 2010). It is an invasive, aquatic weed that floats over fresh water. It reproduces very fast producing over thousands of seeds each year. It has broad, thick, glossy, ovate leaves that distinguish it from other aquatic weeds.

Above water surface, it can grow up to 1 meter and below water surface, the roots can grow up to 80 centimeters. The leaves are 10 to 20 centimeters wide. It has long and spongy stalks. The seeds can stay intact for more than 28 years. In south East Asia, some were found to grow 2 to 5 meters in a day.

Water hyacinth has been listed as one of the 100 most dangerous invasive aquatic species and one of the top 10 worst weeds in the world if not properly managed and converted to use products (Admas et. al., 2017). Water hyacinth obtains all of its nutrients directly from the water that it inhabits. It grows, most efficiently, in habitats with tropic climates and nutrient-enriched water. Their growth in fresh waters causes serious problems for water activities. They affect activities like swimming, boating and fishing. It can block water ways which can interfere with transport and irrigation. This, in turn, affects the economy of the country. So converting them into energy is

one of the many ways of changing this negatively perceived weed into a positive outcome. On the other hand, it also has ecological consequences. Once it grows in an area, it forms a rug of vegetation that is very thick and that covers large surfaces of water. This limits light penetration into the water which reduces the growth of the plants below the water surface. This, together with reduced decomposition of the problematic aquatic weed, affects the distribution of dissolved oxygen in the water body finally causing the other aquatic species to die and perish (Lee and Fagan, 2015).

In Ethiopia, water hyacinth first appeared in 1965 at Koka reservoir and Awash River. Other infested areas in the country were Gambella region, Blue Nile from just below Lake Tana to Sudan and Lake Ellen near alem tena (Admas et. al., 2017). Since a few years ago, water hyacinth has been invading Lake Tana, and other domestic lakes like ziway. Its removal has almost reached to the level of beyond control as the pace of weed removal has been far slower than the rate of weed expansion. Even the region around such as Bahar Dar is prone to the infestation as the weed is progressing at an alarming rate. If proper mitigation measures are not taken, the lakes of Ethiopia are in grave danger.

Different attempts were under way at local and national level in order to mitigate this problem; however, this invading plant is not only a threat but also an opportunity. It can be converted into useful products such as bioethanol as it becomes additional resource to supplement the economy. Therefore, the present research work uses water hyacinth, as a lignocellulosic biomass feedstock, in order to produce bioethanol. This approach has a dual economic and environmental advantage as it mitigates the current risk that Lake Tana, Lake Ziway and other lakes in Ethiopia are facing to continue as a lake due to this invading dangerous water weed. The use of water hyacinth as lignocellulose for the production of ethanol also avoids the controversial debates using food sources for the production of energy.

1.2 Problem statement

Recently, water hyacinth has taken its residence on Lake Tana in Bahir dar and Lake Ziway in Ziway, Ethiopia. This has caused the lake to deplete and lose its aesthetic values. Admas et al. (2017) stated that water hyacinth impacts the physicochemical characteristics of the lake in a way that decreases the temperature, pH, biological oxygen demand (organic load) and nutrient levels which results in the loss of the ecosystem in the water. The ecological problems it is causing are not easy to solve at the moment and getting rid of water hyacinth is very costly. But despite it being an ecological nuisance, water hyacinth has a potential to be used as a biofuel source which can solve two problems with one solution. If the sugar recovery process is optimized, its bioethanol yield can be enhanced.

According to Amriani et al., (2016), water hyacinth was subjected to a physical (chopping and grinding) and biophysical pretreatment (combination of physical and biological pretreatment) in order to degrade the lignin and improve the accessibility of the cellulose to cellulase producing fungi known as *Aspergillus niger* and *Trichoderma reesei.* It was found that lignin was degraded significantly after the biophysical pretreatment. A white rot fungus known as *Ganoderma boninense* was used during the biophysical pretreatment. A good degradation o flignin leads to the accessibility of cellulose. The crystallinity of water hyacinth was also seen to decrease after the biophysical treatment. However, with the decrease in crystallinity came a small amount of cellulase being produced since the motivation of the cellulase producing microbes was the crystallinity. This resulted in the decreased activity of the enzyme. Finally, it was concluded that the physical pretreatment had the best cellulase production and good enzyme activity that could degrade cellulose. Eventhough cellulase production was improved, the effect of the physical pretreatment on the reducing sugar recovery is not done.

Zhang et al., (2016) reported different pretreatments that water hyacinth was subjected to before enzymatic hydrolysis in order to investigate their effect on reducing sugar recovery. Acid hydrolysis (using sulfuric acid), alkaline pretreatment (using sodium hydroxide) and microwave-alkaline combined pretreatments were employed. It was found that acid hydrolysis was the most effective step for higher reducing sugar recovery. Simultaneous saccharification and fermentation (SSF) was

finally employed and ethanol yield of 1.289 g/L. Therefore, separate hydrolysis and fermentation can be employed to compare and contrast.

It was reported by Sornvoraweat & Kongkiattikajorn, (2010) that water hyacinth leaves are good candidates for biofuel biomass since they have good reducing sugar and ethanol yield. During the course of their work, water hyacinth leaves were subjected to a dilute acid hydrolysis pretreatment followed by enzymatic hydrolysis. The highest reducing sugar yield was 8.56±0.12 wt%. After fermentation, the highest ethanol yield was 0.27 g/ g biomass. Accordingly, water hyacinth stalks can be combined with the leaves for more production of reducing sugar for the present work.

Thus, for a maximal reducing sugar recovery, the present work can investigate pretreatments that are environmentally-friendly which can be employed and optimized on the combined leaves and stalks of water hyacinth. separate hydrolysis and fermentation can also be investigated for the respective cellulose degradation and ethanol production.

1.3 Significance of the study

According to the FDRE government report of 2011, Ethiopia plans to attain a middle income status by 2025 while developing a climate resilient green economy. This shows that, as a responsible member of the world, Ethiopia wants to play a major role in the fight against climate change. Ethiopia's green economy plan is made up of four pillars whose core idea is growth with decreased amount of carbon dioxide emission or at least limited to the current value of 150 Mt CO_2 e if possible (FDRE government, 2011). According to a report by UNDP, mean annual temperature rise in Ethiopia has increased by 1.3℃ between the years 1960 and 2006 and it is expected to increase by 1.1 to 3.1℃ by year 2060 (Irish aid, 2017). This means that using sustainable renewable energy should be considered as alternative sources of energy for the sustainable growth. Bioethanol being a biofuel from biomass can contribute in this green economy journey and also benefits the society.

Water hyacinth is known to be an environmental hazard, not only to the water bodies but also to the farmers living in the marginal areas of Ethiopia since it is hard to access the water body once the water hyacinth invades.so changing the weed into biofuel can be a good option for the farmers as well as the country's economy.

The main significance of the study would be establishing the best way of converting this negative ecological problem into a positive solution. If the best possible bioethanol can be produced from water hyacinth, then the problem of it being a nuisance would be solved and this study will be focusing on doing that. Previous researches show that the ethanol yield of water hyacinth varies with the area it grows around. This happens due to the variation in the moisture content of the water hyacinth with the area. So this research will contribute to the determination of the optimum values that could result in an efficient yield of bioethanol corresponding to the one that grows in Ethiopia.

1.4 Objectives of the research

1.4.1 General objectives

The main objective of the research work is to recover reducing sugar from water hyacinth inorder to produce bioethanol, to characterize the produced ethanol and to optimize the process by analyzing the different parameters.

1.4.2 Specific objectives

- To investigate the yield of reducing sugar from water hyacinth during liquid hot water pretreatment
- To investigate the effect of acid hydrolysis on water hyacinth and the affecting parameters like the acid concentration, temperature and residence time of the hydrolysis.
- To investigate the amount of reducing sugar obtained from enzymatic hydrolysis of water hyacinth cellulose
- to investigate the fermentation of sugars recovered by hydrolysis process and to characterize the product of fermentation

1.5 Scope of the research

For the present research work, water hyacinth (*eicchornia crassipes*) was be used as a raw material to produce bioethanol. It was collected from Zeway Lake. Physical, chemical and thermal pretreatments are done before enzymatic hydrolysis takes place. The physical pretreatment is size reduction followed by thermal pretreatment which is only liquid hot water pretreatment. Chemical pretreatment is dilute acid hydrolysis which will use dilute sulfuric acid then sodium hydroxide is used to detoxify and to neutralize the pH of the hydrolysate.

After chemical pretreatment, separate saccharification and fermentation will take place. Saccharification will take place by enzymatic hydrolysis which will use cellulase enzyme that is specific to cellulose. So lignin and hemicellulose have to be removed during the preceding steps. After enzymatic hydrolysis, fermentation will take place with the help of *saccharomyces cerevisae.* The product will be centrifuged or filtered and distilled. Simple distillation but double distillation will be used. The ethanol produced will be analyzed for physicochemical characteristics but not for engine properties. The raw material will be analyzed by FTIR, XRD and SEM. The reducing sugar in the dilute acid and enzymatic hydrolysis filtered liquid products will be analyzed by using the DNSA (dinitrosalicylic acid) method. The product from fermentation will be analyzed by using FTIR, refractometer and densitometer. The present work aims at simply producing bioethanol from water hyacinth.

2 Literature review

The rise in the deterioration of non-renewable energy resources, the rise of oil price and the increase in energy demand has fostered the need for a sustainable and renewable energy during the previous years. In order to solve this, biofuels especially bioethanol has been commercially competitive. This chapter will focus on the review of what is already known about biomass, biofuels (especially bioethanol) and methods of recovering bioethanol from biomass.

2.1 Biofuels

Continuous and excessive use of fossil based fuels increased generation of pollutants and greenhouse gases such as oxides of nitrogen, sulfur and carbon. Fossil fuel is also an energy source that is depleting and resulting in unsustainable energy source for long term energy supply. Renewable energy resources which include biomass and biofuel is the fore becoming the global attention for sustainable development. Biofuels are fuels derived from biomass or materials with biological origin. Biofuels can be classified into three as bioethanol, biodiesel and biogas. Among bioethanol has got increasing attention as transportation fuel followed by biodiesel(Kumar et al., 2019).

Bioethanol is a fuel derived from sucrose and sugar containing feed stocks like sugar cane, maize, sugar beet and any sucrose rich biomass. Biodiesel is derived from animal fats and vegetable oil like soy beans, jatropha, palm and sunflower oil. Finally biogas is derived from municipal solid waste and sewerage. This type of biofuel is applicable in households as well as in large scale(Devarapalli & Atiyeh, 2015).

Currently, there are increasing interests of shifting to biofuels due to rising petroleum based oil prices, global pressure to reduce carbon emission, and energy sources diversification. It was reported that bioethanol is a better alternative to complement or replace gasoline as it is clean and renewable combustible (Akbar et al., 2019). Its combustion causes less emission of toxic substances. Bioethanol use as a transportation fuel reduces the CO_2 emission. Its use reduces CO_2 emission by more than 80% as compared to use of gasoline. It also completely eliminates sulfur dioxide related acid rain. Due to its environment friendly impact and mitigation of worsening of the environment quality and increasing oil price, bioethanol is becoming the promising fuel (Balat & Balat, 2009).

Bioethanol is the most consumable biofuel in the transportation sector. Bioethanol has high octane number that provides higher engine knock resistance. It has an octane rating of 113 that makes it the inexpensive octane source. Hydrocarbon octane sources such as methyl tert-butyl ether (MTBE) and aromatics of benzene are highly toxic and are a source of environmental risks. Due to the aforementioned reasons, R. Ahmad Dar et al. 2017 reported that ethanol global production increased from increased from 13,123 million gallons to 27,023 million gallons.

2.2 Biofuel classification based on generations

The transportation and machinery sector currently uses fossil fuels owing to their high heating power, availability and excellent combustion characteristics. However, the fact that fossil fuels are non-renewable and contributors to GHG emission calls for an alternative sustainable energy source. Biofuels have the potential to be replacements for fossil fuels. The most commonly commercialized biofuels are bioethanol and biodiesel. Bioethanol is further classified into first, second and third generation bioethanol (Ziolkowska, 2014).

2.2.1 First generation biofuel

Bioethanol is a fuel produced from numerous feed stocks. First generation bioethanol are those produced from sugar and starch crops such as sugar cane, corn, sugar beet, etc. 40% of the total ethanol produced is from sugar crops and 60% of it is produced from starch crops (Vohra et al, 2013). These are conventional biofuels produced by conventional technologies like fermentation and distillation. They have detrimental effect on food security because they compete with feed/food production by using the agricultural land required as well as actually using the crops that are food sources in most third world countries. As a result, the food/feed versus fuel debacle was increasing and people had to choose between food and fuel. This problem was magnified during unpredicted weather episodes and climate change (Vohra et al, 2013; Ziolkowska, 2014).

2.2.2 Second generation biofuel

Second generation biofuel can overcome the drawbacks of first generation biofuels. Second generation bioethanol is generated from non-edible Lignocellulosic biomass (LC), residue of forest management or food crop production or whole plant biomass. Lignocellulosic biomass is a composite material comprising of cellulose, hemicellulose and lignin. These fuels do not jeopardize food security. They are produced from feed stocks such as miscanthus, switch grass, poplar, elephant grass etc. the problems with second generation bioethanol production is the difficulty to break down the plant cell wall component i.e. the lignin in order to expose the cellulose and hemicellulose that have the potential to be degraded into fermentable sugar (Vohra et al, 2013; Ziolkowska, 2014).

2.2.3 Third generation biofuel

Third generation biofuels tighten the gap that is created by the first and second generation biofuels. These biofuels are produced from microorganism like microalgae. Microalgae can be cultivated on fresh water, waste water, sea water, as well as brackish water. However the cultivation cost by using either phytobioreactors or open ponds is very high (Li-Beissona & Peltier, 2013). This has 30-100 times more energy productive and effective (Akula, 2013). Unfortunately, the biomass harvest and oil extraction is very costly as well (Li-Beissona & Peltier, 2013).

2.3 Feedstocks for Fuel Ethanol Production

Plant biomasses that are rich in sugar can be an ethanol feedstock. All plants contain sugars, and these sugars can be fermented to make ethanol through biochemical conversion process or any plant material that can be converted using heat and chemicals in a process to ethanol called are source of bioethanol feedstock. Selection of appropriate feedstock depends on many factors, such as crop cultivation practice, geographical location, selection of specific crop, the choice between ethanol for energy and food. Crop residues and wood wastes can also be used as feedstock (Vohra et al., 2013). Among these are residues like straw, rice husk, cobs, stalks, leaves, barks, fruits, cutting vines in addition to animal wastes and plant products. Other biomass types can be energy crops that are purposely planted for the production of this bioenergy i.e. they are not used as a food source.

2.3.1 Bioethanol from sucrose-rich feed stocks

These feed stocks are high on sugar yield per acre and have low cost of conversion. Sources like sugar beets, sweet sorghum and sugar cane are used but seasonal availability of these sources might be a little disadvantageous. In tropical areas like India, Brazil and Columbia, sugar cane is a popular feed stock for bioethanol production(Devarapalli & Atiyeh, 2015).

2.3.2 Bioethanol from lignocellulosic feed stocks

Lignocellulosic Biomass is one of the sources for environmentally clean, renewable energy. They are considered as a carbon neutral source as there is no net gain of carbon into the atmosphere i.e. the carbon released from the conversion process is returned back or absorbed by plants that are growing. It is generally classified in to two: (Akula, 2013)

- Vegetation: like energy crops, wood, agricultural and forest residues and floating plant waste (aquatic weeds).
- Organic wastes: includes animal waste and organic industrial waste

Table 2. 1. Classification of lignocellulosic biomass. Adopted from Obafemi et al. (2018)

Based on Origin	Based on properties
Primary waste, e.g. grain waste, wood waste	Wood and woody biomass (newly harvested soft and hardwood)
Secondary waste, e.g. food processing residue and sawdust.	Agricultural biomass (Stray, stalk, grass, Stover)
Tertiary waste, e.g. wood used in construction	Aquatic biomass (Algae and different water weeds)
Energy crop, e.g. cassava tuber	Animal and human waste (bones, manure, etc.)
	Municipal biomass (solid waste, organic waste, sludge, refuse)

Second generation bioethanol is achieved from these lignocellulosic biomass. The biomass types can be agri-residue, energy crops, human and animal waste as well as invasive aquatic weeds (Vohra et al., 2013).Second generation bioethanol, even though it's sustainable, the ability to breakdown the plant cell wall component, i.e. the lignin, in order to expose the cellulose and hemicellulose that have the potential to be

converted to fermentable sugars. Moreover, the recalcitrance of cellulose makes it difficult to degrade it to its monomers (Ziolkowska, 2014). The most widely used lignocellulosic biomasses in the world are corn Stover, switch grass, miscanthus, elephant grass and popular.

Table 2. 2. Summary of different feedstocks with their respective conversion technology and gap analysis

Feedstock	Conversion technology	Comparison and gap analysis	References
Wheat straw	• Optimized liquid hot water pretreatment (temperature and residence time) followed by enzymatic hydrolysis	• Low reducing sugar recovered after both steps • Gap: additional pretreatment method for higher ssugar recovery	(Pérez et al., 2008)
Poplar	• Liquid hot water pretreatment with different severity factors	• Substantial xylose degradation and delignification • Gap: if used with other sugar recovery methods higher sugar recovery and degradation might be achieved.	(Li et al., 2017)
***Eucalyptus grandis* residue**	• Dilute acid hydrolysis in stainless steel reactor (optimized) for hemicellulose degradation	• Only hemicellulose degradation to xylose was investigated • Gap: total reducing sugar recovery investigation for further processing like ethanol production	(Vieira et al., 2007)
Sugar cane bagasse	• Dilute slufuric acid hydrolysis optimization with conditions such as temperature of 155-175^{o}C, residence time of 10-30 min and acid concentration of 2-6 %(w/v) • Fermentation	• Good reducing sugar yield (22 g/L) with good ethanol yield(16.8 g/L). • Gap: effective cellulose degradation in addition tohemicellulose and lignin should be studied	(Dussán et al., 2014)
Rape seed straw	• Dilute acid hydrolysis optimized in a 15 mL bomb tube reactor • Enzymatic hydrolysis	• High amount of fermentable sugar obtained during the two steps • Gap: no ethanol production performed eventhough high fermentable sugar was obtained	(Jeong et al., 2010)

2.3.2.1 Invasive aquatic weeds

Invasive aquatic weeds are currently being utilized in order to produce bioethanol. Amongst these aquatic weeds are water lettuce, water hyacinth and duckweeds. Water hyacinth, as a lignocellulosic material, has the potential to produce bioethanol. This is due to its high hemicellulose and cellulose amounts that can be reduced to their monomeric states or fermentable sugars and fermented to alcohol (kasthuri et al., 2012).

2.4 Water hyacinth as bioethanol feed stock

Bioethanol can be produced from different sources but the most common ones are from sugar and starch containing feed stocks and Lignocellulosic feed stocks. But most of the sugar and starch containing feed stocks, usually, pose a threat to food security since they are, most of the time, major food sources in third world countries. Therefore, Lignocellulosic feed stocks are the most recommended types of biomass (Vohra et al., 2014).

Lignocellulosic biomasses are the type of feed stocks that are found in abundance, that are sustainable and that are not food sources. Converting these feed stocks into bioethanol is a major solution to the food crisis that is caused due to the use of sugar and starch containing feed stocks. These sources are comprised of pentose and hexose sugar, containing up to 50-60% carbohydrates. Lignocellulosic biomass includes sources like wood residue which are considered the most abundant sources in the bioenergy sector. Other sources are energy crops. These are crops solely dedicated to energy production. They are non-edible and can grow on marginal land. Municipal solid waste and agricultural waste are other types of Lignocellulosic biomass. These sources can solve two problems at once i.e. by creating the opportunity for biofuel production while taking care of waste (Pirzadah et.al., 2014).

These biofuel sources include invasive plants as well. Of these invasive plants, water weeds like duckweed, water hyacinth and water chestnut are potential sources of biofuels. These weeds tend to put the water bodies they invade in danger be it by decreasing the amount of water due to rapid evapo-transpiration or by decreasing the biodiversity inside the lake by decreasing the amount of dissolved oxygen. Water hyacinth, especially, is currently a very problematic weed all over the world but also

has the potential to produce bioethanol. So focus has turned into converting it to a useful product.

In South Africa, an evaluation of water hyacinth as biofuel feedstock was done. After the water hyacinth was dried and collected, it was analyzed for cellulose, hemicellulose and lignin contents. The fact that the water hyacinth had high cellulose and hemicellulose content and low lignin content led to the conclusion that it was suitable for the respective biofuel production (Schabort, 2014).

2.5 Water hyacinth in Africa

WWF-Kenya (2017) reported that a resident and chemistry teacher in Kisumu Count, Richard Ochieng, who is also the founder of center for innovation, science and technology in Africa, has made it his mission to convert this weed into a useful product to community that is being affected by the hyacinth. The water hyacinth was collected, chopped and, through vigorous steps, converted to biofuel. The biofuel produced was a product that could be used for stoves. The product obtained was very clean i.e. it did not produce soot and also didn't produce smell while burning. Also when it comes to carbon emission, it was relatively cleaner when compared to paraffin. Paraffin emits 8 times more carbon per liter than that of 1 liter of the biofuel produced from the water hyacinth. This product helps in controlling the weed propagation on Lake Victoria as well as the community in producing a clean and affordable fuel. "The product also underwent a market acceptability test and the result was overwhelming."(Richard Ochieng). Future plans include improving the capacity of the plant from 300 liters per day to 3000 liters per day.

The Water hyacinth, a perennial aquatic weed, is invading water bodies all over the world. It was originally spotted in Brazil but now it has made an appearance in the continents Asia and Africa since it finds the warm climate comfortable for fast growth. In Egypt, this specific weed appeared and is blocking rivers and channels; it is also reducing aquatic organisms by restricting light and air to reach the underwater environment. Due to the problems it caused, the need for its removal has led the way to find some uses for it. So it was reported that it could be used as animal feed, agricultural fertilizer and enzyme production. But this specific research aims at the production of bioethanol from water hyacinth. The production process they considered is as follows: collection and size reduction, pretreatment and hydrolysis

where the hydrolysis was carried out in three ways; chemical hydrolysis (acid and alkaline), fungal hydrolysis and combined and finally fermentation where bioethanol was recovered as an end product. Results showed that the acid hydrolysis produced more sugar than that of the fungal, alkaline and combined hydrolysis and that the water hyacinth was a suitable raw material for bioethanol production. As conclusion, it was stated that water hyacinth being used as a raw material for bioethanol production not only aids in being an alternative source of energy but also in the protection and well-being of the environment which in turn helps in the community with their economic growth (Madian et al.,2018).

In Zimbabwe, water hyacinth has made its appearance on Lake Chivero. Zimbabwe is a developing country that suffers from shortage of electricity due to the inability of using all the opportunities that are available and also the country imports its petroleum based fuel from foreign countries which is very expensive. The people of Zimbabwe mostly use traditional and inefficient sources of energy that disables them to grow economically and improve their quality of life. Zimbabwe's' rivers, dams and lakes are currently captivated by water hyacinth which calls for research institutes to come up with a solution that would be beneficial in controlling it. The elimination and maintenance of the weed is costly so it was found out that converting the water hyacinth into biogas may be a better choice since it increases the diversity of the types of energy sources as well as improving the environmental sustainability by decreasing the weeds impact on the environment. It was pointed out that biogas can be produced by anaerobically digesting water hyacinth together with fresh rumen residue.it was then concluded that the production of this biogas from water hyacinth not only solves the threat posed by water hyacinth but also improves the energy and power situation in the country (Kunatsa and Mufundirwa, 2013).

2.6 Water hyacinth in Ethiopia

Water hyacinth is a weed that invades fresh water. This aquatic weed, locally known as Emboch, appeared on Lake Tana and now has spread to Ziway and Koka lakes as well as on Boye River found in Jimma town. According to biodiversity director at Oromia regional state, more than 800 hectares of Lake Koka is covered by this invasive weed. Ziway Lake is the third largest lake in Ethiopia covering 434 sq. kilometers. Of this, the invasive weed has possessed 50 meters in width and 1.6 kilometers in length of the lake. In Jimma, 50 hectares of the Boye River is covered

by this water-thirsty Weed. According to the Ziway lake tourist guide associations chair person, the harmful weed is thriving due to solid wastes like trash, litter, garbage and fertilizers originating from the surrounding farms and entering the lake due to runoff during the rainy season.

2.7 Biofuel technologies

The production of advanced biofuels, especially second generation bioethanol, in a way that is cost competitive to fossil fuels is difficult for the biofuel industry due to the struggle with cellulose extraction and conversion to fermentable sugars. Since the process of production is cost-intensive, the end product will be costly as well (Ziolkowska, 2014).

Attempts have been made to produce second generation biofuels in a cost effective way that could compete with fossil fuels or petroleum fuels. But it has been challenging to get an efficient process. The key problem in producing biofuels is acquiring the enzyme that could degrade cellulose and lignin into fermentable sugar. This is an expensive process so the overall cost will also increase as a result and in turn, affects the cost of the biofuel product.

Biomass, in general, can be converted to energy through different routes. These include:

- Thermochemical routes: this involves combustion, gasification and pyrolysis.
- Biochemical routes: this includes anaerobic digestion and fermentation.
- Chemical route: this is mainly transesterification.

These conversion routes can produce either bioethanol, biodiesel or biogas for heat, electricity and fuel purposes but the efficient production of these biofuels depends on the conversion routes we use for the type of biomass we choose. For example, if a thermochemical conversion process is used for wet biomass then the yield will not be as satisfying as a biochemical or chemical process. The amount of energy spent on drying the biomass alone will be high and the outcome will not match the input (Akula, 2013).

All in all, the current trend for the production of second generation bioethanol is following, more or less, the biochemical path due to its inexpensiveness and the fact

that even when being used in large scale, it is still cost effective and efficient (Ziolkowska, 2014).

2.7.1 Bioethanol generation technologies

The bioethanol production process i.e. fermentation has been carried out since early times so the process is a well-known process. The challenge arises when the ethanol is being produced from lignocellulosic biomass due to the recalcitration of the lignocellulosic components. Many researches have been carried out in order to find a cost effective and cost competitive way of producing bioethanol. However, there are still some drawbacks when it comes to its commercial production. Nonetheless, there are two routes for the production of bioethanol. One is a thermochemical conversion route, which is a syngas platform, and the other is a biochemical conversion route, which is a sugar plat form (Vohra et al., 2013).

2.7.1.1 Thermochemical conversion route (syn gas platform)

Lignocellulosic biomass is converted to ethanol by first being converted to syn gas through gasification. Gasification is a process of converting carbon containing materials, be it solid or liquid, to a gaseous product called syn gas or producer gas or synthetic gas by reacting with air, oxygen and/or steam. Syn gas or producer gas is composed of carbon monoxide (CO), hydrogen (H_2), methane (CH_4), carbon dioxide (CO_2), and nitrogen (N_2). After the syn gas is achieved, it is then converted to ethanol, methanol or hydrogen by either metal-catalytic methods or bio-catalytic methods (Vohra et al., 2013).

2.7.1.2 Biochemical conversion route (sugar platform)

The biochemical conversion route can convert lignocellulosic biomass into bioethanol and it is currently the most practical process of converting this feedstock to bioethanol. The biochemical platform consists of 4 main processes, namely, pretreatment, enzymatic hydrolysis, and fermentation. Additionally, detoxification and fermentation of pentose liberated throughout the pretreatment step take place. The cellulose undergoes enzymatic hydrolysis to produce hexoses such as glucose (Vohra et al., 2013).

2.8 Biochemical conversion route: Lignocellulosic material to bioethanol

The general bioethanol production from Lignocellulosic biomass mainly includes 4 steps:

- Pretreatment
- Hydrolysis
- Fermentation
- Product separation or purification

2.8.1 Pretreatment

This is the process of altering the structure of the Lignocellulosic biomass so that during enzymatic hydrolysis, the enzyme would be able to access the cellulose easily and convert it to fermentable sugars. The alteration takes place by breaking down the lignin and exposing the cellulose by ruining the crystalline structure. The aim of pretreatment is to separate the biomass into cellulose, hemicellulose and lignin no matter what type of biomass it is and to increase the surface area of carbohydrate available for enzymatic saccharification, while minimizing the content of inhibitors (Robak and Balcerek, 2018). The more we obtain the three structures in pure form, the more economical feasibility the process would ensure. The pretreatment should be cost effective, should be efficient on different lignocellulosic biomass types and require minimum preparation or handling that would result in high recovery of the desired product (Ewanick and Bura, 2010).

The aim of pretreating lignocellulosic biomass is to solubilize, hydrolyze and chemically modify individual cell wall components like lignin, hemicellulose and cellulose. This helps in increasing the digestibility and fermentability of the treated biomass by decreasing the recalcitration of lignocellulose to biological degradation. Pretreatments could be physical, chemical, thermal and physicochemical.

O. Bani et al. (2015), has done a research on evaluating and selecting the critical process parameters in order to cost effectively obtain bioethanol from water hyacinth. The process focused on pretreatment methods in order to get a much better result at the end of enzymatic hydrolysis and fermentation. To decrease cost, enzyme production was carried out on-site. Four types of pretreatments took place which is namely: liquid hot water, sterilization, dilute acid pretreatment and biological pretreatment. In this particular paper dilute acid pretreatment showed the highest yield of sugar and ethanol. The liquid hot water and simple sterilization were comparable when it came to ethanol and sugar yield, which may have been due to the similar

operation conditions. Finally, biological pretreatment had lower ethanol concentration but a higher sugar yield.

As conclusion, the dilute acid pretreatment was chosen as the ideal pretreatment for bioethanol production from water hyacinth despite reported inhibitor generation and it was also recommended that further research must be done for minimizing production cost.

Rezania et al. (2017) aimed at reviewing the process of producing bioethanol from water hyacinth through different pretreatments and converting the cellulose to fermentable sugars then to ethanol. The pretreatments reviewed were acid hydrolysis, alkaline hydrolysis, ionic liquid pretreatment and combined methods that are micro-wave assisted. These pretreatment methods are evaluated according to their effect on the subsequent enzymatic hydrolysis. The review concludes that acid hydrolysis can lead to a higher glucose and total sugar yield than the other pretreatment processes. But the ethanol concentration was less than that of the alkaline pretreatment and microwave assisted pretreatments.

Pretreatments can be physical, physicochemical and chemical pretreatments.

2.8.1.1 Physical pretreatments

This is, usually, a mechanical comminution mainly aiming at reducing the size of the sample particles and disrupting the crystallinity of the cellulose so that the surface area for enzyme action increases for enzymatic hydrolysis (Monika et al., 2016). This is achieved by different ways: chopping and milling or grinding, extrusion, pyrolysis (Monika et al., 2016) and irradiation (El-Naggar et al., 2014).

Feedstock characteristics: Ewanick and Bura (2010), stated that the primary factors that greatly affect a pretreatment product are pretreatment conditions and raw biomass species. Other factors include harvest and storage before the pretreatment process and particle size. Moisture content of the particle is another factor that affects the pretreatment process. It is high immediately after harvest but decreases significantly during storage. Longer reaction time is required for raw biomass with high moisture content so that the additional water in the cell is heated up. It also consumes up to 50% more steam than dried biomass. This, usually, occurs when there is large particle size. It should be noted that the particle size mustn't be too small as well since the particle may heat up too quickly without the buffering effect of a slower heating,

increasing the hemicellulose degradiation.so the main factors that are important in determining the characteristics of the final product of a pretreatment.

- **Chopping and milling or grinding**

The Lignocellulosic biomass is first chopped here. The particle size we require after chopping is between 10-30mm. then grinding or milling follows. The particle size required after grinding is between 0.2-2mm (Monika et al., 2016).

- **Extrusion**

This type of treatment is achieved by passing the Lignocellulosic biomass through an extruder and subjecting it to heating, mixing and shearing. The screw speed and temperature of the barrel disrupt the Lignocellulosic structure by causing defibrillation, fibrillation and shortening of fibers, which increases the accessibility of carbohydrates for enzymatic hydrolysis (Monika et al., 2016).

- **Pyrolysis**

This is a high temperature process where the biomass is subjected to a temperature above 300℃ and it converts the cellulose into gaseous products and residual char. When pyrolysis and acid hydrolysis are combined, it produces between 80-85% of cellulose that can be converted to fermentable sugar (Monika et al., 2016).

- **Irradiation**

This is the use of irradiations like gamma rays and microwaves that can improve the enzymatic hydrolysis process. If combined with other methods like acid pretreatment, it helps in speeding up the enzymatic hydrolysis. According to a study done on pretreatment of bagasse, it was found that irradiation affected the enzymatic hydrolysis in a way that it doubled the glucose yield (El-Naggar et al., 2014).

2.8.1.2 Physicochemical pretreatment

These pretreatments include ozonolysis, liquid hot water, ultrasound, steam explosion, alkaline wet oxidation, supercritical CO_2, ammonia recycle percolation and ammonia fiber explosion (Lugani et al., 2017). Of these, the liquid hot water and steam explosion pretreatments are collectively known as hydrothermal pretreatments because they only use water or steam at high temperature (Ewanick and Bura. 2010).

- **Hydrothermal pretreatment**

These pretreatments are carried out at high temperatures. Since physical comminution is not enough to get a complete fractionation of a biomass to a digestible and

fermentable product, it is further required for certain chemical reactions to occur. So pretreatments that use steam or liquid water at high temperatures are employed. These pretreatments are collectively known as hydrothermal pretreatments. These pretreatments have the advantage of being environmentally sound i.e. they do not need any chemicals and they are less corrosive for equipment (Ewanick and Bura. 2010).

Steam pretreatment is strongly comparable to the dilute acid method. It uses a high-pressure saturated steam in order to dismantle or disturb the structure of the Lignocellulosic biomass. The feed stock is subjected to high pressure steam at a temperature of between 180 and 260℃ for 1 to 15 minutes (Monika et al., 2016)

According to Taharzadeh and Karimi (2008), the steam explosion process is a relatively lower cost process when it comes to energy expenses. The temperature, at high pressure, that the feed stock was subjected to in this research was 160-260℃ for about 1-20 minutes. It was concluded that that the increase in the temperature will significantly release hemicellulosic sugar but if there is a substantial sugar loss, then it will result in the loss of total sugar recovery.

- **Liquid hot water (LHW) pretreatment**

The LHW pretreatment uses no other chemicals except aqueous water at high temperature and pressure liquid hot water pretreatment reduces the recalcitrance of the biomass structure increasing the accessibility of cellulose which ensures an effective enzymatic hydrolysis. The alterations expected after this pretreatment process is partial delignification, hemicellulose dissolution, small cellulose deconstruction and minimal carbohydrate degradation due to the water acting as both solvent and catalyst. Hemicellulose dissolution occurs at a temperature of 150-170 ℃ for a time range of 18-70 minutes (Li et.al, 2017).

Michellin and tiexiera (2016) analyzed the effect of using a common pretreatment method with a condition of 190 ℃ for 30 minutes on 4 different feed stocks namely corn husk, corn cob, wheat straw, brewers spent grain and luffa sponge. This was carried out in a cylindrical reactor with some working volume. The product was then filtered and insoluble were separated from the slurry. The insoluble were then dried and further used for product recovery. As a result, it was found that the liquid hot

water treatment did remove a huge portion of the hemicellulose from the feed stocks. While the cellulose and lignin amounts increased.

According to Wang et al. (2016), liquid hot water pretreatment of alkali lignin at a temperature of 180℃ and a pressure of 4 MPa for 20 minutes redistribute the structure of the lignin resulting in a structure favorable to influence by enzymatic hydrolysis. The surface area, total pore volume and average pore size increased. The increase in surface area meant more contact area and adsorption of cellulase enzyme onto the substrate. As a result, better enzymatic hydrolysis of cellulose was obtained.

2.8.1.3 Chemical pretreatment

Chemical pretreatment is a type of pretreatment that involves chemicals and as a result chemical reactions occur between the feedstock and the chemical added. Rezania et al. (2017) stated that 4 types of chemical pretreatment were available: Acid pretreatment, alkali preatreatment, ionic liquid pretreatment and microwave-assisted pretreatment. According to the author, eventhough each one of the pretreatments had their own benefits, the most efficient was dilute acid pretreatment. This is also an environmentally sound process.

- **Acid hydrolysis**

Acid hydrolysis is a process where Lignocellulosic structure is dissembled by the use of acid, either in its concentrated form or diluted form. Dilute acid hydrolysis is used in order to break down or degrade hemicellulose into fermentable sugar because hemicellulose is soluble in acid. After hydrolysis of hemicellulose, the cellulose will be exposed for degradation by enzymatic hydrolysis (Balat and Balat, 2009). Since hemicellulose is highly soluble in acid, the recovery of a high hemicellulose amount (>90%) is possible with this process. Its effectiveness in fermentable sugar recovery (both xylose and glucose) makes it preferable. But disadvantages like formation of inhibitors and the need for neutralization of the pH for enzymatic hydrolysis also exist. It is also not effective in dissolving lignin but it can weaken its structure and cellulose can be accessed by enzymatic hydrolysis (Rezania et al., 2017). According to Kang et al. (2014), the US national renewable energy laboratory suggests the hydrolysis of Lignocellulosic biomass in acid with a concentration of 0.5-1%, and at a temperature of 160-190℃ for about 10 minutes.

The most applied type of acid is sulfuric acid but hydrochloric acid and nitric acid can also be used. The dilute acid hydrolysis functions under high temperature but low concentration (El-Naggar et al., 2014). Amin et al., (2017) suggests that dilute acid with concentration ranging from 1-4% (w/w) is also applicable. It should be noted that if high temperature is applied the hydrolysis time will be shorter and vice versa.

- **Alkaline hydrolysis**

Alkaline pretreatment, as the name indicates, is a pretreatment that uses base as the chemical for structure disruption. The most commonly used bases are ammonia, sodium hydroxide and calcium hydroxide. The lignocellulosic structure recalcitrance is reduced as a result of saponification of hemicellulose acetyl and lignin-carbohydrate complex linkages as well as the result of ammonolysis in the presence of liquid ammonia. Ammonia fiber expansion (AFEX) is a process of recalcitrance reduction by utilizing concentrated liquid ammonia and water mixture at a temperature of 60-140℃. AFEX is a dry-to-dry process i.e. there will be no liquid to solid separation. Since ammonia is volatile, it is relatively easier to recover and reuse(Limited, 2010).

2.8.2 Hydrolysis

Hydrolysis, usually known as enzymatic hydrolysis, is an important step in bioethanol production from Lignocellulosic biomass because it is a step that determines how much glucose will move on to the fermentation step. Due to different pretreatment methods, monomers of cellulose and hemicellulose are recovered which then will move onto the hydrolysis step so that they are converted to fermentable sugars by using enzymes. Enzymatic hydrolysis breaks down the cellulose due to the unified action of multiple enzyme components having different mechanisms of action. Hydrolytic enzymes like celluclast, novozyme188, accellerase, cellic ctec2, speczyme cp and cytolase CL are some of the commercially prepared ones.

Cellulose enzyme is composed of components like endoglucanases, exoglucanases and β-glucosidases and each components fraction depends on the sources of the enzyme. These components degrade the cellulose into glucose step by step:

- Endoglucanases bind to cellulose to expose reducing and non-reducing ends forming cello oligomers as a result.

- Exoglucanases bind to reducing and non-reducing ends of the cello oligomers and converts it to cellobiose.
- The β-glucosidase then converts the cellobiose to glucose.

Inhibition of the hydrolysis process could be caused by the accumulation of cellubiose due to insufficient amounts of β-glucosidases in the enzyme. Other inhibitors could be xylose, glucose and cello oligomers. It is also important to maintain optimal temperature and pH with values that ranges between 40 and 55℃ and 4.5 to 5.5, respectively (Shastri, 2016).

2.8.3 Fermentation

This is the conversion of glucose sugar ($C_6H_{12}O_6$) to alcohol (C_2H_5OH) and carbon dioxide gas (CO_2) constitutes the overall chemistry process of fermentation (Balat and Balat, 2009). Bioethanol fermentation uses yeast for the fermenting process. Here, the cellulose is converted or fermented to ethanol at optimum temperature of 32-35℃. The most commonly used yeast is *saccharomyces cerevisae* but other yeasts like *candida shehatae*, *zymomonas mobilis*, *pichia stipilis* etc.… can also be used as needed (Manzanares, 2010).

2.8.4 Product separation and dehydration

This is an important step because, at the end of the day, the bioethanol produced will contain some amount of water and will form an azeotropic mixture (Busic, 2018). An azeotropic mixture is a mixture of liquids that boil at constant temperature like pure liquid and possess same composition of components in liquid as well as in vapor state (emedical prep, n.d.). Usually, distillation is used for solutions that have one part more volatile than the other. But in azeotropic mixtures, the vapor and fluid concentrations are the same so it will not work here. If a mixture deviates from raoult's law, which states that "a solvent's partial vapor pressure in a solution (or mixture) is equal or identical to the vapor pressure of the pure solvent multiplied by its mole fraction in the solution", then azeotropes are formed. There are different types of azeotropic mixtures: minimum boiling azeotropes (negative azeotrope), maximum boiling azeotrope (positive azeotrope), heterogeneous and homogeneous azeotropes and azeotropes based on the number of constituent i.e. binary, ternary and so on. The mixture of ethanol and water we find at the end of bioethanol production is a binary, maximum boiling, homogeneous azeotropic mixture. So to separate this type

of mixture, conventional distillation is not effective rather we use azeotropic distillation, where a new component known as entrainer is added in order to form a lower boiling azeotrope of the heterogeneous solution with one or more of the feed component (byju's the learning app, 2020).

Table 2. 3. Summary of water hyacinth pretreatment and conversion technologies with their respective advantages and gap analysis

Pretreatments	Conversion technology	Advantages and gap analysis	References
Size reduction Dilute acid hydrolysis (optimized)	• Dilute acid hydrolysis for fermentable sugar recovery	• High reducing sugar yield with high lignocellulosic structure degradation • Gap: bioethanol production after fermentable sugar recovery	(Reales-Alfaro et al., 2013)
Dilute acid hydrolysis Alkaline pretreatment Heat treatment Combined pretreatment	• Enzymatic saccharification • Fermentation	• The best pretreatment was acid hydrolysis at high temperature • Gap : investigating pretreatments that are less environmentally detrimental	(Das et al., 2016)
Alkaline pretreatment	• Enzymatic Hydrolysis • Fermentation	• Lignin was removed after alkaline pretreatment which improved cellulose degradation • Gap: hemicellulose degradation can increase the reducing sugar yield.	(Das et al., 2015)
Dilute acid hydrolysis using different acids Alkaline pretreatment using different bases	• Reducing sugar recovery	• Highest reducing sugar yield was obtained by dilute acid hydrolysis. • Gap: integration of low cost pretreatments would have higher sugar yield.	(Awasthi et al., 2013)
Acid hydrolysis	• Simultaneous saccharification and fermentation	• The merging of the processes results in lower cost and higher yield but the process is easily contaminated • Gap: separate hydrolysis and fermentation with other pretreatments	(Zhang et al., 2016)

Acid pretreatment using H_2SO_4	• Simultaneous saccharification and fermentation • Addition of different enhancers such as fermented malt extract (FME), fermented barley extract (FBE), horse dung (HD), saccharomyces cerevisae (SC) and mixture of all enhancers	• High sugar consumption and ethanol yield was obtained when mixture of all the enhancers was used. • Gap: focus on microbial action might be a bottleneck due to its longer time requirement so a more controllable process can be employed and investigated	(Rezania, 2018)
Simple sterilization Dilute acid hydrolysis Liquid hot water pretreatment Biological pretreatment	• Simultaneous saccharification and fermentation • On-site enzyme production from *Trichoderma reesei* (TR) and *Aspergillus niger* (AN)	• In dilute acid hydrolysis, high sugar recovery was obtained. • Lower sugar yield in LHW and simple sterilization • Gap: investigation of combining two steps to have higher sugar recovery	(Bani, 2015)
Alkali pretreatment	• Separate saccharification by *Aspergillus niger* • Fermentation by *Saccharomyces cerevisae*	• High yields of ethanol but very time consuming since microbes are being using. • Environmentally friendly processes • Gap: investigation of fermentation yield by using SC	(Shah et al., 2014)

3 Materials and Methods

3.1 Overview of bioethanol production process from water hyacinth

During the production of bioethanol from water hyacinth, 5 general process steps were followed for the present research work. The main process steps were collection, preparation, pretreatment, saccharification and fermentation and distillation. Figure 3.1 shows the detailed process steps followed in order to obtain bioethanol from water hyacinth.

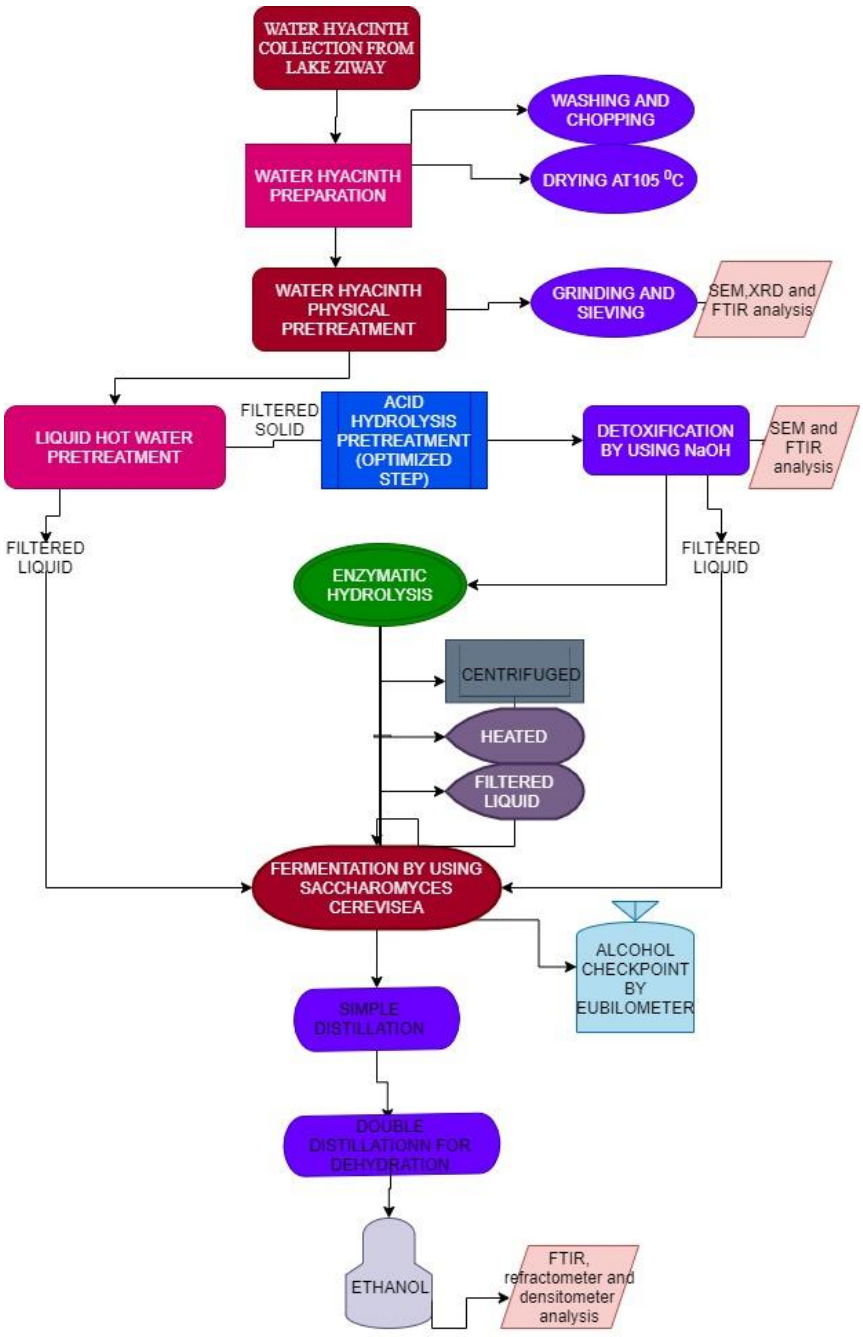


Figure 3. 1. Overall summary of the methodology

3.2 Materials and chemicals

The raw material used in the present research is water hyacinth which is collected from Ziway lake water body located at 7.933 Latitude and 38.716 Longitude east shewa zone in Oromia regional state 168 km from Addis Ababa. It was collected manually and kept in poly bag with internal lining and brought to chemical engineering department postgraduate laboratory for further preparation.

The chemicals used in the present study are reagent grade chemicals and involve acetone (≥ 99.5 %v/v, RANCHEM), cellulose'ono zuka', ex. trichoderma virde, 10 units/mg, dextrose anhydrous (NEOLAB), 3,5-dinitrosalicylic acid (reagent grade), sodium bisulfate, sodium-potassium tartarate (NEOLAB), ethanol (≥ 99.5 %v/v, RANCHEM), d-glucose (LOBACHEMIE), magnesium sulfate, heptahydrate (≥ 99 %w/v, NEOLAB), sodium acetate anhydrous (≥ 99 %w/v, CENTRAL DRUG HOUSE), sodium hydroxide (≥ 99 %w/v, NEOLAB), starch soluble (ex. potato), sulfuric acid (≥ 98 %v/v, CARELABMEB), urea (≥ 99 %w/v, NEOLAB), yeast extract (≥ 99 %w/v, LOBACHEMIE) and yeast powder (≥ 99.5 %w/v, FINKEM).

Different laboratory equipment and instruments are utilized in this research. The major equipment and apparatuses are muffle furnace (MV 106, NUVE), UV-Visible Spectrometer (UV-1800, SHIMADZU), scanning electron microscope (SEM) (FEI, INSPCT-F50, Germany), fourier transform infrared radiation (FTIR) (Thermo Scientific is50 ABX), x-ray diffractometer (XRD), soxhlet extraction unit (BST/SXM-6, BIONICS), EBULLIO meter (Bulteh 2000, EON TRADING), density meter (Densito 30 PX), refractometer (DE 75167706, KRUSS optronic), dry oven (TD-1315, GENLABPRIME), centrifuges (Pro-Analytical C2004, , CENTURION scientific), autoclave, analytical Balance (AD300-3), sieve shaker (IC-205/EV), grinder (NIMA, Japan /220v/50-60Hz), magnetic stirrer with hot plate (MS-H280-PRO), vacuum pump (MZ 2C NT), water bath (BM 30) and other glass ware like beaker, flask etc.

3.3 Raw material treatment and preparation

In the preparing of water hyacinth, the roots were removed by using a knife since only the shoot and leaves were used for the research work. The shoots and leaves were then washed by tap water to remove all unwanted dirt and other materials. After cleaning, it is chopped into an average of 2 cm length in order to enhance moisture removal during oven drying as presented in Figure 3.1 (a). The pieces of chopped water hyacinth was put in an oven adjusted to 105± 3℃ until the mass of the precursor is remained unchanged that takes three to four hours as illustrated in Figure 3.1 (b). The oven dried water hyacinth is further ground to about 0.4 – 1 mm using a grinder (NIMA, Japan /220v/50-60Hz). The size reduced and dried water hyacinth is ready for further analysis.

Figure 3.2. Size reduced water hyacinth (a) before and b) after drying

3.4 Proximate analysis of water hyacinth

The lignocellulosic feedstock needs to be analyzed for its suitability to be used as a bioethanol fuel source before further processing. This analysis involves the determination of the amount of moisture, volatile matter, ash and fixed carbon. This was done by following standard procedure from the ASTM standards (D 3173- D 3175). The bulk density was also investigated.

3.4.1 Moisture content determination

In order to determine the moisture content, the sample tray was dried separately in order to remove any moisture adhering on the tray so that only the moisture from the water hyacinth can be investigated. 15 g of chopped water hyacinth sample was measured and placed on the tray. The sample was prepared by removing off the roots and chopped into smaller pieces. It was then put on a tray and put into an oven adjusted to a temperature of 105±3℃ for 24 h. The mass of sample was measured

every 4 h in order to use gravimetric method to determine the moisture content according to ASTM D3173 as shown in equation (3.1)(ASTM standard D 3173 – 03, 2013).

Moisture Content (MC) = $\frac{M_i - M_f}{M_i}$ 3. 1

Where:M_i: initial mass when putting in the oven (before being dried).

M_f: Final mass after drying the biomass for 24 h at 105±3^{0}C.

3.4.2 Volatile matter determination

The investigation of volatile matter was carried out by measuring 1.5 g of prepared water hyacinth sample. Ceramic crucible was used as sample tray. Any moisture contaminant in the crucible was removed by putting the crucible in the muffle furnace for about 30 min until constant mass is obtained. Then the measured sample was placed into the crucibles and put in the muffle furnace. The muffle furnace was set to a temperature of 950±50^{o}C and the sample was left in there for 7 min. The crucible was then taken out of the furnace and put into a desiccator in order to avoid moisture contamination (absorption) by the sample during cooling. This analysis was done in triplicates. It was then weighed and recorded. The volatile matter was determined using equation (3.2) (ASTM standard D 3175 – 89a, 2013).

$$VMC\% = \frac{W_{ods} - W_{mfs}}{W_{ods}} \times 100\% \quad 3.\ 2$$

Where: VMC%: percentage volatile matter content

W_{ods}: weight of oven dried sample

$W_{mfs:}$ weight of the sample after it is burnt in the muffle furnace

3.4.3 Ash content determination

Investigation of ash content was conducted by taking 1.5 g of water hyacinth sample. The sample was put into a crucible and placed in the muffle furnace. The muffle furnace was set at a temperature of 550±50^{0}C and left for 3 h. After the 3 h, the crucible was taken out of the furnace and placed in the desiccator for cooling and avoiding moisture contamination. After the sample was cooled to room temperature, the sample was weighed and recorded. This was also done in triplicates as well. The ash content was determined using equation (3.3)(ASTM standard D 3174 – 02, 2013)

$$AC\% = \frac{W_{AR}}{W_{ods}} \times 100\% \quad 3.3$$

Where AC%: percentage ash content

W_{AR}: weight of ash residue

3.4.4 Fixed carbon determination

The percentage of fixed carbon composition was also investigated by subtracting the percentage of moisture content, volatile matter and ash content of the sample from the total composition of the sample using ASTM standard D 3172 – 89 (Reapproved 2002)(ASTM standard D 3172 – 89 R02, 2002).

3.4.5 Investigation of Bulk density

According to the standard method set by ASTM 7481, bulk density was measured by using a glass measuring cylinder of 50 milliliters. The empty measuring cylinder was weighed first. Then the water hyacinth powder was packed into the measuring cylinder without leaving any space for air and weighed again. Finally the bulk density was determined by using the density formula i.e. the mass divided by the volume as shown in equation (3.4).

$$\rho\left(\frac{kg}{m^3}\right) = \frac{M_{pc} - M_{ec}}{V_f} \quad 3.4$$

Whereρ: is the bulk density

M_{pc}: is the weighed mass of the packed cylinder (Kg)

M_{ec}: is the weighed mass of the empty cylinder (Kg)

V_f: is the volume of the volumetric flask (m^3)

3.5 Determination of Lignocellulosic components (cellulose, hemicellulose, lignin and extractives)

The determination of bioethanol potential of a Lignocellulosic biomass relies on the amount of cellulose, hemicellulose, lignin as well as extractives (fats, proteins, waxes, chlorophyll and so on) and ash present in the biomass. Therefore, it is important to investigate the composition of each component in the water hyacinth (Malveaux, 2013).

3.5.1 Extractives determination

Determining the extractives in the specific biomass helps in the determination of other constituents of lignocellulosic biomass. Extractives were determined by using a Soxhlet apparatus in two consecutive stages. 5 g of prepared sample of water hyacinth was placed into a filter paper. The sample was put into the Soxhlet chamber equipped with a condenser on top of it and a three necked round bottom flask on the bottom as presented in Figure 3.2. The extraction was conducted using ultra-purified water for 5 hours. The heating mantle was used to supply heat for the extraction and the temperature inside the solution was maintained near the boiling point of water (Toribio-cuaya et al., 2014). The remaining un-extracted sample was recovered from the filter paper, dried and measured to record the amount of extracted matters using water as a solvent. The dried sample was then further extracted using acetone as a solvent in Soxhlet apparatus. The extraction process using acetone as a solvent, the temperature in the solution is adjusted around the boiling point of acetone (70^{o}C). The extraction was conducted for duration of 4 h. After the extraction, the remaining biomass was sun dried to let acetone evaporate from the sample for about 1 h. The sample was further dried in an oven until it reached a constant weight. The amount of extractives was determined by subtracting the mass of remaining biomass after both water and acetone solvent extraction from the original sample mass(Ayeni et al., 2015).

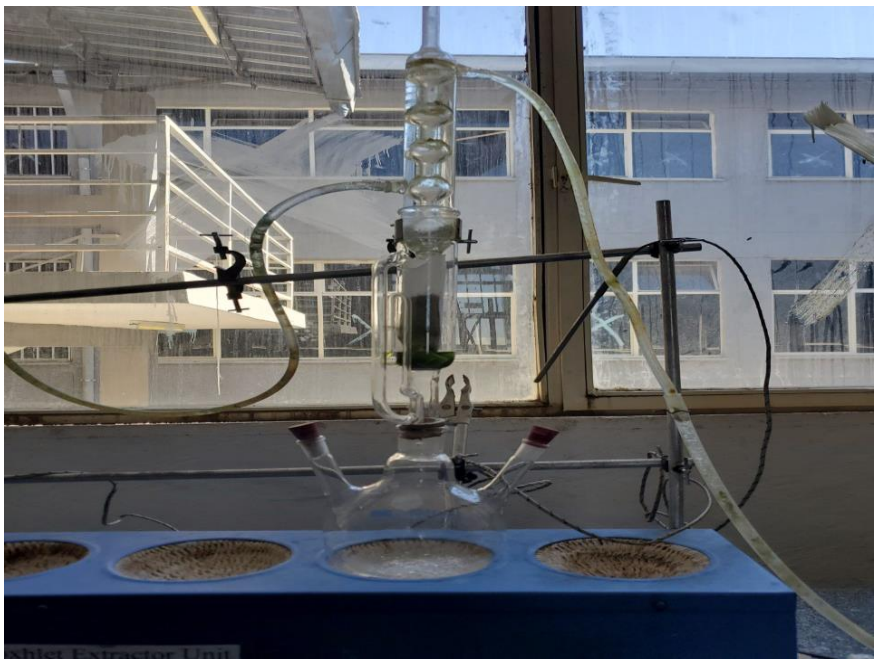


Figure 3. 3. Soxhlet extraction process of extractives

3.5.2 Hemicellulose

In the investigation of hemicellulose, 2 g of biomass recovered after extraction was place inside a 250 mL Erlenmeyer flask. Then 200 mL of sodium hydroxide solution at a concentration of 500 mol/m^3 was added to the flask and boiled for 3.5 h. After boiling, it was cooled to room temperature and filtered using a vacuum filter. The biomass solid recovered after filtration was dried in an oven adjusted to a temperature of 105^0C until constant mass was achieved. Finally the hemicellulose amount was determined by subtracting the final mass of the solid from the original extractive-free biomass(Ayeni et al., 2015).

Figure 3. 4. Hemicellulose determination using a hotplate and an Erlenmeyer flask

3.5.3 Lignin

Investigation of the lignin amount was done by using a 2 step hydrolysis process. A gram of the extractive-free biomass was measured and transferred into a 250 mL Erlenmeyer flask. Then, 10 mL of 72 %(v/v) sulfuric acid was added into the flask to produce slurry. It was kept at room temperature of 25^oC for 2 h while shaking with 30 min interval for complete hydrolysis. In the second step, diluted acid hydrolysis was started by adding 280 mL of distilled water into the slurry in order to reduce the concentration of acid solution to 4 %(v/v). The solution was autoclaved at a temperature of 120^0C for 1 h. The liquid hydrolysate was cooled to room temperature

and filtered by using an ash less filter paper. After filtering out, the solid residue was dried until constant mass was achieved in an oven at 105^0C. The final mass was measured and recorded. The ash was then determined by incinerating the solid residue in a muffle furnace at a temperature of 575^0C for 3 h. Lignin was investigated as acid soluble lignin and acid insoluble lignin.

The acid insoluble lignin is a residue recovered as a solid. Acid insoluble lignin is further incinerated for the investigation of ash in the insoluble lignin which is in turn used to analyze the inorganic components of lignocellulosic biomass. The liquid hydrolysate was analyzed by the UV-spectrophotometry. The absorbance was measured at a 320 nm wave length. The total lignin amount was determined as the summation of the acid soluble lignin and acid insoluble lignin according to the national renewable energy laboratories (NREL) laboratory analytical procedure (LAP) (Sluiter et al., 2012).

3.5.4 Cellulose

The amount of cellulose was investigated by simply subtracting the amount of extractives, hemicellulose, lignin and ash obtained from the original mass used as the quantity other constituents are assumed to be insignificant(Ayeni et al., 2015).

3.6 Pretreatment of water hyacinth

3.6.1 Liquid hot water pretreatment

Hot water pretreatment is used to expose the cellulose for enzymatic hydrolysis by removing the hemicellulose and lignin present in the water hyacinth by rupturing and loosen the cellulose structure resulting in dissolving of suture constituents into the hot water**.** 50 g of prepared water hyacinth sample was put into a 2000 mL Erlenmeyer flask and mixed with 1750 mL of distilled water; the flask was covered by aluminum foil for pretreatment in autoclave. The sample is then placed in autoclave with hot water that was adjusted to a temperature of 160**°**C for 2 h. The flask was withdrawn from the autoclave and cooled at room temperature. The sample solution was filtered using vacuum filtration. The glucose present in the filtrate solution was investigated and recorded. The liquid solution was then put into a 250 ml sample holder bottle screw caped and stored in the refrigerator adjusted at 4^0C for further use during investigation of fermentation process. The solid recovered after filtration was dried using an oven set at 105^0C for dilute acid hydrolysis.

3.7 Dilute acid hydrolysis

In the dilute acid hydrolysis, sulfuric acid at a concentration of 98 % (v/v) was diluted to a lower concentration of 1 to 2.5 %(v/v). The diluted acid hydrolysis was conducted by varying the acid concentration from 1 to 2.5%(v/v) at the interval of 0.25%, hydrolysis time from 75 to 105 min at the interval of 5 min and hydrolysis temperature of 115 to 145°C at the interval 0f 5°C as indicated in Table 3.1. The effect of each variable was investigated while keeping the other variables constant.

Table 3. 1. Levels of variables for the one variable at a time analysis

Variables	Levels						
Acid concentration (%)	1	1.25	1.5	1.75	2	2.25	2.5
Hydrolysis temperature(°C)	115	120	125	130	135	140	145
Hydrolysis time (min)	75	80	85	90	95	100	105

3.8 Investigation of the individual and parametric interaction effect on dilute acid hydrolysis and statistical optimization

Investigation of the individual and cross effect of parametric interaction is near impossible using conventional method as well as the optimum operating condition due to cross influence of different variables. The implementation of statistical methods helps in understanding interactions among variables with minimum number of experiments that need to be performed. It also used to identify the optimum process variables. Response surface methodology (RSM) is one such widely applied statistical tool for experimental design and identification of optimal condition. In the present work technique of RSM with central composite design (CCD) was used for experimental design to study the individual and interaction effects of reaction variables and determine the optimum reaction condition.

The experimental results were fitted using a polynomial quadratic equation in order to correlate the response variables. As three factors were under investigation, a quadratic model is adequate enough to investigate the interaction of squared terms and parametric cross effects. The general form of the polynomial quadratic equation shown in equation (3.5) was used to develop a model that predicts (estimates) the response at designed variable combination.

$$Y_i = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} X_i X_j \quad 3.5$$

Where: Y_i is the predicted response and X_i is the input variables. The term β_o is the offset term (intercept), β_i is the linear terms, β_{ii} is the squared terms and β_{ij} is the interaction terms and X_j is the cross term to represent two-parameter interactions (Satari Baboukani et al., 2012;).

The variable X_i was coded according to equation (3.6).

$$x_i = \frac{X_i - X_i^*}{\Delta X_i} \quad 3.6$$

Where: x_i is the coded value of the i^{th} variable, X_i is the natural value of the i^{th} variable, X_i* is the central value of X_i in the investigated area, and ΔX_i is the step size.

The statistical significance of the mathematical model equation was tested using analysis of variance (ANOVA) with 95% confidence intervals. In the present study the parameters used for the diluted acid hydrolysis were acid concentration (X_1), hydrolysis temperature (X_2) and hydrolysis time (X_3). The statistical tools of Design expert version 12 was used for the experimental design. The minimum and maximum values of the factors were determined from the results obtained during conventional experimental investigation of the effects of these factors as presented in section 3.6. Table 3.2 illustrates the minimum, center point and maximum value of the parameter.

Table 3. 2. The minimum, center point and maximum value of the parameter

Factors			**Levels**		
Code	**Name**	**Units**	**Low**	**Central**	**High**
A	acid concentration	%	1.50	1.75	2.00
B	hydrolysis time	min	85.00	92.50	100.00
C	hydrolysis temperature	ºC	125.00	132.50	140.00

In this design, full factorial of 2^n+2n+c was used where c is the number of center points and n is the number of factors. A total of 20 experiments was design as shown in Table 3.3. In this experiment 6 center points were repeated to observe the repeatability of the experiment (Benjamin et al., 2014).

Experiments carried out as a function of the un-coded variables driven by central composite design technique along with the observed response for the experimental investigation is presented in Table 3.3.

Table 3. 3. Experimental design matrix of the parameters with Central Composite Design

Run	A:acid concentration %(v/v)	B:hydrolysis time (min)	C:hydrolysis temperature (°C)
1	1.5	100	125
2	1.75	92	132
3	1.75	105	132
4	1.33	92	132
5	2.17	92	132
6	2	100	125
7	1.75	92	132
8	1.75	92	120
9	2	85	140
10	2	85	125
11	1.5	85	140
12	1.75	92	132
13	1.75	80	132
14	1.5	85	125
15	1.75	92	132
16	1.75	92	132
17	1.5	100	140
18	1.75	92	132
19	1.75	92	145
20	2	100	140

In order to optimize the factors affecting the dilute acid hydrolysis process, every experiment stated in Table 3.4 was performed by measuring a gram of the LHW pretreated water hyacinth in to a 250 mL Erlenmeyer flask and adding 35 mL of diluted acid specified by each trial. The solid to liquid ratio used was 1:35 since a slurry-like mixture was found at this combination. A fresh batch of dilute acid solution was used for every run. Finally, the product was filtered and the glucose concentration in the liquid filtrate after each run was determined by using DNSA method (Chiaramonti et al., 2012).

3.9 Determination of reducing sugar amount

Reducing sugars are monosaccharide carbohydrates that can be fermented into bio-ethanol. These monosaccharaides are needed to be present in adequate amount in order to have an ethanol yield of ≥ 5%v/v. Therefore, in this work, to investigate the amounts of reducing sugar present in the hydrolysate solution, the dinitrosalicylic acid method was used (Miller, 1959).

The DNSA reagent was composed of 2 g of 3, 5-dinitrosalicylic acid, 2 g of sodium hydroxide, 0.05 g of sodium sulfite and 4 g of sodium potassium tartarate (also known as Rochelle's salt) and a 100 mL of the reagent was prepared (Miller, 1959).

A liter of 1 mg/mL glucose stock solution was prepared by mixing 1 g of glucose with 1 L of distilled water. In the preparation of standard solution, 40, 80, 120, 140 and 200 μL of glucose stock solution was transferred to a test tube and mixed with distilled water until all test tube become 200 μL. After the preparation of the standard solution, similarly the main hydrolysate sample was prepared. The preparation was conducted by transferring 200 μL hydrolysate sample into labeled test tube. 0.5 mL of the DNSA reagent was added to all standard solution and hydrolysate samples in the tubes. The samples were well mixed to achieve homogeneous mixture. The mixture is then placed in a water boiling bicker for 5 min. After cooling the samples, UV-spectrophotometer (UV-1800) was used to measure the absorbance of the sample in each test tubes at 540 nm (Zhang et al., 2016). From the absorbance of each test tube, the reducing sugar was estimated by using the "Trend" function in Microsoft excel.

3.10 Detoxification after Acid Hydrolysis

Detoxification is the neutralization of the acid that was used during the dilute acid hydrolysis in order to avoid further degradation of the glucose and other reducing sugar, obtained in the liquid hydrolysate, into furfural and hydroxymethyl furfural which could inhibit the fermentation process(Dussán et al., 2014). The liquid hydrolysate was detoxified by using 2 M sodium hydroxide to a neutral pH of 7(Amit Ganguly et al., 2013).

3.11 Enzymatic hydrolysis or saccharification

During the enzymatic hydrolysis or saccharifcation, cellulase enzyme was used to break down the cellulose in the pretreated water hyacinth. Since the cellulase enzyme was available in solid form and in small amount (1 g), diluting it was necessary using sodium acetate buffer (1 M and pH= 4.5). The sodium acetate buffer was prepared by adding 1.969 g (0.0327 M) of glacial acetic acid to 800 mL of distilled water and then by adding 5.524 g of sodium acetate. The pH of the solution was adjusted to 4.5-5.5 and mixed well. . The sodium acetate buffer and the materials to be used were sterilized in an autoclave for 15 min at 121^0C. After it was cooled down to room temperature, 0.1 g of an antibiotic (doxycycline) was added to the sodium acetate buffer to avoid contamination of the enzyme by bacteria and other microbes.

The acid pretreated and detoxified water hyacinth was washed three times by the acetate buffer in order to bring the pH of the sample to about 5. The washed water hyacinth was dried at 105^0C until constant weight was achieved. The celluase enzyme (Cellulose'ono zuka', ex. *Trichoderma virde*, 10,000 Units/g of biomass or 10 Units/mg) was then prepared with the concentration of 0.1 %(w/v). 20 g of the dried water hyacinth was mixed with 400 mL of enzyme solution and the mixture was put in a shaker incubator at 50 $\pm 2^0$C for 72 h at 100 rpm. A small sample was withdrawn at every 12 h interval for reducing sugar determination(Aswathy et al., 2010; Kumari et al., 2014). After 3 days of saccharification, the slurry was sterilized at 121^0C for 15 min in order to deactivate the cellulase enzyme. The mixture was then filtered and the liquid was collected to analyzed glucose using DNSA method(saha et al., 2014).

3.11.1 DNSA method for reducing sugar determination in enzymatic hydrolysis

When investigating the amount of reducing sugar produced during the enzymatic hydrolysis, it is necessary to generate a standard glucose curve. The DNSA reagent (Miller, 1959) and the sodium acetate buffer (1 M and pH=4.5-5) were prepared. The enzyme was diluted by using 1 mL of the acetate buffer in order to prepare the enzyme control solution that is useful in avoiding the reading of the enzyme effect in the sample that is measured. The glucose stock solution was readied by adding 1 g of anhydrous glucose to 1 L distilled water(Adney & Baker, 2008).

Table 3.3. Preparation of glucose standards, control and enzyme solution for standard curve generation

Test tubes	Volume of glucose added(mL)	Volume of acetate buffer added(mL)	Volume of DNSA added (mL)
Reagent blank	0	1.5	1
Enzyme control	0.5	1.0	1
1	0.2	1.3	1
2	0.4	1.1	1
3	0.6	0.9	1
4	0.8	0.7	1
5	1	0.5	1
1.5 mL of Samples with unknown concentration of glucose to be determined and 1 mL DNSA reagent are added to each.			

The glucose standard solutions were prepared by transferring 0.2, 0.4, 0.6, 0.8 and 1 mL in different test tubes and adding acetate buffer to each tube to make up 1.5 mL of the solution as shown in Table 3.6. Then 1 mL of the DNSA reagent was added to each test tube. The glucose standard solution test tubes as well as the sample test tubes were boiled for exactly 5 min after screw capping them. They were then cooled to room temperature and transferred into cuvettes so that the absorbance of each solution was measured at a wave length of 540 nm. The measured absorbance was used to estimate the unknown concentration using Microsoft excel (Adney & Baker, 2008).

3.12 Active yeast media preparation (Yeast inoculation)

Yeast inoculation is a primary task in the fermentation of fermentable sugar into bioethanol. In this task, the preparation of the active yeast media for *saccharomyces cerevisae* was started by sterilizing necessary equipment in an autoclave set at 121℃ for 15 min in order to kill micro-organisms attached in the equipment as well as prevent any microbial contamination that may lead to unnecessary side product. After sterilization of equipment and apparatus, the yeast culturing and growth medium was prepared. In the preparation of yeast culturing medium, 2.5 g of peptone that served as nutrient for the yeast (nitrogen source), 5 g of dextrose that served as sugar source, and 2.5 g *saccharomyces cerevisae* (commercial yeast extract) were placed in 250 mL distilled water. Supplementary nutrient sources of 2 g magnesium sulfate heptahydrate and 2 g urea were used to enhance their growth and activity in the

extraction of enzyme zymase. This solution was then stirred at 120 rpm and autoclaved at 120^0C for 15 min. After cooling it down to room temperature, 1 g of the yeast powder was added in order to initiate the preferment ion processes which was termed as pre-fermented mesh, it was incubated for 24 h in a shaker incubator adjusted at 150 rpm.

3.13 Fermentation for ethanol production and distillation processes

After the preparation of the mesh, 250 mL borosilicate bottle with a screw cap was used as a fermentation tank after sterilization. In the fermentation tank, 50 ml of treated liquids that were recovered during the liquid hot water pretreatment, dilute acid hydrolysis (after neutralization) and enzymatic hydrolysis were added to the fermentation broth. The liquid was then placed in autoclave adjusted to 120^oC for 15 min in order to kill and avoid microbial activities. The liquid was then cooled to room temperature and 5 ml of doxycycline as antibiotic solution and 20 mL of prepared mesh from pre-fermenter which serves as activated *saccharomyces cerevisae* that was used to convert fermentable sugar into ethanol were added. Stochiometrically, from one mole of reducing sugar, two mole of ethanol and two moles of carbon dioxide is generated. This reaction is exothermic reaction and hence heat is generated. On the other hand, the yeast is active in the temperature range 28^oC to 38^oC. Therefore, generated heat need to be continuously removed to maintain the temperature in the required range through cooling processes. In this work, the mixture was put in a shaker incubator adjusted at 30 ± 2^0C and 150 rpm (Idrees et al., 2013) in which fermentation was conducted. The conversion of sugar into ethanol was checked every 24 h to observe the rate of converting sugar into ethanol. It was observed the fermentation processes was completed after 72 h. The bioethanol concentration produced during fermentation was measured using eubliometer after the broth was centrifuged and the aliquot was recovered. The eubliometer was first standardized by distilled water in order to make it auto-zero and then alcohol percent in the fermentation broth was measured by using their boiling point. After the alcohol content of the broth was determined, the fermented mesh was ready for distillation process. The fermented and centrifuged liquid is then double distilled in laboratory distiller (simple distillation column). Ethanol recovered was then put in screw caped sample holder for further investigation.

3.14 Characterization for the raw material, intermediate product and final product

During the preparation of raw material and after each step of bioethanol production, analysis of what is present should be done. This is where characterization comes in handy.

3.14.1 Scanning electron microscope

SEM was employed, during this specific work, to see the surface morphology change of the water hyacinth before and after pretreatment. The water hyacinth before and after pretreatment was each plated on an aluminum stub coated with a carbon conductivity tape sample holder. The sample holder was then inserted into the racks of the SEM (FEI, INSPCT-F50, and Germany). The images were generated with 20 kv power, 12.2 mm working distance, magnifications of 3944x and 7234x and scales of 20 and 40 μm.

3.14.2 X-Ray Diffraction analysis of raw water hyacinth

The crystallinity of raw water hyacinth was measured after physical pretreatment (size reduction) by using an x-ray diffractometer (XRD) with voltage and current of 40 kv and 30 A, respectively. Scan range was between 5 and 50 degrees 2θ. The scan speed was 2 degrees/min while the sampling pitch was 0.02 deg. The crystallinity index, crystallite size and the d-spacing were determined using the equations (3.7-3.9), respectively

$$CI(\%) = \frac{(I)_C - (I)_{amp}}{(I)_C} * 100 \qquad 3.7$$

$$\beta = \frac{(k)(\lambda)}{(D)(\cos\theta)} \qquad 3.8$$

$$d = \frac{\lambda}{(2)(\sin(0.5\theta))} \qquad 3.9$$

Where, in equation (3.6), CI is the crystallinity index, $(I)_c$ and $(I)_{amp}$ is maximum intensity of crystalline peak and maximum intensity of amorphous peak, respectively. In equation (3.7), β is the crystalline size, k=0.94 is the scherrer constant, λ=0.154 nm, D is the particle thickness and θ is half of the 2θ where the crystalline peak

occurs. In equation (3.8), d is the d-spacing, λ=0.154 nm and θ is half of the 2θ where the crystalline peak occurs.

3.14.3 Fourier transform infrared radiation (FT-IR) analysis

The raw water hyacinth, pretreated water hyacinth and the ethanol were analyzed by using FTIR (Thermo Scientific iS50 ABX) in order to determine the changes that are occurring on the functional groups. The detection was carried out at a wave number between 400 and 4000 cm^{-1} with a detector at 4 cm^{-1}.

3.14.4 Concentration and density determination of ethanol

The final ethanol product was further investigated by using densitometer (densito 30 px) in order to determine its concentration, density and specific gravity at the national alcohol and liquor factory laboratory. The instrument pipettes out about 2 mL of the ethanol product and measures its density by using the oscillating tube method which employs extremely fine capillaries (U-tubed) that oscillate upon addition of sample and induction of piezoelectric or magnetic oscillation. The apparatus can measure density in the range of 0-2 g/cm^3 with a resolution of 0.0001 g/cm^3 and accuracy of $\pm$0.001 g/cm^3. After the density measurement, it estimates the concentration, specific gravity and other properties like API (American petroleum institute) gravity.

3.14.5 Refractive index measurement and brix determination

The refractive index and degree brix of the ethanol and the fermentation broth was determined by using a refractometer (DE 75167706). Approximately 0.3 mL of each solution was, simultaneously, put on the prism sample holder of the refractometer. The refractive index and ^{0}brix were determined by using LED light source and photodetector.

4 Result and Discussion

4.1 Proximate analysis and lignocellulosic component determination of raw water hyacinth

4.1.1 Proximate analysis of water hyacinth

The moisture content of water hyacinth was investigated and found to be 92.78%. The dry mass of water hyacinth recovered after 24 h was 1.084 g which indicates that a large amount of the raw material is required.

The volatile matter present in water hyacinth was investigated. On average, 84.44±3.14 wt.% volatile matter was obtained. The result demonstrated that water hyacinth volatile matter is in accordance with investigations conducted somewhere else(Melane et al., 2017). High volatile matter increases the energy consumption as combustion need to be conducted for extended time to achieve maximum weight loss. The maximum temperature is the parameter used in measuring the reactivity of the sample as it helps to evaluate the anaerobic digestion rate of biomass in the process of biogas production (Cavalaglio et al., 2020). High volatile matter in water hyacinth is an indication of low carbon content and energy content if it is utilized as solid fuel since it produces a lot of smoke(Jimoh & Academy, 2016). Processing of this biomass for liquid fuel such as bioethanol is the preferred way of its utilization.

The ash content of water hyacinth was investigated and the sample was measured to have 6.807 wt.% ash content on average. The ash content is required to be as low as possible since it is the part of the biomass that will not be converted into energy such as bioethanol due to its inorganic nature. Higher amount of ash present in a biomass is an indication of low energy content of the precursor. As compared to similar water weeds such as water lily (15%) and water lettuce (25%), the ash content of water hyacinth is low (Jimoh & Academy, 2016). Other lignocellulosic biomass such as agricultural residues has an ash content that goes up to 15% (Jönsson & Martín, 2016). This indicated that water hyacinth is a good candidate to be used as energy sources such as for the production of bioethanol.

Fixed carbon content is the non-volatile carbon present in the biomass precursor after the removal of volatile mater and ash content. It is an indication of the amount of carbon present in the biomass precursor that can be utilized as energy source. In the

present study, the fixed carbon content of water hyacinth was investigated and found to be 8.75±3.178%. Kang et al. (2014) has conducted the investigation of various biomass fixed carbon including water hyacinth and reported that the fixed carbon of biomass ranges from 5-25 wt.%. They pinpointed that the variation in fixed carbon might be mainly attributed to environmental impact such as climatic and weather condition, nutrient values in the water body at different location, harvesting time and practice and the type of species. This exhibits that the fixed carbon content of the present study is within the limit to be used as energy sources after maximizing its energy value such as bioethanol.

The bulk density of dry and prepared water hyacinth was investigated and found to be 0.435±0.052 g/cm^3. The lower bulk density of dry and prepared water hyacinth is due to the removal of large quantity of water during moisture removal. The lower bulk density of dry and prepared water hyacinth is a good indication of low material handing cost in the processing of bulk density of dry and prepared water hyacinth to useable products such as bioethanol.

Table 4. 1. Proximate analysis results of water hyacinth from the current work and other references

Proximate analysis	This work	A.O. jimoh et al.	Melane et al.
Moisture content (%)	92.35±0.441	82.96-84.21	94.7±0.1
Ash content (%)	6.81±0.123	13.93-19.80	17.1±1.2
Volatile matter (%)	84.44±3.174	57.67-67.08	85.5±0.8
Fixed carbon (%)	8.75±3.178	18.98-22.53	-
Bulk density (g/cm^3)(dry basis)	0.435±0.0519	-	0.0442

4.1.2 Determination of lignocellulosic components

Lignocellulosic feed stocks are composed of extractives, hemicellulose, lignin and cellulose. Depending on the environmental impact such as harvesting time, climatic conditions and nutrient values of the water body, water hyacinth has different proportions of cellulose, hemicellulose, lignin and extractives. Investigation of the amounts of extractives, hemicellulose, lignin and cellulose is essential in the

bioethanol production process as these components are broken into simple sugar or fermentable sugars such as glucose, fructose, xylose, galactose etc. Water hyacinth in the present study was investigated for its constituents of extractives, hemicellulose, lignin and cellulose. The results obtained are 8.92 wt.% extractives, hemicellulose 45.137 wt.%, lignin 12.43 wt.%, and cellulose 33.513 wt.% as illustrated in Figure 4.4. Isikgor & Becer (2015) reported that lignocellulosic biomass generally composed of 35-50% cellulose, 10-25% lignin and 20-30% hemicellulose. This shows that the values of extractives, hemicellulose, lignin and cellulose obtained in the present study are within the range reported by Isikgor & Becer, 2015. It was also reported that the hemicellulose could reach up to 50 wt.% depending in the biomass(Gandhimathi, 2017). Similarly the proportions of extractives, hemicellulose, lignin and cellulose in water hyacinth can vary due to environmental impact such as climatic and weather condition, nutrient values in the water body at different location, harvesting time and practice and the type of species.

As reported by haghighi et al. (2013), the proportion of the lignocellulosic constituent of a biomass plays an important role in the efficiency of pretreatment, enzymatic hydrolysis and bioethanol production processes. The authors stated that there should be less lignin and extractives present in the lignocellulosic biomass. In this particular work, it is proven that the relative amounts of hemicellulose and cellulose in water hyacinth is greater than that of extractives and lignin which entails there is more potential for the water hyacinth to be broken down to reducing sugar than that of other undesirable products which in turn favors that production of bioethanol(Haghighi et al., 2013).

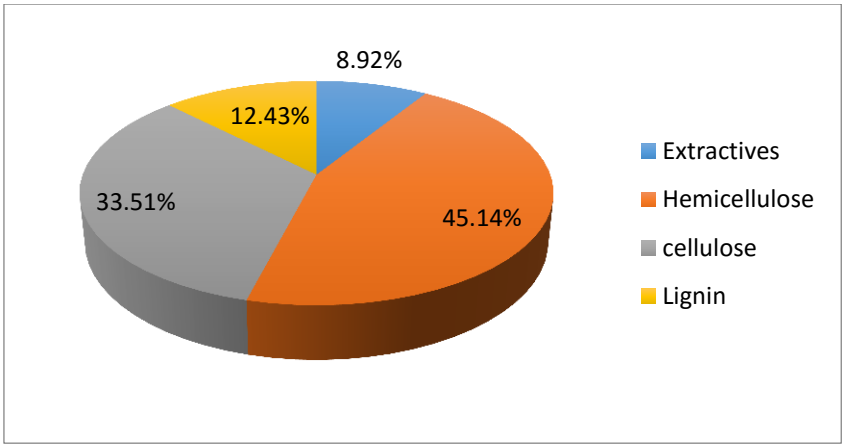


Figure 4. 1. The proportions of the lignocellulosic constituents of water hyacinth in (wt.%)

Table 4. 2. Compositional analysis of water hyacinth

References	Cellulose (wt.%)	Hemicellulose (wt.%)	Lignin (wt.%)
Present work	33.513	45.137	12.43
Kumar et al., 2019	32-54	11-37	17-32
Gandhimathi, 2017	30	50	5
Sagar and kumari, 2013	20	33	10
Schabort,2014	17.8-28.9	18.4-49.2	2.8-10
Kurchania et al., 2016	22.11	16.61	9.60
Das et al., 2016	24.7	32.2	3.2
Pattra and Sittijunda, 2015	27.55	39.88	14.96

4.2 SEM analysis of the raw water hyacinth

The raw water hyacinth was analyzed by scanning electron microscope to see its morphological characteristics of its starch granules. Figure 4.2(A) shows the overall surface morphology of the raw water hyacinth. The starch granules shown in Figure 4.2(B, C, and D) are from the SEM analysis of dried and size reduced water hyacinth. As seen on the figure, there are some bits of materials that need to be removed so that the structure will be a smooth, spherical granule.

According to Sparla et al.(2014), the surface morphology of barley starch granules were analyzed by using a scanning electron microscope. The starch granules extracted from the barley had regular spherical shapes. The starch was extracted in 70 mL Extraction Buffer that was composed of 55 mMTris-HydroChloric acid with pH 6.8, 2.6% Sodium Dodecyl Sulfate, 10% glycerol and 2% ß-mercaptoeth-anol. It was then shaken for 48 h while replacing the extraction buffer solution every 24 h. It was then washed and filtered then suspended again in acetone. So the regular spherical shape might be the result of the extraction process which might have removed the unwanted material in the raw barley(Sparla et al., 2014).

The analysis of surface morphology of starch granules of cow peas, black beans and carioca beans(raw and cooked materials) was done by De MIRANDA et al.(2019) using a scanning electron microscope (SEM) and the author stated that starch granules have an ellipsoidal or spherical shape. The raw cow pea appeared to have spherical granules but with impurities on it while the cooked one had a regular spherical shape. The raw black bean, on the other hand, had an ellipsoidal starch granule with bits on it but the cooked one had an ellipsoidal granule with cracks on it due to bond breakage. Lastly, the raw carioca bean had ellipsoidal shaped starch granules while the cooked ones had ellipsoidal shaped granules with gelatinization i.e. the cleavage of intermolecular bonds in the presence water and heat(MIRANDA et al., 2019). This indicates that the starch granules with impurity in water hyacinth need to undergo further pretreatment i.e. chemical, physicochemical or thermal pretreatment in order to facilitate the reduction of the starch into reducing sugar.

Figure 4. 2. The Scanning Electron Microscope (SEM) analysis of raw water hyacinth (A) at 100 µm, (B) at 40 µm, (C) at 20 µm, (D) at 5 µm

4.3 X-ray diffraction (XRD) analysis of untreated water hyacinth

The crystallite analysis of untreated water hyacinth was analyzed by using X-ray analysis. The scanning angle used is from 5-50^0. The raw material has a narrow peak as well as wide ones as illustrated in Figure 4.3. The wide or broader peak is created by either the particle size being very small (usually in Nano size),by the presence some impurities of amorphous structure or by the instrumentation as well (Holder & Schaak, 2019; Speakman & Ph, n.d.).The figure also shows that there is a presence of both crystalline and amorphous structure. The amorphous structure is due to the presence of hemicellulose and lignin while the crystalline structure is cellulose(Netai et al., 2016). The strongest peaks appeared around 28.6694^0, 22.2768^0 and 22.5561^0 2 thetas where the first value represents the narrow peak and the latter two represent the wider peaks.

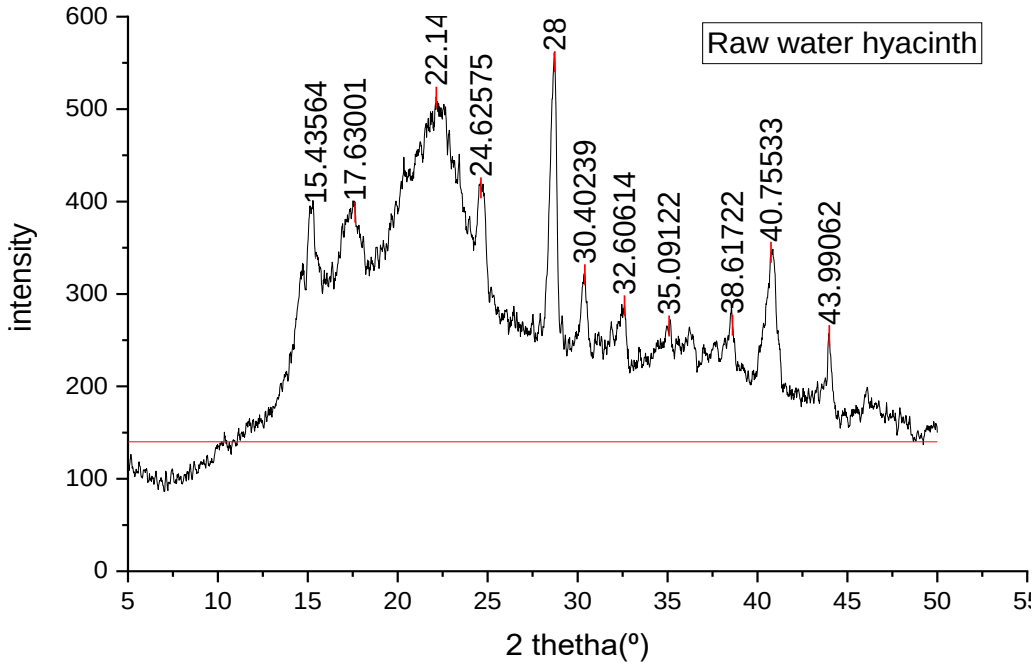


Figure 4. 3. The XRD refractogram of raw water hyacinth

The XRD characteristics such as crystalline size, crystalline index and the d-spacing were calculated using the Seagel and Scherrer equations as well as Bragg's law as stated in Table 4.3.

Table 4. 3. The water hyacinth characteristics obtained by the data analyzed with XRD refractogram

XRD characteristics	Raw water hyacinth
Crystalline index (%)	75.536
Crystalline size(nm)	19.655
D-spacing(nm)	17.665

4.4 Pretreatments of water hyacinth

4.4.1 Liquid hot water pretreatment of raw water hyacinth

The pretreatment process of lignocellulosic biomass is important in order to make enzymatic hydrolysis and fermentation efficient. In the present work, the reducing sugar concentration in the liquid recovered after hot water treatment was investigated in duplicate by DNSA method and found to be 3.55±0.137 g/L. After hot water pretreatment, the yield of reducing sugar is 12.43% (g/g). This shows that good amount of reducing sugar was recovered in the pretreatment steps. Therefore, choosing a suitable pretreatment process is important in ensuring enhanced digestibility and decreased limitation of the enzymatic hydrolysis due to the exposure of cellulose(Rezania et al., 2017). Herbaceous feed stocks require lower temperature and energy relative to woody biomass(Zhuang et al., 2016).

According to zhuang et al. (2016), during liquid hot water pretreatment, 2 things mainly happen; hemicellulose degradation and delignification. The hemicellulose will be decomposed into reducing sugar and some of the lignin also depolymerizes upon heating. During this pretreatment, up to 61.7% of the total reducing sugar in the feedstock can be recovered from the liquid fraction (Zhuang et al., 2016).

4.4.2 Dilute sulfuric acid pretreatment of water hyacinth

The dilute acid pretreatment process was carried out using sulfuric acid concentration of 1 – 2.5 %v/v. In the dilute acid pretreatment process the parameters investigated were hydrolysis temperature, dilute acid concentration and hydrolysis time. The diluted acid hydrolysis was conducted at a temperature 115 – 145^{o}C at the interval of 5^{o}C, dilute acid concentration of 1 – 2.5 %v/v at the interval of 0.5 %v/v and dilute acid concentration of 75 – 105 min at the interval of 5 min. The effect each parameter was investigated as presented in section 4.4.2.1 to 4.4.2.3.

4.4.2.1 The effect of dilute acid concentration in water hyacinth hydrolysis

Dilute acid concentration is one of the parameters that affect the acid hydrolysis pretreatment of the hemicellulose and lignin into fermentable sugars. This process, usually, takes place by using acid concentration between 0.5 and 6%(Timung et al., 2016). So for this particular work, concentrations between 1 and 2.5% were analyzed. As illustrated in Figure 4.4, we can see that the reducing sugar concentration increased from 3.2 to 11.56 g/L as dilute acid concentrations increases from 1 to 1.75%. This is due to the sole presence of the cleavage of β-1, 4 linkages(Ganguly et al., 2012). However, reducing sugar concentration decreases from 11.56 to 2.74 g/L. This is because there might be the formation of different types of inhibitors like carboxylic acids, furans and phenolic compounds. At relatively higher temperatures and concentrations of acid, glucose and xylose can be degraded into furfural and hydroxymethylfurfural, respectively(Chiaramonti et al., 2012). So to make the range a little more accommodating, concentrations between 1.5 and 2% v/v are optimized for the best results when interaction occurs with the other parameters.

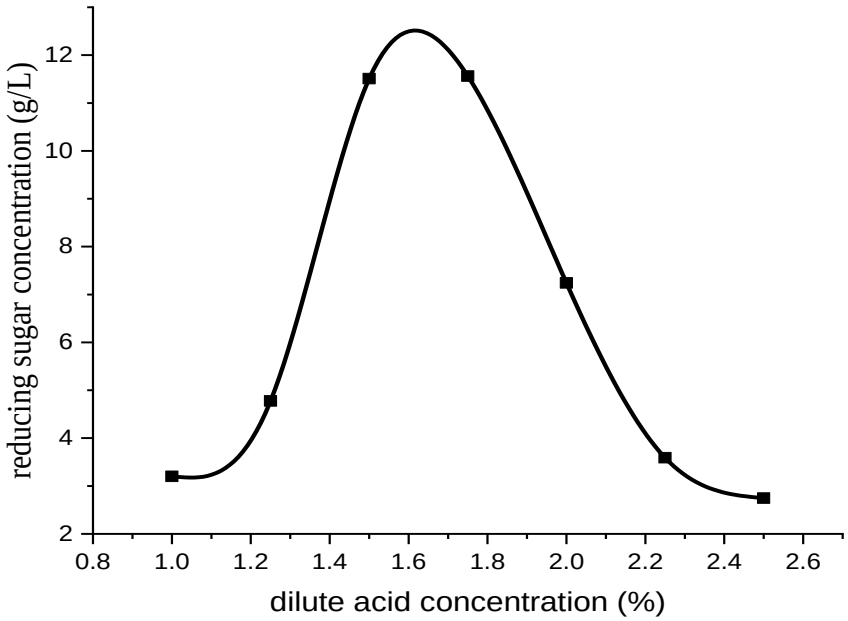


Figure 4. 4. Effect of dilute acid concentration on the reducing sugar yield

4.4.2.2 The effect of hydrolysis temperature in water hyacinth hydrolysis

The variation of reducing sugar concentration as a function of hydrolysis temperature is another concern during the dilute acid hydrolysis pretreatment of water hyacinth. Figure 4.5 shows that the reducing sugar concentration increased from 11.52 to 11.79 g/L as the temperature increased from 115 to 140^{0}C. This is due to the reduction of the polysaccharides of the hemicellulose and lignin into the respective monosaccharaides like glucose, xylose and others. During this work, the temperature range between 130 and 140^{0}C was observed to produce more reducing sugar as glucose. Nevertheless, the reducing sugar concentration decreased below 11.35 g/L as the temperature increased beyond 140^{0}C which might be due to the further degradation of the glucose and xylose into other unwanted products(Idrees et al., 2013). The temperature at which the hydrolysis takes place has a strong relationship with the hydrolysis time (residence time). But for its individual effect, the reducing sugar yield increased while the temperature increased. According to Satari Baboukani et al.(2012), Increasing the temperature while lowering the pH of the solution is known to have a positive effect on the hydrolysis process i.e higher recovery of fermentable sugars. The decrease in reducing sugar from 145^{0}C up to 160^{0}C might be caused because of the longer residence time it is being subjected to.

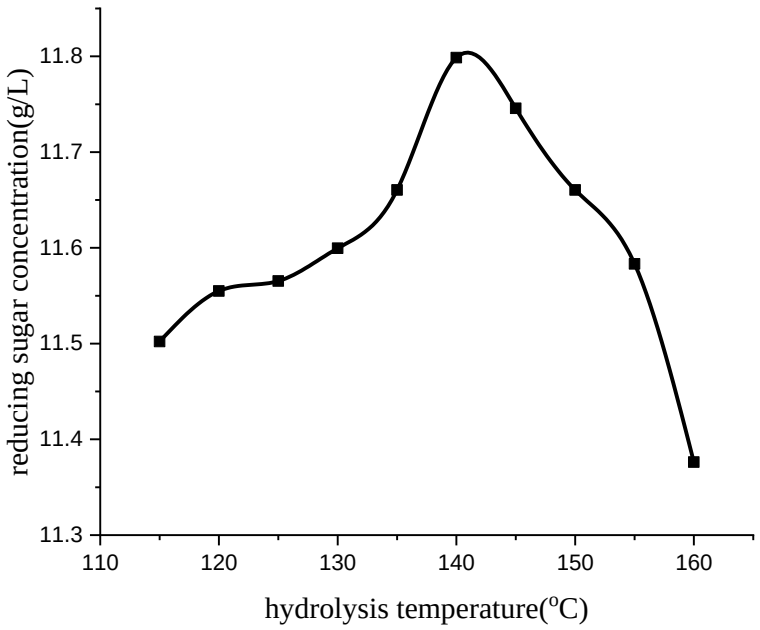


Figure 4. 5. Effect of hydrolysis temperature on the reducing sugar yield

4.4.2.3 The effect of hydrolysis residence time in water hyacinth hydrolysis

During the dilute acid hydrolysis pretreatment of water hyacinth, the reducing sugar concentration after hydrolysis varies as the function of the residence time. In Figure 4.6 , it can be observed that the reducing sugar concentration increases from 11.5 to 11.84 g/L while the residence time Increased from 75 up to 90 min which is due to the reduction or hydrolysis of the hemicellulose and cellulose into glucose and other reducing sugar(Idrees et al., 2013). Nonetheless, as the residence time increased from 95 to 105 min, the reducing sugar concentration decreased from 9.76 to 8.79 g/L. This may be due to the further degradation of the reducing sugars(glucose, xylose, arabinose…) into furfural(Idrees et al., 2014). The longer the residence time, the more time the glucose is subjected to the hydrolysis process which could lead to the degradation of the glucose itself into hydroxyl-methyl furfural, acetic acid, formic acid, levulinic acid and others. The same degradation occurs to xylose and other reducing sugars. These inhibit the fermentation and their removal would be an extra cost in the process(Ganguly et al., 2012).

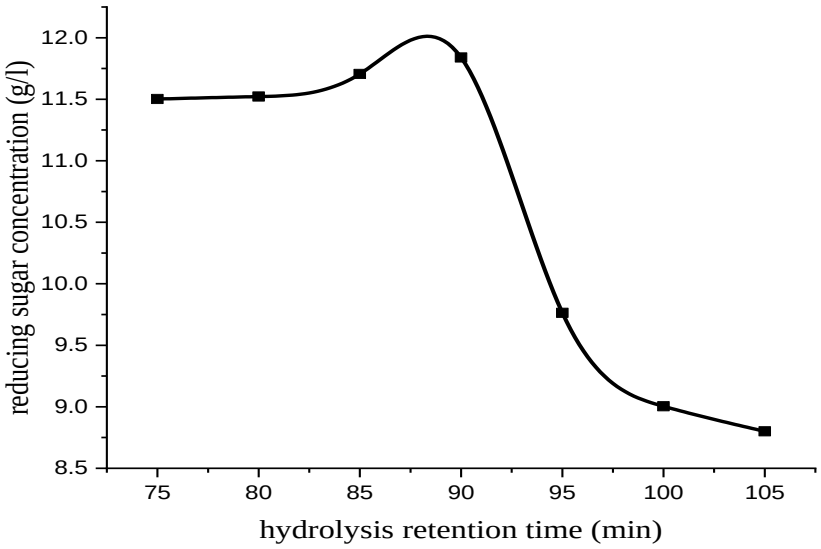


Figure 4. 6. Effect of hydrolysis residence time on the reducing sugar yield

4.4.3 Investigation of the interaction effect of parameters and parametric optimization

The optimization of the dilute acid pretreatment was carried out by using Design Expert (version 12) software. Response surface methodology (RSM) - central composite design (CCD) was used because the process to be optimized has more than one factor and the central composite design replicates the center point 6 times. It is a

collection of statistical and mathematical methods that are useful in analyzing and modeling problems with a response being affected by several factors and the aim is optimizing the response. It also reckons the relationship between the major input factors and the measured responses(Noordin et al., 2004). There were 20 runs performed with 6 center points. The main factors affecting the dilute acid hydrolysis process were considered to be acid concentration, hydrolysis temperature and hydrolysis residence time and the conditions for them were set.

4.4.3.1 ANOVA of the optimization process

The design model that was found after analyzing the response data was a quadratic model. The model equation for the estimation of the reducing sugar concentration was given as follows by using the coded coefficients.

$$\mathrm{RSC} = +11.45 + 0.2500A + 0.0463B - 0.0935C + 0.1970AB - 0.1264AC - 0.4255BC$$
$$+0.0174A^2 + 0.0326B^2 - 0.0125C^2 \qquad 4.1$$

Where, RSC is reducing sugar concentration

The statistical significance of Equation (4.1) is tested by the analysis of variance (ANOVA) results obtained from the quadratic CCD model. The low probability value of the model (p-value) is expected to be less than 0.05, which in this case is <0.0001, so it is evidenced that the model is highly significant for this study. The P-value is a quantitative measurement for reporting the result of a tested hypothesis.

The other analysis done by the software according to the data entered is the lack-of-fit test. This test is important in determining whether or not the suggested model has a significant lack-of-fit(Balcha, 2019) which are summarized in Table 4.4. The model summary statistics, on the other hand, analyzes the standard deviation, the r-squared value and the press of the model. The selected model has a lower standard deviation, higher R-squared value and a lower press value. The R-squared value is needed to be as close to unity (1) as possible, which in this case it is. The predicted R-squared value indicates the closeness of the data while the standard deviation indicates the difference between the factors.

Table 4. 4. ANOVA for quadratic model

Source	Sum of squares	df	Mean square	F-value	P-value	
Model	2.91	9	0.3235	84.58	< 0.0001	significant
A-acid concentration	0.8536	1	0.8536	223.21	< 0.0001	
B-hydrolysis time	0.0292	1	0.0292	7.64	0.0200	
C-hydrolysis temperature	0.1193	1	0.1193	31.19	0.0002	
AB	0.3104	1	0.3104	81.17	< 0.0001	
AC	0.1278	1	0.1278	33.42	0.0002	
BC	1.45	1	1.45	378.71	< 0.0001	
A^2	0.0044	1	0.0044	1.14	0.3100	
B^2	0.0153	1	0.0153	4.01	0.0730	
C^2	0.0022	1	0.0022	0.5842	0.4623	
Residual	0.0382	10	0.0038			
Lack of fit	0.0274	5	0.0055	2.52	0.1667	Not significant
Pure Error	0.0109	5	0.0022			

The analysis of variance (ANOVA) of a model is done to shows the adequacy of the model for the specific data. The larger the F value is and the smaller the P value is, the more significant the model(Balcha, 2019). In this specific work, the model is found to be significant or adequate for the respective data. The model F-value of 84.58 implies the model is significant. P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, BC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The Lack of Fit F-value of 2.87 implies the Lack of Fit is not significant relative to the pure error. There is a 13.04% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good as can be seen in Table 4.4.

The Multiple regression coefficients R^2 was found to be 0.9794, as stated in Table 4.5. This implies that the predicted values of the glucose concentration are close to the values found experimentally(Wang et al., 2013). The R^2 values being closer to unity (1) means the model is strong and that its ability in predicting the response is stronger. The Predicted R^2 of 0.9188 is in reasonable agreement with the Adjusted R^2 of 0.9699; i.e. the difference is less than 0.2. Adeq Precision measures the signal to noise

ratio. A ratio greater than 4 is desirable. The ratio of 47.770 indicates an adequate signal. This model can be used to navigate the design space. The coefficient of variation (CV) indicates the precision with which the experiments were conducted. The lower it is the better. The CV of this work was found to be 0.596% which is low.

Table 4. 5. Result of R-squared test

Sr. no.	Determination of coefficient	Values
1	R^2	0.9794
2	Adjusted R^2	0.9699
3	Predicted R^2	0.9188
4	Adeq Precision	47.7698

As shown in Table 4.6, the factors that were optimized in the conducted experiments are sulfuric acid concentration (1.5-2 %(v/v)), hydrolysis temperature (125-140℃) and hydrolysis residence time (85-100 min). After conducting the experiments, the reducing sugar concentration from the actual experiments were found to be very close to the predicted values by the model.

Table 4. 6. CCD matrix of independent variables used in RSM with the actual and predicted values

Run	A:acid concentration $\%(\frac{V}{V})$	B:hydrolysis time (min)	C:hydrolysis temperature (℃)	Reducing sugar concentration (g/l)	
				Actual	Predicted
1	1.50	100	125	11.40	11.48
2	1.75	92	132	11.50	11.45
3	1.75	105	132	11.64	11.62
4	1.33	92	132	11.09	11.08
5	2.17	92	132	11.87	11.92
6	2.00	100	125	12.66	12.62
7	1.75	92	132	11.50	11.45
8	1.75	92	120	11.56	11.57
9	2.00	85	140	11.78	11.70
10	2.00	85	125	11.28	11.29
11	1.50	85	140	11.82	11.84
12	1.75	92	132	11.48	11.45
13	1.75	80	132	11.40	11.46
14	1.50	85	125	10.98	10.93
15	1.75	92	132	11.48	11.45
16	1.75	92	132	11.49	11.45
17	1.50	100	140	10.71	10.69
18	1.75	92	132	11.48	11.45
19	1.75	92	145	11.23	11.25
20	2.00	100	140	11.29	11.33

The normal probability plot of the residuals is a plot that shows if the residuals are following a normal distribution and are lying on a straight line. Scatter is expected on some level but it should be a random scatter(*Stat-Ease » V11 »* Tutorials *» Response Surface,* n.d.). If the points lie on the line, transformation is not required. As can be seen in Figure 4.7, the scatter is more or less random**.**

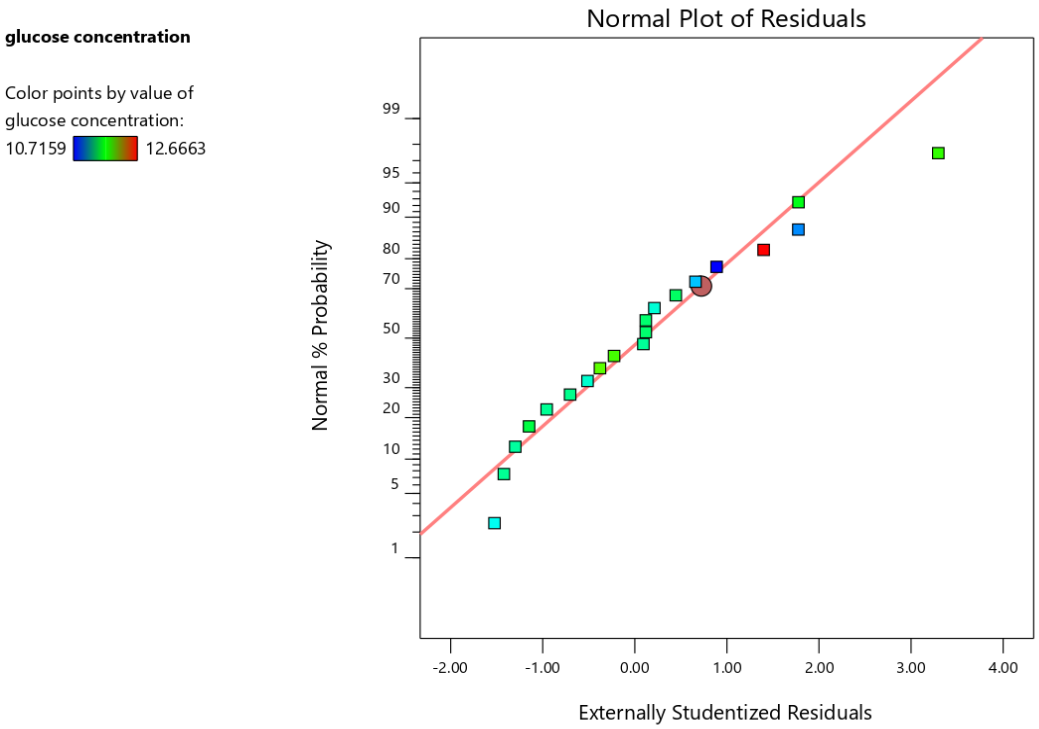


Figure 4. 7. Normal plot of residuals of experimental values

The other diagnostic plot is the actual vs. predicted value plot that plots the experimental values with the values expected or predicted by the model(*Stat-Ease » V11 » Tutorials » Response Surface*, n.d.). Figure 4.8 shows that the predicted values are very close to the experimental values in the way that the values are fitting on the straight line. The residual vs. run number is a plot of the residuals versus the experimental run order. It checks for lurking variables that may have influenced the response during the experiment as shown in Figure 4.9. The plot should show a random scatter. Trends indicate a time-related variable lurking in the background(*Stat-Ease » V11 » General Sequence of Analysis » Diagnostics » Diagnostics Plots*, n.d.).

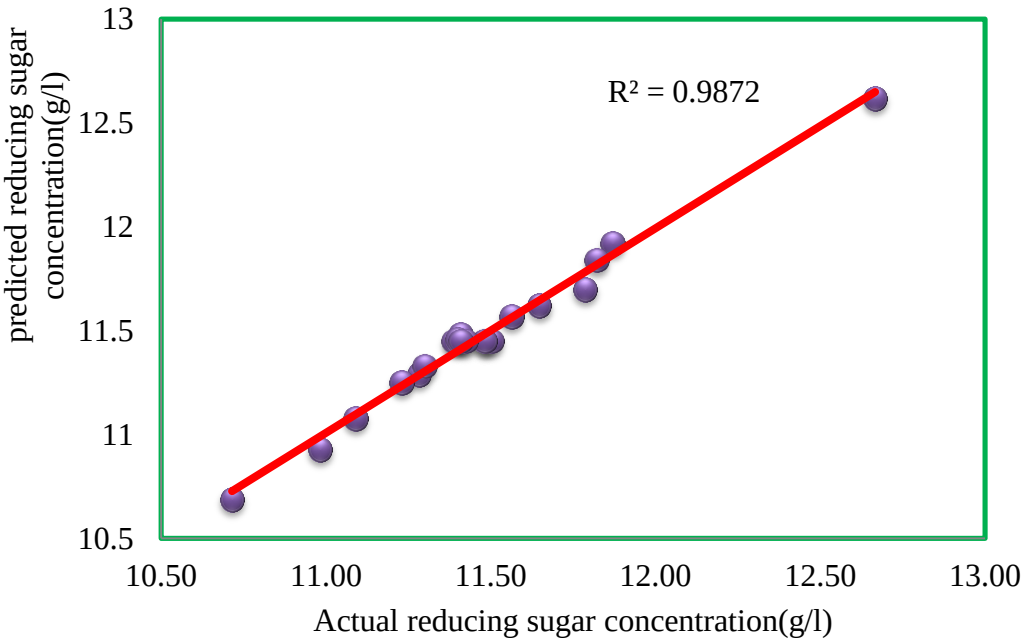


Figure 4. 8. The experimental values vs. model predicted values of the reducing sugar concentration

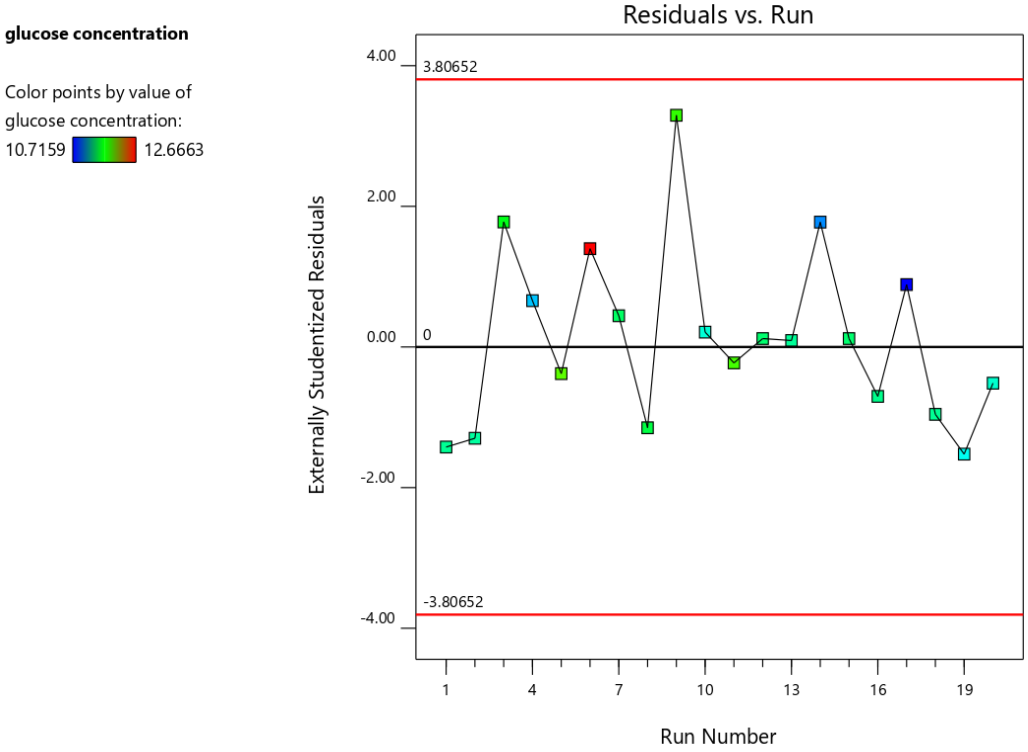


Figure 4. 9. Residual vs. run plot

4.4.3.2 Interaction effect of the factors affecting dilute acid hydrolysis

The factors affecting dilute acid hydrolysis process have individual effects and it has been proved in the previous subtopics. However, the fact is that they do not only have individual effects but also interactive effects. These interactive effects bring changes to the response indefinitely. The effect of the factors in dilute acid hydrolysis process has been presented on Figure 4.10 (a, b and c) by using their respective 3D response surface representation in order to show their impact on the reducing sugar concentration.

The solid residue from liquid hot water pretreatment, which contains some amount of lignin and hemicellulose together with the recalcitrant cellulose, is seen to be converted into reducing sugar concentration of about 12 g/L as demonstrated in Figure 4.10 (a-c). As can be seen on Figure 4.10 (a), it presents the interactive effect of dilute acid concentration and the hydrolysis time on the reducing sugar concentration at a hydrolysis temperature of 132.5^0C.

The reducing sugar concentration from the hydrolysis of the lignocellulosic components increased from 11.1 to 11.52 g/L as the acid concentration increased from 1.5 to 1.9 %(v/v) and as the hydrolysis time decreased from 100 to 87 min. This occurred because the increase in acid concentration at a lower time is a favorable condition for the hydrolysis process so that the acid could breakdown a good amount of reducing sugar from the lignocellulosic materials without further degrading the reducing sugars like glucose and xylose since it was not given time to do so (Idrees et al., 2013) and also due to the presence of higher acid catalyst which leads to unwanted product formation(Reales-Alfaro et al., 2013). The more time the acid is given, the more chance there is for the reducing sugar to be degraded to other products like furfural. Nonetheless, as the acid concentration increased from 1.9 to 2 % (v/v) and as the hydrolysis time decreased from 87 to 85 min, the glucose concentration decreased from 11.52 to 11.48 g/L. This might be due to the fact that it was not given enough time to produce more glucose.

According to Reales-Alfaro et al.(2013), optimization of acid hydrolysis of water hyacinth was carried out by considering acid concentration (1-3% (v/v)), hydrolysis time(15-25 min) and solid concentration(10-12.5% (w/v)) as the parameters affecting the process. As the acid concentration was increased from 1 to 2.5 %(v/v), it was

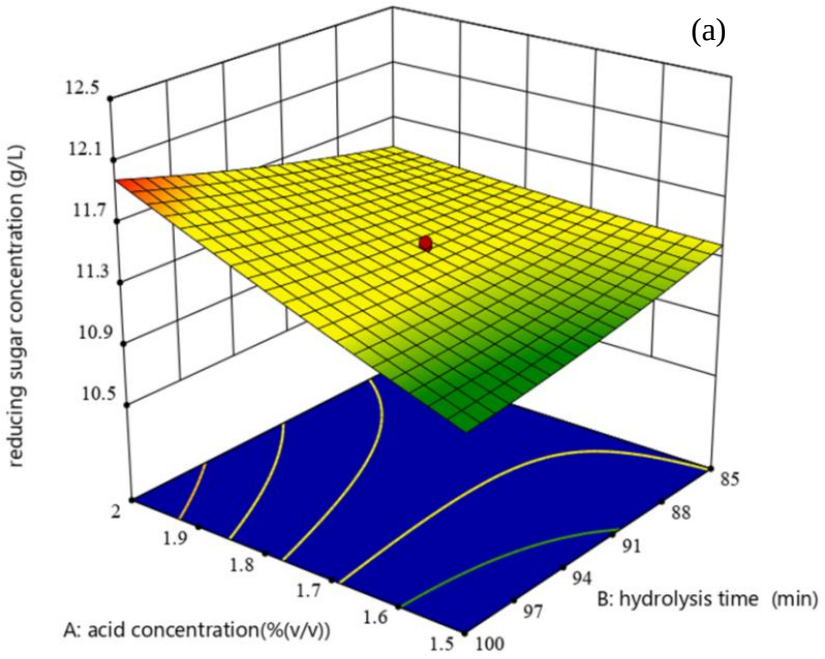
observed that the reducing sugar concentration was increasing with increase in reaction time until a certain point. Then the reducing sugar concentration started to decrease with the further increase in acid concentration and reaction time. This was because of the existence of higher acid catalysis which increases the reducing sugar concentration at first but then decreases it due to the degradation of the reducing sugar into other products.

On the other hand, for instance, when increasing the hydrolysis time from 85 to 100 min at given acid concentration of 1.9 %(v/v), the reducing sugar concentration increases from 11.4 up to 11.8g/L. Similarly, if the acid concentration is increased from 1.5 to 2 %(v/v) at a given hydrolysis time of 100 min, the reducing sugar concentration increases from 11.01 to 12 g/L. therefore, it is safe to conclude that the model is significant enough to represent the experimental data since the conditions stated above are coherent with the one factor analysis done.

Figure 4.10 (b) shows that the reducing sugar concentration increases from 11.2 to 11.5g/L as the acid concentration increases from 1.5 to 1.9 %(v/v) and as the temperature increases from 125 to 137℃ at a hydrolysis time of 92.5 min. this is due to the fact that only the breakdown of lignocellulosic materials into their respective monomers happens, i.e hexose and pentose sugar. However, as the acid concentration and temperature further increased from 1.9 to 2 %(v/v) and 137 to 140℃, respectively, it was observed that the concentration of the reducing sugar decreased from 11.5 to 11.48 g/L. This is because of the conversion or further degradation of the hexose and pentose sugar into inhibitors like furans. On another note, it can clearly be seen that the reducing sugar concentration increases from 11.2 to 11.9 g/L as the dilute acid concentration increases and as the hydrolysis temperature decreases. This is due to the resistance of reducing sugar against the degradation at lower temperature(Satari Baboukani et al., 2012). In order to have reducing sugar degradation into 5-hydroxymethylfurfural, the temperature should increase with increase in acid concentration or hydrolysis time. Generally, the best reducing sugar yield is obtained at a lower acid concentration and increasing the hydrolysis temperature(Satari Baboukani et al., 2012).

The interactive effects of hydrolysis temperature and hydrolysis time are shown on Figure 4.10 (c). As depicted on the graph, at an acid concentration of 1.75% (v/v), the reducing sugar concentration increased from 11.1 to 11.45 g/L while the hydrolysis

temperature increased from 125 to 132℃ and the hydrolysis time increased from 85 to 92 min. But as both the hydrolysis temperature and hydrolysis time are further increased from 132 to 140 ℃ and from 92 to 100min, respectively, the reducing sugar concentration was observed to decrease from 11.45 to 11.03 g/L. this is because during the first 92 minutes only reducing sugar was being produced but the more temperature and time the process is given, the more susceptible the reducing sugars are to being converted into unwanted materials like furfural and 5HMF(Wei et al., 2012). Another observation is that as the hydrolysis time increases from 85 to 100 min at a temperature of, for example, 136℃, the reducing sugar concentration decreased from 11.6 to 11.2 g/L. similarly, at a hydrolysis time of 100 min, the reducing sugar concentration was seen to decrease from 12 to11 g/L with increase in hydrolysis temperature from 125 to 140℃. This shows these properties are in line with the one factor a property which indicates the significance of the model in representing the data points.



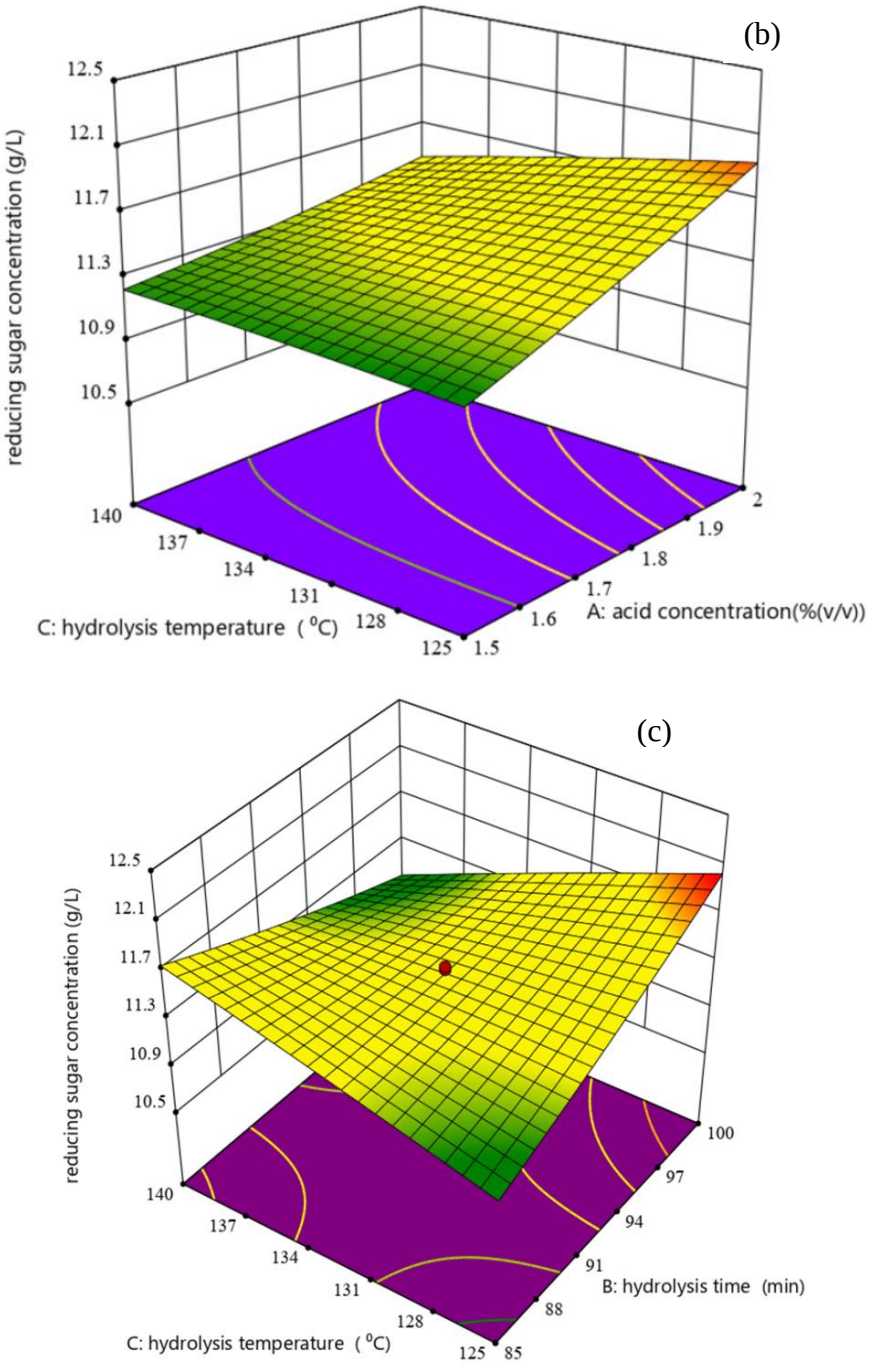


Figure 4. 10. Interaction effects of the different parameters affecting the dilute acid hydrolysis process: (a) the interactive effect of acid concentration and hydrolysis time on glucose concentration, (b) the interactive effect of hydrolysis temperature and acid concentration on glucose concentration, (C) the interactive effect of hydrolysis temperature and hydrolysis time on glucose concentration.

4.4.3.3 Statistical optimization using response surface optimizer

The parameters affecting the dilute acid hydrolysis were optimized in order to get a desirable amount of reducing sugar concentration. Response surface methodology with central composite design was used to optimize the factors. The parameters affecting the process were considered to be dilute acid concentration, hydrolysis time and hydrolysis temperature. The criteria of the optimization was set to maximize the reducing sugar concentration while minimizing the dilute acid concentration and the hydrolysis time and while the hydrolysis temperature was kept in range between the levels it was given, as stated on Table 4.7. With the criteria set in Table 4.7, the desirable and optimum value for reducing sugar concentration was obtained to 11.844 g/L at a hydrolysis temperature and time of 140℃ and 85 min, respectively with 1.5%(v/v) of dilute acid concentration. This combination had a desirability of 0.833.

Table 4. 7. Numerical optimization constraints of the parameters

	Parameters			
	Dilute acid concentration	Hydrolysis time	Hydrolysis temperature	Reducing sugar concentration
Goals	Minimize	Minimize	In range	Maximize
Lower limit	1.5	85	125	10.7
Upper limit	2	100	140	12.6
Importance	3	3	3	3

The values predicted by the model to be optimum were validated by carrying out a triplicated experiment by using the conditions of the parameters that are specified in Table 4.8. The actual and predicted values of reducing sugar concentration have a 0.039% difference between them. This shows that the model is capable of predicting the values of the response i.e. reducing sugar concentration.

Table 4. 8. Validation of the model predicted values with experimental values

	Dilute acid concentration	Hydrolysis time	Hydrolysis temperature	Reducing sugar concentration
	(%(v/v))	(Min)	(℃)	(g/L)
Predicted	1.5	85	140	11.844
Experimental	1.5	85	140	11.884±0.205

After the desirable process was performed with 30 g of solid and 1050 mL liquid, the final slurry was filtered and the liquid was stored in the refrigerator while the solid was further hydrolyzed by using cellulase enzyme. The liquid stored in the refrigerator will later be used in the fermentation process together with the liquid from liquid hot water pretreatment and enzymatic hydrolysis. The reducing sugar yield of the dilute acid hydrolysis process is about 41.594 %(w/w) or 12.4782 g of reducing sugar is produced from 30 g of water hyacinth.

A total of 20.545 g/L reducing sugar concentration was obtained from the pretreatment processes i.e. liquid hot water pretreatment and dilute acid hydrolysis pretreatment. A combined reducing sugar yield of 71.906% (w/w) was obtained from the two pretreatments.

4.5 Enzymatic saccharification

The enzymatic hydrolysis was carried out by using 0.1(%(w/v)) of cellulase enzyme extracted from *Trichoderma virde* with activity of 10 units/ mg of biomass. The water hyacinth was hydrolyzed for 72 h. The hydrolysate, separated by filtration from the solid residue, was analyzed for reducing sugar concentration by using DNSA method with the help of a UV-spectrophotometer.

As observed from Figure 4.11, it shows that the glucose concentration increases, exponentially, from 0 to 10.9554g/L with increase in the saccharification time from 0 to 72 h but the reducing sugar concentration decreases when hydrolysis time further increases from 72 to 84 h. The glucose yield of this specific process was obtained to be 219.108 mg/g of WH and the rate of glucose yield form cellulose hydrolysis was acquired to be 58.8% (w/w). Assuming all the cellulose and hemicellulose are completely degraded, the theoretical maximum reducing sugar yield was found to be 88.6% (w/w). The actual or experimental glucose yield, on the other hand, was attained to be 58.8%. Finally, the efficiency of the enzymatic hydrolysis was found to be 66.36%.

The saccharification was increasing exponentially during the first 24 and 48 h which might be mainly due to the adsorption of cellulase enzyme on the surface of the cellulose. This process is irreversible which resulted in the slowing down of the hydrolysis process as of the reduction in the enzyme activity(Karri et al., 2011). After the parametric optimization of enzyme loading (cellulase and xylanase enzymes) and

substrate loading in enzymatic saccharification of water hyacinth, a reducing sugar yield up to 432 mg/g can be obtained by incubating at a pH and temperature of 5 and 50°C, respectively(Ganguly et al., 2013). The present study only used cellulase enzyme and optimization was not performed. Therefore, the result obtained is an indication of the potential of water hyacinth to be used for ethanol production.

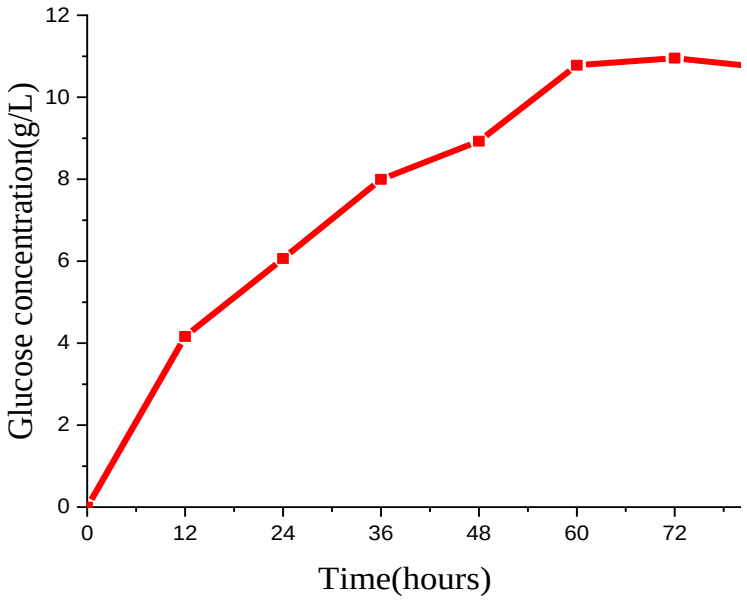


Figure 4. 11. Effect of time on the glucose concentration during saccharification

4.6 Fermentation and distillation results

The fermentation process took place after preparation of the active yeast media. Commercial *saccharomyces cerevisae* was used after being activated for 24 h. The fermentation mixture was then divided into two. The first was a mixture of the liquids from liquid hot water pretreatment, dilute acid hydrolysis and enzymatic hydrolysis but the second was a mixture of only the liquids from the pretreatment steps i.e. liquid hot water pretreatment and dilute acid hydrolysis. This was done in order to determine effect of the enzymatic hydrolysis in the fermentation process, as illustrated in Figure 4.12. The fermentation took place for 72 h.

The maximum bioethanol concentration in the fermentation broth was measured to be 7.18% (v/v) after 72 h of fermentation as demonstrated in Figure 4.13. The bioethanol produced was obtained to be 10.5mL or 8.4g from the 150mL fermentation mixture. From 1 g of water hyacinth, 0.168g or 168mg of ethanol was acquired. The bioethanol yield was calculated to be 22.13% (v/v) per 1g water hyacinth. The fermentation process had the efficiency of 43.3%. The volumetric ethanol productivity was

computed in terms of the ethanol concentration from fermented broth as well as the fermentation duration and it was obtained that 0.778 g/L of ethanol was produced per hour. The highest amount of the ethanol was obtained from the fermentation of the mixture involving the liquids from all the three steps.

Figure 4. 12. (A) Fermentation result and (B) simple distillation setup

Das et al. (2015) reported that the highest ethanol yield obtained from water hyacinth was investigated and found through fermenting using three different yeast such as *pichia stipitis, candida shehatae* and *saccharomyces cerevisae*. *Pichia stipitis* had an ethanol yield of 10.44 g/L equivalent to 0.1044 g/g while *candida shehatae* and *saccharomyces cerevisae* had ethanol yields of 8.24 g/L (0.0824 g/g) and 6.76 g/L (0.0676 g/g), respectively. The lower yield of ethanol by SC is due to the fact that SC only utilizes hexose sugar but PS uses both hexose and pentose sugars for the production of ethanol since both sugars exist in the fermentation broth. In the current research work, SC was used and an ethanol yield of 0.168g/g was obtained which is higher than that of the literature. This might be due to the amount of reducing sugar yield after the two-steps pretreatment processes.

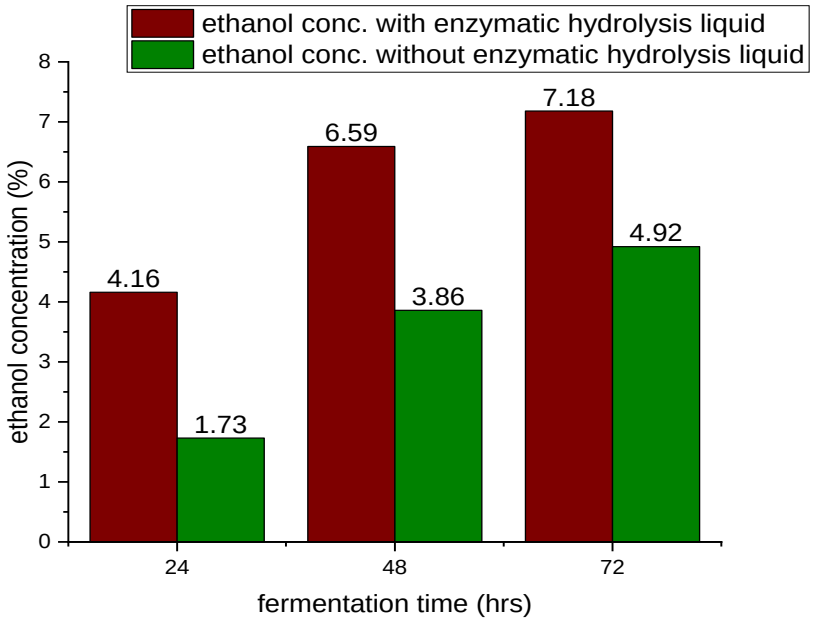


Figure 4. 13. Ethanol concentration in the fermentation broth measured every 24 h

4.7 Physicochemical characterization of the bioethanol

After the double distillation and collection of the bioethanol, its physicochemical characteristics were investigated in order to identify the potential pros and cons of the bioethanol for use such as a fuel and so on. The first investigation made was the determination of the refractive index and brix of the bioethanol hoping to determine the concentration of the ethanol from the refractive index using a standard curve but instead of the concentration, the refractive index was helpful to conclude that the alcohol product that was obtained was indeed bioethanol. In the present work the refractive index and the ^{0}brix were found to be 1.3640 and 20.11, respectively. In comparison with other literature values, the value obtained during this work proves that the produced alcohol is bioethanol. The refractive indices of different types of alcohol are listed below in Table 4.9.

Other physico-chemical properties like such as density, specific gravity and API (American petroleum institute) gravity were also investigated using a densitometer. The density of the 72%(V/V) bioethanol was found to be 0.877 g/cm^3, stated in table 4.9, while the specific gravity and API gravity were found to be 0.8805 and 0.8850(15^{0}C), respectively.

The final property measured was the pH of the bioethanol which was found to be 4.701.

Table 4. 9. Refractive indices and density of different types of alcohol

Types of alcohol	Refractive index	Density(g/cm^3)	reference
Ethanol(72%)	1.3640	0.877	present work
Methanol	1.3264	0.786	(Herr & Belda, 2006)
Ethanol	1.3592	0.784	(Cristina et al., 1996)
1-Propanol	1.3847	0.804	(Herr & Belda, 2006)
1-Butanol	1.3979	0.810	(Herr & Belda, 2006)

4.8 FT-IR analysis of untreated water hyacinth, treated water hyacinth and bioethanol

The Fourier transform-infrared radiation of the raw water hyacinth, the hydrolyzed water hyacinth and the final ethanol product was done. When it comes to identifying the peaks in the FT-IR spectroscopy, it must be known that there are two designations. They are designated as the fictional group section which is found in the range of 1500-4000 cm^{-1} wavenumber and the fingerprint section which is found in the range of 400-1500 cm^{-1} as can be seen in Figure 4.16.

In all three spectra, we can see that there are peaks appearing at around 3200 - 2700 cm^{-1}. They are weak and broad peaks that show the presence O-H stretching representing for primary alcohol and water (Anjos et al., 2016; *IR Spectrum Table & Chart Sigma-Aldrich*, n.d.). When looking at the spectra of the raw water hyacinth and the hydrolyzed water hyacinth, it can be seen that the O-H stretch has lower intensity than in the ethanol spectrum. This indicates that the product being characterized shows the presence of primary alcohol and in the hydrolyzed water hyacinth, it shows the removal of unwanted water molecules from the raw water hyacinth.

In the raw water hyacinth spectrum, it is observed that there is a peak with 2917 cm^{-1} wavenumber. The peak lies in the frequency range of 3000 - 2500 cm^{-1}. This represents the presence of C-H stretching of alkanes. But on the spectrum of the hydrolyzed water hyacinth, it is visible that this peak has disappeared showing that the hydrolysis process is yet again successful. The other peak in the raw WH sample exists at 1610 cm^{-1} with medium strength appearance. This shows the presence of amines due to an N-H bending. Whereas in the hydrolyzed WH, the single peak dissolved into 2 peak at wavenumbers 1550 and 1633 cm^{-1} and these indicate the

presence of an N-O stretching representing nitro groups and a C=C stretching representing conjugated alkenes, respectively. These groups might be present due to the presence of extractives. The amide group might be dissolved into the Nitro groups and alkenes due to hot water pretreatment but some extractives cannot be hydrolyzed or dissolved by acids because sulfuric acid is a polar solvent(*IR Spectrum Table & Chart Sigma-Aldrich*, n.d.).

In the fingerprint region, raw water hyacinth has peaks at 1315 cm^{-1} indicating the presence of O-H bending representing carboxylic acid salts and at 1020 cm^{-1} indicating the presence of C-N stretch representing aliphatic amines. On the other hand, the hydrolyzed WH spectrum shows the presence of carboxylic acid salts at 1408 cm^{-1}. It also shows the presence aliphatic amines but at a shorter peak. This means there is some degradation of the aliphatic amines by dilute acid hydrolysis(Fallis, 2013; *IR Spectrum Table & Chart Sigma-Aldrich*, n.d.; Sataloff et al., n.d.).

The ethanol spectrum has peaks at 2975 and 1657 cm^{-1}. These values indicate that there is a presence of a C-H stretch accounting for the existence of alkanes and the presence of C-H bending which represents aromatic compounds. In the finger print section; there is a major signal at 1043 cm^{-1} and a minor signal at 1075 cm^{-1}. These values indicate the presence of a C-O stretch which represents for primary alcohol class. For pure ethanol, the minor and major peaks exist at 1087 and 1047 cm^{-1}, respectively(Coldea et al., 2013). The fact that the alcohol product from this work has both minor and major peaks at similar wave numbers and the fact that it also includes a primary alcohol indicates that the product is ethanol. The values of the peak wave numbers are also similar. Some deviations might occur due to the presence of water and other impurities.

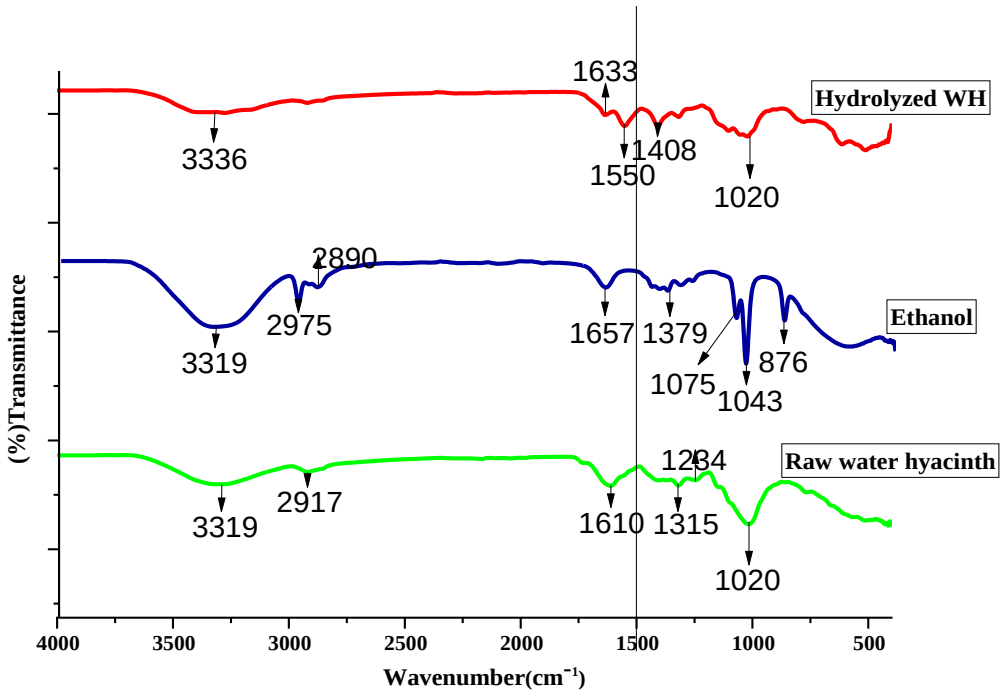


Figure 4. 14. The FT-IR spectroscopy of WH and products

5 Conclusion and recommendations

5.1 Conclusion

The production of bioethanol was carried out by using a two-step pretreatment process followed by enzymatic hydrolysis and fermentation. The two-step pretreatment processes were liquid hot water hydrolysis and dilute acid hydrolysis. The dilute acid hydrolysis process was optimized in order to have an optimum yield of reducing sugar that can enhance the fermentation process and to make the cellulose in the lignocellulosic structure most susceptible to enzymatic hydrolysis by reducing the hemicellulose as much as possible. The parameters that were optimized were the dilute acid concentration, hydrolysis temperature and hydrolysis time. The optimum condition that yielded the maximum amount of reducing sugar (11.884 g/L) were a dilute concentration of 1.5 %(V/V), hydrolysis temperature of 140^0C and hydrolysis time of 85 min. The yield of reducing sugar was obtained to be 415.94 mg per gram of water hyacinth. It can be concluded that the amount obtained is a good amount that can enhance the fermentation process.

The enzymatic hydrolysis step was a very important step where the recalcitrant cellulose was broken down into glucose and other hexose sugar. This step was carried out using a cellulase enzyme (activity: 10 U/mg) that was extracted from *trichoderma virdie.* The reducing sugar obtained was 10.9554 g/L and the yield was obtained to be 588 mg of reducing sugar per gram of water hyacinth. The theoretical yield of enzymatic hydrolysis was 886 mg of reducing sugar per gram of water hyacinth and in turn the efficiency was obtained to be 66.36%. This shows that more than 50 % of the theoretical yield was obtained.

During the fermentation process the liquids filtered from both the pretreatments and the enzymatic hydrolysis was utilized. *Saccharomyces cerevisae* was used for the production of ethanol from the fermentable sugars. The bioethanol obtained in the fermentation broth was 7.18 %(v/v). After distillation, 72 %(v/v) was recovered and used for further characterization by FTIR and other physic-chemical characteristics such as density, API gravity, specific gravity, refractive index and pH. The bioethanol yield of the fermentation process was found to be 168mg of ethanol per gram of water hyacinth. The theoretical yield was 511mg per gram of water hyacinth. Finally the efficiency of the fermentation was 43.3% which is low. It was concluded that this might be due to the use of commercial yeast.

5.2 Recommendation

The following recommendations are suggested to further improve the bioethanol production from water hyacinth.

- Optimization can be carried out on the liquid hot water pretreatment process to increase the reducing sugar yield
- Optimization can be carried out on the enzymatic hydrolysis process to increase the reducing sugar yield
- Optimization can be carried out on the fermentation process to have a better yield of bioethanol
- Other types of yeasts can be tested out on the fermentation process and do a comparison
- Rather than buying the cellulase enzyme, the microbe that produces the enzyme can be tested out for breaking done of cellulase enzyme into hexose sugars

References

Adney, B., & Baker, J. (2008). *Measurement of Cellulase Activities Laboratory Analytical Procedure (LAP) Issue Date : 08 / 12 / 1996 Measurement of Cellulase Activities Laboratory Analytical Procedure (LAP). January*.

Akbar, D., Subedi, R., Rolfe, J., Ashwath, N., & Rahman, A. (2019). 16 - *Reviewing commercial prospects of bioethanol as a renewable source of future energy-an Australian perspective. In Advances in Eco-Fuels for a Sustainable Environment.* Elsevier Ltd. https://doi.org/10.1016/B978-0-08-102728-8.00016-4

Amriani et al. (2016). *Physical and Biophysical Pretreatment of Water Hyacinth Biomass for Cellulase Enzyme Production*. *30*(2), 237–244. https://doi.org/10.15255/CABEQ.2015.2284

Anjos, O., Santos, A. J. A., Estevinho, L. M., & Caldeira, I. (2016). *FTIR – ATR spectroscopy applied to quality control of grape-derived spirits*. *205*, 28–35. https://doi.org/10.1016/j.foodchem.2016.02.128

ASTM standard D 3172 – 89 R02. (2002). *Standard Practice for Proximate Analysis of Coal and Coke 1*. *89*(Reapproved), 1–2.

ASTM standard D 3173 – 03. (2013). *Standard Test Method for Moisture in the Analysis Sample of Coal and Coke 1*. 7–9.

ASTM standard D 3174 – 02. (2013). *Standard Test Method for Ash in the Analysis Sample of Coal and Coke from Coal 1*. *14*.

ASTM standard D 3175 – 89a. (2013). *Standard Test Method for Volatile Matter in the Analysis Sample of Coal and Coke 1*. *Reapproved 1997*, 1–3.

Aswathy, U. S., Sukumaran, R. K., Devi, G. L., Rajasree, K. P., Singhania, R. R., & Pandey, A. (2010). *Bio-ethanol from water hyacinth biomass : An evaluation of enzymatic saccharification strategy. Bioresource Technology*, *101*(3), 925–930. https://doi.org/10.1016/j.biortech.2009.08.019

Awasthi, M., Kaur, J., & Rana, S. (2013). *Bioethanol production through water hyacinth , eichhornia crassipes via optimization of the pretreatment conditions*. *3*(3), 42–46.

Ayeni et al. (2015). *Compositional analysis of lignocellulosic materials : Evaluation of an economically viable method suitable for woody and non-woody biomass American Journal of Engineering Research (AJER)*. *4*, 14–19.

Balat, M., & Balat, H. (2009). Recent trends in global production and utilization of bio-ethanol fuel. *Applied Energy*, *86*(11), 2273–2282. https://doi.org/10.1016/j.apenergy.2009.03.015

Balcha, M. A. (2019). *Synthesis and Characterization of Cellulose Nanocrystals From Water Hyacinth Using Acid* (Issue June).

Bani, O. (2015). *Process selection on bioethanol production from water hyacinth (eichhornia crassipes) 2 . Materials and Methods*. 29–39.

Benjamin, Y., Cheng, H., & Görgens, J. F. (2014). *Optimization of Dilute Sulfuric Acid Pretreatment to Maximize Combined Sugar Yield from Sugarcane Bagasse for Ethanol Production*. 610–630. https://doi.org/10.1007/s12010-013-0545-z

Cavalaglio, G., Cotana, F., Nicolini, A., Coccia, V., Petrozzi, A., Formica, A., & Bertini, A. (2020). *Characterization of Various Biomass Feedstock Suitable for Small-Scale Energy Plants as Preliminary Activity of Biocheaper Project*. 1–10.

Chiaramonti, D., Prussi, M., Ferrero, S., Oriani, L., Ottonello, P., Torre, P., & Cherchi, F. (2012). *Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. Biomass and Bioenergy*, *46*, 25–35. https://doi.org/10.1016/j.biombioe.2012.04.020

Coldea, T. E., Socaciu, C., Fetea, F., Ranga, F., Pop, R. M., & Florea, M. (2013). *Rapid quantitative analysis of ethanol and prediction of methanol content in traditional fruit brandies from romania, using FTIR spectroscopy and chemometrics*. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *41*(1), 143–149. https://doi.org/10.15835/nbha4119000

Cristina et al. (1996). *Refractive Indices , Densities , and Excess Properties for Binary Mixtures Containing Methanol , Ethanol , 1 , 2-Ethanediol , and 2-Methoxyethanol*. *9568*(95), 685–688.

Dale. (2019). *BP Statistical Review of World Energy Statistical Review of World*.

Das et al. (2015). *Sustainable Materials and Technologies Optimization of enzymatic sacchari fi cation of water hyacinth biomass for bio-ethanol : Comparison*

between arti fi cial neural network and response surface methodology. SUSMAT, *3*, 17–28. https://doi.org/10.1016/j.susmat.2015.01.001

Das, Ghosh, P., Paul, T., & Ghosh, U. (2016). P*roduction of bioethanol as useful biofuel through the bioconversion of water hyacinth (Eichhornia crassipes). 3 Biotech*. https://doi.org/10.1007/s13205-016-0385-y

Das, S., Ganguly, A., Dey, A., & Ting, Y. (2014). *Journal of Chemical , Biological and Physical Sciences Characterization of Water Hyacinth Biomass and Microbial Degradation of the Biomass under Solid State Fermentation Using a Lignocellulolytic Fungus (Alterneria Spp NITDS1). August*.

de MIRANDA, J. A. T., de C*ARVALHO, L. M. J., Vieira, A. C. de M., & de CASTRO, I. M. (2019). Scanning electron microscopy and crystallinity of starches granules from cowpea, black and carioca beans in raw and cooked forms. Food Science and Technology*, *39*, 718–724. https://doi.org/10.1590/fst.30718

Devarapalli, M., & Atiyeh, H. K. (2015). *A review of conversion processes for bioethanol production with a focus on syngas fermentation*. *7*, 268–280. https://doi.org/10.18331/BRJ2015.2.3.5

Dussán et al. (2014). *Dilute-acid Hydrolysis of Cellulose to Glucose from Sugarcane Bagasse*. *38*, 433–438. https://doi.org/10.3303/CET1438073

Fallis, A. . (2013). FTIR spectrum analysis. Lecture notes. *Journal of Chemical Information and Modeling*, *53*(9), 1689–1699.

Gandhimathi, A. (2017). *Enhanced biofuel production from Water hyacinth*. *23*(3), 1586–1590.

Ganguly, A, Chatterjee, P. K., & Dey, A. (2012). Studies on ethanol production from water hyacinth — A review. *Renewable and Sustainable Energy Reviews*, *16*(1), 966–972. https://doi.org/10.1016/j.rser.2011.09.018

Ganguly, Amit, Das, S., Bhattacharya, A., Dey, A., & Chatterjee, P. K. (2013a). *Enzymatic hydrolysis of water hyacinth biomass for the production of ethanol : Optimization of driving parameters*. *51*(July), 556–566.

Ganguly, Amit, Das, S., Bhattacharya, A., Dey, A., & Chatterjee, P. K. (2013b). Enzymatic hydrolysis of water hyacinth biomass for the production of ethanol: Optimization of driving parameters. *Indian Journal of Experimental Biology*,

51(7), 556–566.

Haghighi, S., Hossein, A., & Tabatabaei, M. (2013). *Lignocellulosic biomass to bioethanol , a comprehensive review with a focus on pretreatment*. *27*, 77–93.

Herr, J. V, & Belda, R. (2006). *Refractive Indices , Densities and Excess Molar Volumes of Monoalcohols + Water Refractive Indices , Densities and Excess Molar Volumes of Monoalcohols + Water*. *May 2014*. https://doi.org/10.1007/s10953-006-9059-4

Holder, C. F., & Schaak, R. E. (2019). *Tutorial on Powder X-ray Diffraction for Characterizing Nanoscale Materials. ACS Nano*, *13*(7), 7359–7365. https://doi.org/10.1021/acsnano.9b05157

Idrees et al. (2013a). *Optimization of dilute acid pretreatment of water hyacinth biomass for enzymatic hydrolysis and ethanol production. EXCLI Journal*, *12*, 30–40. https://doi.org/10.17877/DE290R-5398

Idrees et al. (2013b). *Optimization of dilute acid pretreatment of water hyacinth biomass for enzymatic hydrolysis and ethanol production Original article : January*. https://doi.org/10.17877/DE290R-5398

Idrees, M., Adnan, A., Bokhari, S. A., & Qureshi, F. A. (2014). *Production of fermentable sugars by combined chemo-enzymatic hydrolysis of cellulosic material for bioethanol production*. *31*(02), 355–363.

IR Spectrum Table & Chart Sigma-Aldrich. (n.d.).

Jeong, T., Um, B., & Kim, J. (2010). *Optimizing Dilute-Acid Pretreatment of Rapeseed Straw for Extraction of Hemicellulose*. 22–33. https://doi.org/10.1007/s12010-009-8898-z

Jimoh, A., & Academy, N. D. (2016). *Assessment of the chemical constituents of freshwater biomass in the Assessment of the chemical constituents of freshwater biomass in the production of Biofuel*. *August*.

Jönsson, L. J., & Martín, C. (2016). Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresource Technology*, *199*, 103–112. https://doi.org/10.1016/j.biortech.2015.10.009

Karri, S., Raveendran, S., Parameswaran, B., & Pandey, A. (2011). *Bioethanol*

production from acid pretreated water hyacinth by separate hydrolysis and fermentation. *May 2014*.

Kumar et al. (2019). *Lignocellulosic Biomass for Bioethanol Production Through Microbes : Strategies to Improve Process Efficiency Lignocellulosic Biomass for Bioethanol Production Through Microbes :* (Issue April). Springer International Publishing. https://doi.org/10.1007/978-3-030-14463-0

Kumari, N., Bhattacharya, A., Dey, A., Ganguly, A., & Chatterjee, P. . (2014). *BIOETHANOL PRODUCTION FROM WATER HYACINTH BIOMASS USING ISOLATED FUNGAL STRAIN FROM LOCAL ENVIRONMENT*. *2*(2), 516–522.

Li, M., Cao, S., Meng, X., Studer, M., Wyman, C. E., & Ragauskas, A. J. (2017). *Biotechnology for Biofuels The effect of liquid hot water pretreatment on the chemical – structural alteration and the reduced recalcitrance in poplar*. 1–13. https://doi.org/10.1186/s13068-017-0926-6

Limited, W. P. (2010). *Bioalcohol production Related titles :*

Malveaux, C. C. (2013). *Coastal plants for biofuel production and coastal preservation*.

Melane, M., Ham, C., & Meincken, M. (2017). Characteristics of selected non-woody invasive alien plants in South Africa and an evaluation of their potential for electricity generation. *Journal of Energy in Southern Africa*, *28*(3), 92–98. https://doi.org/10.17159/2413-3051/2017/v28i3a1896

Miller, L. G. (1959). *Use of DinitrosaIicyIic Acid Reagent for Determination of Reducing Sugar*. *Analytical Chemistry*, *lll*, 426–428.

Netai, M. M., Jameson, K., & Mark, F. Z. (2016). S*urface composition and surface properties of water hyacinth (Eichhornia crassipes) root biomass: Effect of mineral acid and organic solvent treatment. African Journal of Biotechnology*, *15*(21), 897–909. https://doi.org/10.5897/ajb2015.15068

Noordin, M. Y., Venkatesh, V. C., Sharif, S., Elting, S., & Abdullah, A. (2004). *Application of response surface methodology in describing the performance of coated carbide tools when turning AISI 1045 steel*. *145*, 46–58. https://doi.org/10.1016/S0924-0136(03)00861-6

Pattra, S., & Sittijunda, S. (2015). *Optimization of Factors Affecting Acid Hydrolysis of Water Hyacinth Stem (Eichhornia Crassipes) for Bio-Hydrogen Production.* In *Energy Procedia* (Vol. 79). Elsevier B.V. https://doi.org/10.1016/j.egypro.2015.11.574

Pérez, J. A., Ballesteros, I., Ballesteros, M., Sáez, F., Negro, M. J., & Manzanares, P. (2008). *Optimizing Liquid Hot Water Pretreatment Conditions to Enhance Sugar Optimizing Liquid Hot Water pretreatment conditions to enhance sugar recovery from wheat straw for fuel-ethanol production*. *December*. https://doi.org/10.1016/j.fuel.2008.06.009

Reales-Alfaro, J.-G., Daza, L. T., Arzuaga-Lindado, G., Castaño-Peláez, H.-I., & Polo-Córdoba, Á.-D. (2013). *Acid hydrolysis of water hyacinth to obtain fermentable sugars*. *June*. https://doi.org/10.29047/01225383.60

Rezania, S. (2018). Ethanol Production from Water Hyacinth (Eichhornia crassipes) Using Ethanol Production from Water Hyacinth (Eichhornia crassipes) Using Various Types of Enhancers Based on the Consumable Sugars. *Waste and Biomass Valorization*, *9*(6), 939–946. https://doi.org/10.1007/s12649-017-9883-3

Rezania, S., Mohamad, S. E., Sohaili, J., Taib, S. M., Badruddin, M., Yusof, M., Kamyab, H., Darajeh, N., & Ahsan, A. (2017). *com Review on Pretreatment Methods and Ethanol Production from Cellulosic Water Hyacinth*. *12*, 2108–2124.

saha, P., Alam, M. F., Baishnab, A. C., Khan, M. R., & Islam, M. A. (2014). Fermentable Sugar Production and Separation from Water Hyacinth Using Enzymatic Hydrolysis. *Sustainable Energy*, *2*(1), 20–24. https://doi.org/10.12691/rse-2-1-4

Sataloff, R. T., Johns, M. M., & Kost, K. M. (n.d.). *infrared spectroscopy*.

Satari Baboukani, B., Vossoughi, M., & Alemzadeh, I. (2012). Optimisation of dilute-acid pretreatment conditions forenhancement sugar recovery and enzymatic hydrolysis ofwheat straw. *Biosystems Engineering*, *111*(2), 166–174. https://doi.org/10.1016/j.biosystemseng.2011.11.009

Shah, S., Alvi, M., Ali, M. N., Mohiuddin, M., & Munawer, M. (2014). *Original*

Research Article Eichhornia crassipes - a potential substrate for biofuel production. *3*(10), 618–627.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Nrel, D. C. (2012). *Determination of Structural Carbohydrates and Lignin in Biomass Determination of Structural Carbohydrates and Lignin in Biomass*. *April 2008*.

Sornvoraweat, B., & Kongkiattikajorn, J. (2010). *Separated hydrolysis and fermentation of water hyacinth leaves for ethanol production*. *15*(9), 794–802.

Sparla, F., Falini, G., Botticella, E., Pirone, C., Talamè, V., Bovina, R., Salvi, S., Tuberosa, R., Sestili, F., & Trost, P. (2014). New starch phenotypes produced by TILLING in barley. *PLoS ONE*, *9*(10). https://doi.org/10.1371/journal.pone.0107779

Speakman, S. A., & Ph, D. (n.d.). *Estimating Crystallite Size Using XRD*.

Stat-Ease » v11 » General Sequence of Analysis » Diagnostics » Diagnostics Plots. (n.d.). https://www.statease.com/docs/v11/contents/analysis/diagnostics/diagnostics-plots/#diagnostics-plots

Stat-Ease » v11 » Tutorials » Response Surface. (n.d.).

Timung, R., Deshavath, N. N., Goud, V. V, & Dasu, V. V. (2016). *Effect of Subsequent Dilute Acid and Enzymatic Hydrolysis on Reducing Sugar Production from Sugarcane Bagasse and Spent Citronella Biomass*. *2016*.

Toribio-cuaya, H., Pedraza-segura, L., & Macías-bravo, S. (2014). Journal of Chemical , Biological and Physical Sciences Characterization of Lignocellulosic Biomass Using Five Simple Steps. *Journal of Chemical, Biological and Physical Sciences*, *4*(5), 28–49.

Vieira et al. (2007). *Optimization of acid hydrolysis from the hemicellulosic fraction of Eucalyptus grandis residue using response surface methodology*. *98*, 422–428. https://doi.org/10.1016/j.biortech.2005.12.012

Wang, L., Luo, Z., & Shahbazi, A. (2013). Optimization of simultaneous saccharification and fermentation for the production of ethanol from sweet sorghum (Sorghum bicolor) bagasse using response surface methodology. *Industrial Crops & Products*, *42*, 280–291.

https://doi.org/10.1016/j.indcrop.2012.06.005

Wei, W., Wu, S., & Liu, L. (2012). Bioresource Technology Enzymatic saccharification of dilute acid pretreated eucalyptus chips for fermentable sugar production. *Bioresource Technology*, *110*, 302–307. https://doi.org/10.1016/j.biortech.2012.01.003

Zhang, Q., Weng, C., Huang, H., Achal, V., & Wang, D. (2016). *Optimization of Bioethanol Production Using Whole Plant of Water Hyacinth as Substrate in Simultaneous Saccharification and Fermentation Process*. *6*(January), 1–9. https://doi.org/10.3389/fmicb.2015.01411

Zhuang, X., Wang, W., Yu, Q., Qi, W., Wang, Q., Tan, X., Zhou, G., & Yuan, Z. (2016). Liquid hot water pretreatment of lignocellulosic biomass for bioethanol production accompanying with high valuable products. *Bioresource Technology*, *199*, 68–75. https://doi.org/10.1016/j.biortech.2015.08.051

Appendices

Appendix A: data for proximate and physicochemical analysis

- Results from volatile matter analysis

Table 6. 1. Data obtained during volatile matter measurement

Initial weight in grams of the moisture content removed water hyacinth(± 0.1)	Final mass of the water hyacinth after being incinerated(± 0.1)	Volatile matter removed (%w/w)	Average volatile matter in %w/w
1.5	0.2	86.67	84.44
1.5	0.2	86.67	
1.5	0.3	80	
			Stdev = 3.14

- Results from ash content analysis**:**

Table 6. 2. Data obtained during ash content measurement

Weight of the water hyacinth before being incinerated (in grams) with crucible	Weight of the residual after incineration (in grams) with crucible	Ash content analyzed in % w/w	Average Ash Content in % w/w
35.9	33.5	6.68523677	6.80751478
34.4	32	6.97674419	
35.5	33.1	6.76056338	

- Results for the fixed carbon determination

The fixed carbon was analyzed according to Equation 3.4 and the results obtained were:

- FC_1:6.648097
- FC_2:6.356589
- FC_3:13.23944

- Results of bulk density of water hyacinth

Table 6. 3. Data obtained during bulk density determination

Mass of powdered water hyacinth(g)	volume of measuring cylinder (mL)	Bulk density (g/mL)	Average bulk density (g/mL)
24.7	50	0.494	0.43533333
20.83	50	0.4166	
19.77	50	0.3954	

- Result from extractives amount determination

Table 6. 4. Data obtained during the extraction of extractives

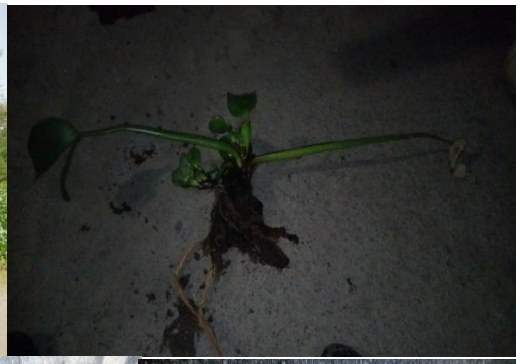
Initial weight of dried water hyacinth(grams)	Weight of water hyacinth after extraction by water (grams)	Weight of water hyacinth after acetone extraction (grams)	Amount of extractives (%(w/w))	average extractives amount (%(w/w))
5	4.872	4.596	8.08	8.54
5	4.791	4.55	9	

- Hemicellulose determination results

Table 6. 5. Data obtained during the determination of hemicellulose amount

extractive free water hyacinth (grams)	residual after cooking WH with NaOH (grams)	Hemicellulose amount (%(w/w))	average hemicellulose amount(%(w/w))
1	0.831	16.9	20.8
1	0.669	33.1	
1	0.876	12.4	

Appendix B: Pictures taken during the present work

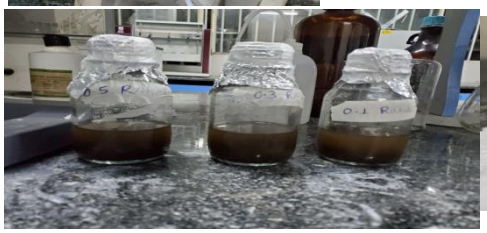








Autoclave 008


100

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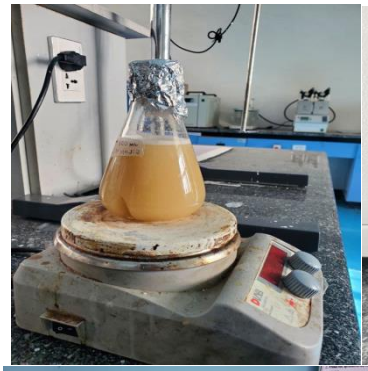
SRL

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