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MICROWAVE ASSISTED ALKALINE PRE-TREATMENT TO IMPROVE TOTAL REDUCING SUGAR YIELD OF RICE HUSK FOR PRODUCTION OF BIOETHANO

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By:

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July, 2021
Bahir Dar, Ethiopia

MICROWAVE ASSISTED ALKALINE PRE-TREATMENT TO IMPROVE TOTAL REDUCING SUGAR YIELD OF RICE HUSK FOR PRODUCTION OF BIOETHANOL

BY:

ELSA ZERAY

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF BAHIR DAR INSTITUTE OF TECHNOLOGY, BDU IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN THE SUSTAINABLE ENERGY ENGINEERING.

ADVISOR: DR. NIGUS GABBIYE (ASSOCIATE PROFESSOR)

JULY, 2021
BAHIR DAR, ETHIOPIA

DECLARATION

I, the undersigned, declare that the thesis comprises my own work. In compliance with internationally accepted practices, I have acknowledged and refereed all materials used in this work. I understand that non-adherence to the principles of academic honesty and integrity, misrepresentation/ fabrication of any idea/data/fact/source will constitute sufficient ground for disciplinary action by the University and can evoke penal action from the sources that have not been properly cited or acknowledged.

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Place: Bahir Dar

This thesis has been submitted for examination with my approval as a university advisor.

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Approval of thesis for defense result

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

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As members of the board of examiners, we examined this thesis entitled "Microwave Assisted Alkaline Pre-Treatment to Improve Total Reducing Sugar Yield of Rice Husk for Production of Bioethanol" by Elsa Zeray. We hereby certify that the thesis is accepted for fulfilling the requirements for the award of the degree of Masters of Science in "Sustainable Energy Engineering".

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ABSTRACT

Lignocellulosic biomass is becoming a primary candidate for bio-fuels production, due to the energy and food security concerns compared to starch and sugar based feed stocks. However those biomasses has limited digestibility due to high lignin content which is reluctant to microbial action in the subsequent step to bioethanol production such as hydrolysis. This study evaluates the effect of Microwave assisted alkali compared to alkali alone pretreated rice husk feedstock on the improvement of lignin removal, cellulose yield and total yield of reducing sugar for production of bioethanol. The pretreatment were carried out using concentration of 3.5% NaOH at temperature of 40, 60, 80 °C, and MW irradiation power of 300, 450, 600 W at pretreatment time of 15, 30, 60 min. The chemical compositional analysis of raw and treated rice husk was characterized using NREL and ASTM procedures in identify the biomass has a potential for fermentable sugar production and The pretreated biomass was evaluated for its reducing sugar yield via dilute acid hydrolysis (at 1.5% concentration) at a temperature of 131°C and contact time of 24 min using DNS method. It was found that the raw rice husk used for this study has a composition of 33.97% cellulose, 30.48% hemicellulose and 24.13% lignin. A maximum lignin removal of 47.57% and cellulose recovery of 66.52% were achieved with microwave assisted alkali pretreatment at microwave power of 600W and contact time of 30min in contrast to 25.15% lignin removal and 55.78% cellulose content recovery for in alkali pretreatment at a temperature of 80°C and contact time 60 min. The maximum reducing sugar (glucose) was observed for sample pretreated with MW assisted alkali pretreated (at 600W microwave power and 30 min contact time) which was 380.2 mg/g compared to alkali pretreated alone which was 270.3mg/g at 80°C, 60min.

Keywords: lignocellulose biomass, Rice husk, alkali pretreatment, microwave pretreatment, reducing sugar.

TABLE OF CONTENT

LIST OF TABLE

LIST OF FIGURE

LIST OF APPENDIX TABLE AND FIGURE

Appendix 1: Effect of Alkali (3.5%NaOH) pretreatment on the composition of Rice husk

Appendix 2: Effect of MW Assisted Alkali (3.5%NaOH) pretreatment on the composition of Rice husk

Appendix 3: Effect Of Pretreatments on the percentage weight loss/recovery of Rice husk

Appendix 4: effect of pretreatments on lignin removal and total reducing sugar concentration

Appendix 6: % of lignin removal with NaOH treatment by ANOVA

Appendix 7: Total reducing sugar with NaOH treatment by ANOVA

Appendix 8: % of lignin removal with MW Assisted NaOH treatment by ANOVA

Appendix 9: Total reducing sugar with MW Assisted NaOH treatment by ANOVA

Appendix 10: comparison of total reducing sugar yield between the two methods (with and without Microwave Assisted Alkali Pretreatments)

LIST OF ABBREVIATION

RH	Rice husk
MW	Microwave
SSF	Simultaneous Scarification and Fermentation
SHF	Separate Hydrolysis and Fermentation
ASTM	American Society for Testing and Materials
MC	Moisture content
AC	Ash content
FC	Fixed carbon content
VM	Volatile mater
AIL	Acid insoluble lignin
ASL	Acid soluble lignin
GHG	Greenhouse gas
NREL	National renewable energy laboratory
UV-VIS	Spectrometer Ultra violate -Visual spectrometer

CHAPTER ONE

1. INTRODUCTION

1.1 Background

The fossil fuels are considered to be the main source of energy today, but there are some concerns regarding their sustainability due to an increase demand and finite resources of natural reserves. Their expected reserves are depleting with the rapid explorations in order to meet the growing energy needs of the world [Matsakas et al., 2014]. The pollutants and other extractives from these fuels are also harmful for the environment. Both these considerations demand an energy source, which will be available in abundant quantity and sustainability, and that must also be environmental friendly. This reason drives the world interest on other alternative, non-fossil fuel sources of energy [Montoya G, 2014]. Agricultural wastes (biomass) are viewed as a good alternative energy sources with an estimated worldwide annual production of 140 billion metric tons which have the potential to produce an energy equivalent of 50 tons of oil [UNEP, 2009].

As a result of their accessibility, sustainability, reduction of GHG emission, opportunity for rural manufacturing jobs, regional development, decrease of importing fuels and bio degradability, liquid biofuels derived from lignocellulosic biomass are now getting worldwide attentions [Demirbas A., 2009]. Mostly liquid bio fuels derived from lignocellulosic biomass are used for transportation and house hold cooking.

Bio-ethanol is one of the liquid fuels with feed stocks of: sucrose-containing materials (sugar cane, sugar beet, sweet sorghum etc.), starchy materials (corn, wheat, rice, barley etc.), and lignocellulosic biomass (wood, straw, husk etc.). At this time bio-ethanol production processes utilize first generation feed stocks which are more easily degradable biomass such as cereals and sugarcane juice but the utilization of these agricultural crops for energy production are highly conflict with food security [Ikechukwu, 2012]. Therefore this enforces the world to shift their focus on utilization of second generation feed stocks which are nonedible crops and agricultural waste (lignocellulosic biomass). Lignocelluloses wastes (LCW) refer to plant biomass wastes that are composed of cellulose, hemicellulose and lignin. They can be categorized as: wood residues (including sawdust and paper mill discards), grasses, waste paper, agricultural residues (including straw, husk, peelings, cobs, stalks, nutshells etc), nonfood seeds, domestic wastes (lignocelluloses garbage and sewage), food industry residues, municipal solid wastes [Rodríguez et al., 2008].

Ethiopia is well known for agriculture having overall favorable climate condition like Land, Labor and Organizing Skills to adopt rice as a food/commercial crop. Rice cultivation is well-suited to countries and regions with low labor costs and high rainfall, as it is labor-intensive to cultivate and requires sufficient water. Rice can be grown practically anywhere, even on a steep hill or mountain's areas. It is mainly cultivated by small farmers in developing countries. The consumption of rice is also readily stood at third place after Teff and Wheat in the country. Rice has become a commodity of strategic significance across much of Ethiopia for domestic consumption as well as export market for economic development. The estimated yields of rice in Ethiopia for 2006 are 2.32 tons per hectare compared to 5.80 tons in Europe, 4.93 in the Americas and 4.22 in Asia [Sreepada Hegde et.al; 2013]. There was 0.46 ton per hectare (46kg/ha) of rice husk released to the environment in 2006.

Some researches had been conducted on optimization of acid hydrolysis conditions of rice husk fermentable sugar products [Mustafa Germec et al., 2016], improvement of bioethanol production from rice husk through simultaneous hydrolysis and fermentation (SSF) and simultaneous hydrolysis followed by fermentation (SHF) by pretreated under mild condition with NaOH [Ana Maria

Arismendy et al., 2020], conversion of rice husk in to fermentable sugar by two stage hydrolysis [M N Salimi et al., 2017] and delignification of rice husk by microwave assisted chemical pretreatment [S.M. Laghari et al., 2018]. Microwave irradiation has been widely used in many areas because of its high heating efficiency and easy operation. Microwave irradiation could be accelerates the chemical reaction rate and reduction of overheating effect. In this work, the microwave assisted alkali pretreatment of rice husk and dilute acid hydrolysis were investigate and compare with alkali pretreatment alone.

1.2 Statement of the problem

Currently the requirement of fuel has increased significantly with the increasing human population. The energy from the fossil fuels are getting unable to fulfill the demand, this led to the large increment of price as well as there is a significant amount of CO_2 emission to the environment due to the consumption of these fuels. To resolve the aforementioned problem producing bio energy such as bio ethanol from lignocellulosic materials could be a sustainable option [Hahn-Hagerdal et al., 2006].

When it comes to Ethiopia, fossil fuel is imported mainly for transportation. Thus, the issues of fossil fuel highly affect its economy and foreign currency shortage. At this time Ethiopia is working on reducing imported fuel by blending it with ethanol. This ethanol is coming from sugar factory. To increase ethanol production we need to see the other alternative feed stocks. Since Ethiopia is agriculture based country, there are residues leftover which are not properly handled. Rice husk is one of them, which is a by-product of rice milling process, and it represents approximately 20% by weight of rough rice [Hashim et al., 1996]. Dawit, 2015 studied that, in Ethiopia the total national volume of production during 2013 cropping seasons was 184,210 ton (1,842,100 quintal) of paddy in 58,806 ha and 36,842 ton (368,420 quintal) of estimated rice husk released in to the environment in 2013. Most of this rice husk is discarded as waste which results in waste disposal problem and methane emission. It is also light weighted due to its low density which causes breathing problem if/when inhaled.

Though few studies are available, As far as my review through MW assisted alkali pretreatment of rice husk via acid hydrolysis is lacking. Thus this research is aiming on improving the reducing sugar yield of rice husk for ethanol production with microwave assisted alkali pretreatment.

1.3 Objectives

1.3.1 General objective

The general objective of this work to study the effect of microwave assisted alkaline pretreatment on the improvement of total reducing sugar yield of agricultural residue (rice husk) for production of bioethanol.

1.3.2 Specific objectives

- To characterize the physical and chemical properties of raw and pretreated rice husk following NREL method and ASTM and determine the reducing sugar using UV-Vis Spectrophotometer.
- To investigate the effect of operating parameters (temperature, microwave power and contact time) on delignification of rice husk with and without microwave assisted alkali pretreatment.
- To conduct acid hydrolysis at optimized operating parameters on pretreated rice husk.

1.4 Significance of the study

Due to the growing energy demand, running down of fossil fuels, raising fuel prices and concerns about global warming, motivates the world to shift its energy source to alternative renewable energy sources[Heike Kahr et al; 2013]. One of the renewable fuels expected to replace petroleum is bio-ethanol. Countries like

Ethiopia which depend on importing fuel spend huge amount of foreign currency for importing which is highly/significantly affect the economy.

Therefore this study has a great significant in assuring the production of an alternative form of energy from rice husk which is locally available and also substitute's fossil fuel. Rice husk is one of a renewable and non-food competitive feedstock raw material desirable for the production of alternative fuel oil such as bio ethanol.

1.5 Scope of the Study

Rice husk consisted of known amount of cellulose (36.70%), hemicellulose (20.05%) and lignin (21.3%) (Parajo et al., 2004), can use for different productions. Consequently, in this study rice husk collected from Fogera area around Bahr Dar, was used for total reducing sugar production by removing the hemicelluloses and lignin content in the pretreatment step. The pretreatment is not only needed to discharge the hemi-cellulose and lignin from the cellulose but also to reduce cellulose crystallinity and to increase cellulose porosity.

The main scope of this study was to improve total reducing sugar yield of rice husk for bioethanol production using with and without microwave assisted alkaline pretreatment methods. The experiments were analyzed the effect of process parameter (time, temperature and power) on lignin removal and the yield of total reducing sugar. The pretreated rice husk was hydrolyzed using dilute acid hydrolysis to produce glucose. Following hydrolysis, the identification of the reducing sugar of the hydrolyzed rice husk was conducted using DNS method.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Biomass

According to the international energy agency biomass is defined as any organic matter that comes from biogenic sources and it's available on a renewable basis. This includes animals and plant sourced by wood and agricultural crops, organic waste from municipal and industrial sectors. Biomass resources can be classified according to their origin: (1) agricultural, (2) lignocellulosic plants and (3) organic residue and waste. Lignocellulosic biomass is an abundant biopolymer in nature as a by-product of agricultural industry which offers a cheap source of sugar and bio-fuel which makes it a promising feedstock for bio-ethanol production [Dalgaard et. al., 2006]. Lignocellulose, the most abundant renewable biomass on earth, is composed mainly of cellulose, hemicellulose and lignin. Both cellulose and hemicellulose fractions are polymers of sugars and there by a potential source of fermentable sugar. Lignin can be used for production chemicals, combined heat and power or other purposes. They are also categories as wood residues, grasses, waste paper, agricultural residues, nonfood seeds, domestic wastes, food industry residues and municipal solid wastes (Rodríguez G et al., 2008].

2.2 Bio ethanol application

USA and Brazil are the largest bio-ethanol producers in the world, using cornstarch and sugarcane juice as their main substrate for bio-ethanol production. Ethanol is used in the transportation sectors by blending in various ratios with normal gasoline. Ethanol is an excellent additive for preventing engine knock and overheating of the engine valves. Ethanol has higher octane number (96- 113) than conventional gasoline (86-87) and thus, blending it will increase the octane number and reducing the need for toxic, octane enhancing additives. It enables combustion engines to run at a higher compression ratio and therefore provides a net performance gain of nearly 15% w/w (Ikechukwu, 2012). And also bio-ethanol used in house hold, chemical industries for the production of pharmaceutical products, dyestuffs, perfumes and numerous products. The main chemical industries that utilize ethanol industry are: solvents and alcoholic for beverages. According to (Vincent, 2012), about 73% of produced ethanol worldwide is used as fuel ethanol; while the rest goes to the beverage and industrial sectors.

2.3 Over view of ethanol production in Ethiopia

There is a large interest in bio fuels in Ethiopia as a substitute to petroleum-based fuels with a purpose of enhancing energy security and promoting rural development. Ethiopia has announced a national bio fuel production in the GTP in order to secure energy in the rural part and urban of Ethiopia. Its implications need to be studied intensively considering the fact that Ethiopia is a developing country with high population density and large rural population depending upon land for their livelihood. Ethiopia plan to reduce importing oil, since the imported oil has huge potential of polluting the environment and spent large amount of foreign currency. Therefore, since bio fuel is free from pollutants and found in abundant; it is the main energy source for Ethiopia in the near future [Abreham Berta et.al; 2015]. In Ethiopia currently ethanol is produced from sugar cane and bio-ethanol has been starting to be mixed with petrol at the rate of 5% since October 2008. Ethanol is more efficient than wood and dung, so it is good to promote ethanol usage at home as a short term measure. Also, a joint venture of American and Ethiopian companies is producing a cooking stove using ethanol, which may gradually increase the domestic demands (Embassy of Japan in Ethiopia, 2008).Some are already using bio-ethanol for cooking at home but due to shortage of ethanol production it is not going as expected.

2.4 Feed stocks for bio ethanol production

Bio-ethanol can be produced from different biomass feed stocks: sucrose rich feed stocks (sugar cane, sugar beet, fruits etc) , starchy materials (corn grain, sorghum grain, wheat grain, rice etc) and lignocellulosic biomass (corn stove, wheat straw, rice straw, rice husk etc.).

2.5 Physical and chemical characteristics of Lignocellulosic biomass

2.5.1 Composition

Lignocellulosic plant biomass is considered as an important renewable carbon resource for the bio refinery industries and also considered as a sustainable and environmental friendly alternative to control current petroleum platform. No matter the species of the plant, lignocellulosic biomass is mainly composed of a mixture of cellulose, hemicellulose and lignin but their constituent may vary from one plant species to another (Table2.1).

Table 2.1: Composition data of different lignocellulosic material for bioethanol production

Feed stock	Content dry wt %			
	Cellulose	Hemicellulose	Lignin	Reference
Hard woods				
Eucalyptus Globules	37.5	23.6	21.90	Neiva, 2018
Acacia dealbata	43.00	19.00	25.90	S. Ferreira et al., 2011
Black locust	41.46	33.16	19.73	Frantisek, K. et al., 2016
Soft woods				
Salix	42.50	25.00	26.00	Sassner et al., 2008
Spruce	44.00	24.60	27.50	Sassner et al., 2008
Agro-industrial residues				
Corn stover	38- 40	28.00	7- 21	Saini, J.K et al., 2015
Corn cobs	38.08	44.04	11.09	M. Pointner et al., 2014
Rice husks	37.01	29.04	24.01	Kalita et al., 2015
Barley straw	33.00	26.00	19.00	Duque, A., et al.,2020
Rye straw	41.10	30.20	22.90	Gullon et al., 2010
Oat straw	39.40	27.10	17.50	Nigam et al., 2009
Rice straw	38.02	18.30	21.60	Tsegaye, B. ga et al., 2019
Wheat straw	32.90	24.00	8.90	Nigam et al., 2009
Corn stalks	35.00	16.80	7.00	Nigam et al., 2009
Cotton stalks	43.07	12.05	28.50	K Shahzad et al., 2019
Soya stalks	44.02	15.09	19.02	Seonghun Kim et al.,2018
Sunflowers stalks	41.83	25.49	20.12	V. Babash et al., 2016
Sugarcane bagasse	45.05	27.00	21.01	Rocha et al., 2011
Ethiopian Mustard	32.70	21.90	18.70	Gonzalez-Garcia et al., 2010
Flax shives	47.70	17.00	26.60	Gonzalez-Garcia et al., 2010
Hemp hurds	37.40	27.60	18.00	Gonzalez-Garcia et al., 2010

2.5.2 Internal structure physical property

The physical properties of each of the components of lignocellulose, and how each of each component contributes to the behavior of the complex structure as a whole. This study is oriented towards breaking down the complex of lignocellulose and utilizing the components to produce reducing sugar.

Cellulose:

Cellulose is the fiber material that gives flexibility and strength to the cell wall. It is represented by generic formula (C_6 H_{10} O_5) n. Cellulose is a long chain polysaccharide formed by D–glucose units, linked by β–1, 4 glycosidic bonds: its structure has crystalline part and amorphous ones.

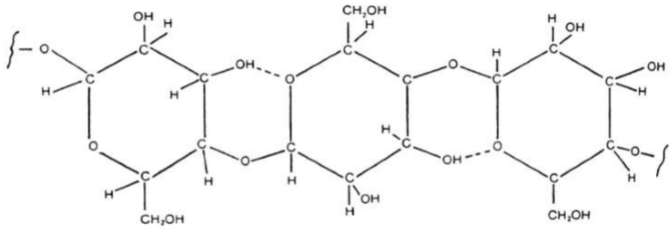


Figure 2.1 Cellulose Molecular structure

Cellulose is relatively hygroscopic material absorbing 8-14% water under normal atmospheric conditions (20°C, 60% relative humidity). Nevertheless it is insoluble in water, where it swells, also insoluble in dilute acid solution at low temperature. The solubility of the polymer depends on the degree of hydrolysis achieved. At higher temperature it becomes soluble, as the energy provided is enough to break down the hydrogen bond that holds the crystalline structure of the molecule [P.F.H Harmsen et al., 2010].

Hemicellulose:

Hemicellulose is a highly branched hetero-polymer containing sugar residues such as hexoses (D-galactose, L-galactose, D-mannose, L-rhamnose, L-fucose), pentoses (D-xylose, L-arabinose), and uronic acids (D-glucuronic acid). It is represented by the generic formula (C_5 H_8 O_4) n. It has a lower molecular weight than cellulose and its role is to connect lignin and cellulose fibers.

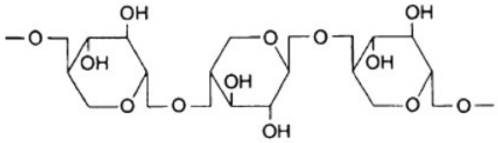


Figure 2.2 Hemicelluloses Molecular structure

Hemicellulose is insoluble in water at low temperature. Though, its hydrolysis starts at low temperature than cellulose. The presence of acid highly improves the solubility of hemicellulose in water [P.F.H Harmsen et al., 2010].

Lignin:

Lignin is the most abundant aromatic polymer in nature, highly branch polymer of phenyl propane and is an integral part of the secondary cell walls of plants. It is primarily a three dimensional polymer of 4-propenyl phenol, 4-propenyl2-methoxy phenol, and 4-propenyl-2.5-dimethoxyl phenol. Lignin is an amorphous polymer made by different phenolic compounds and is the main component of cell walls. Lignin holds together cellulose and hemicellulose fibers and gives support, resistance and impermeability to the plant.

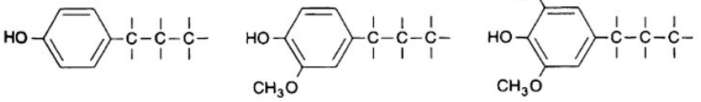


Figure 2.3 Lignin Molecular Structure

Low molecular alcohols, dioxane, acetone, pyridine and dimethyl sulfoxide are solvents that have been significantly dissolved lignin. Also it has been observed that at elevated temperature, thermal softening of lignin takes place, which allows depolymeristation reaction of acidic or alkaline nature to accelerate (O'Connor et al., 2007).

2.5.3 Chemical interaction between compounds

There are four main types of bonds in the lignocellulose complex which provide linkage with in the individual components of lignocellulose (intrapolymer linkage) and connect the different components to form the complex (interpolymer linkage).

Table 2.2: Overview of linkages between the monomer units that form the individual polymers lignin, cellulose and hemicellulose, and between the polymers to form lignocellulose [Paulien et, al., 2010].

Bonds with in individual components (Intrapolymer linkage)	
Ether bond	Lignin, (hemi)cellulose
Carbon to carbon	Lignin
Hydrogen bond	Cellulose
Ester bond	Hemicellulose
Bonds connecting different components (interpolymer linkage)	
Ether bond	Cellulose – Lignin
	Hemicellulose – Lignin
Ester bond	Hemicellulose – Lignin
Hydrogen bond	Cellulose – Hemicellulose
	Hemicellulose – Lignin
	Cellulose – Lignin

Ether bond is the most predominant bond in the lignin polymer and it is the bond that holds the glucose monomers in a polymer chain (glucosidic linkage). Therefore the cleavage of the ether bond can lead to separation of lignin from polysaccharides matrix and degradation of the polymers to monomer sugars and lignin fragments.

Ester bond is the bond between lignin and polysaccharides as well as with in hemicellulose polymers. Hydrolysis is performed to break the ester bond and results carboxyl and hydroxyl groups. The traction is reversible and endothermic. To increase the rate of reaction either acid or alkaline catalysts can be used. The most prominent difference with the acid catalyzed reaction route is that it leads to irreversible hydrolysis of the ester.

Hydrogen bond is considered to be responsible for the crystalline fibrous structure of cellulose. It is a bond connecting lignin with cellulose and with hemicellulose respectively as well as with in the cellulose polymer chains. Breaking of hydrogen bond can be accomplished by applying high temperature to the solution and a physical destruction of the cellulose molecules or by chemically producing cellulose derivatives such as cellulose acetate (Bochek, 2003).

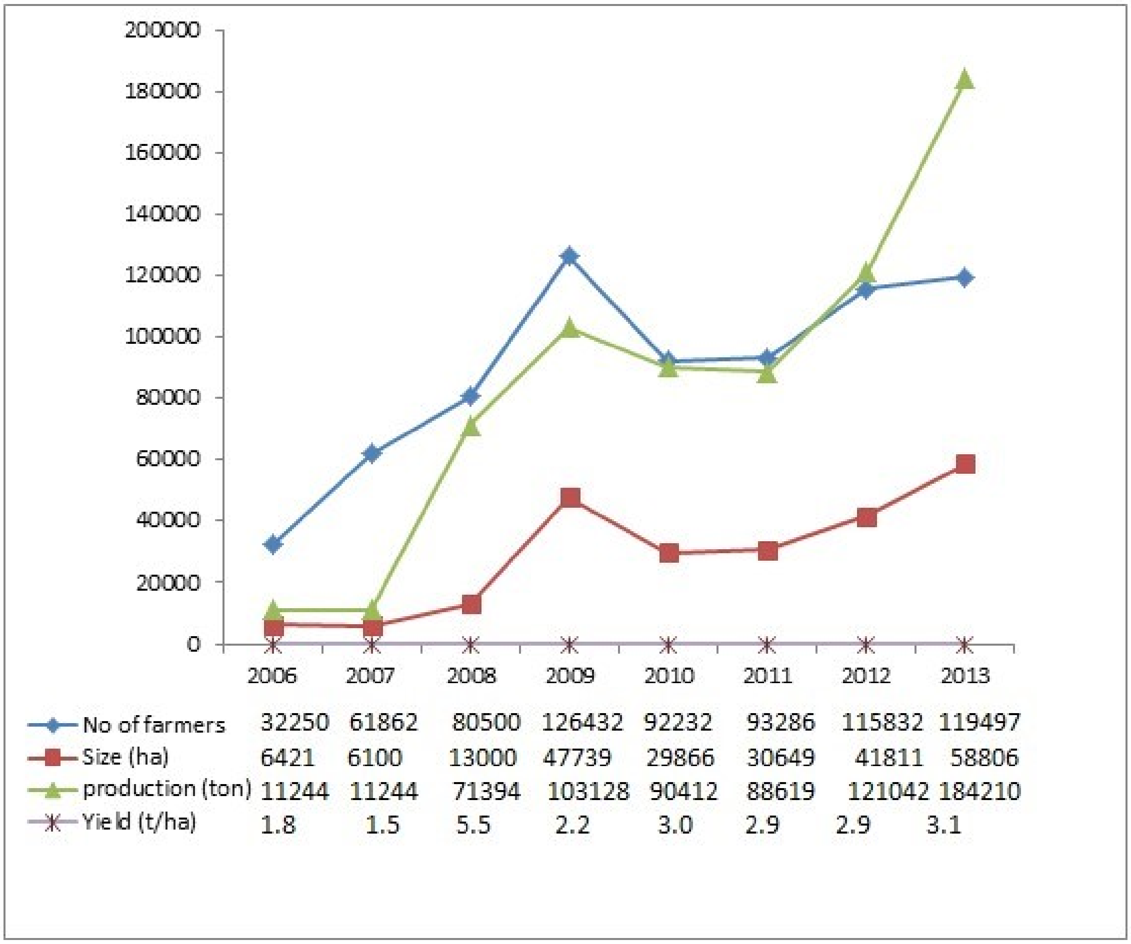
2.6 Rice husk as a feed stock for ethanol production

Rice husk is the hard protecting coverings of rice grains and most widely agricultural wastes in different rice producing countries in world. RH has a potential of fermentable sugar (glucose) for ethanol production. RH is currently not widely collected simply left it on the open field dump as a waste. However, this biomass could be used for lignocellulosic bio-ethanol production. As a renewable raw material, one of the usages of RH is its potential feedstock for the production of bio-ethanol to fulfill the increasing demand for bio-fuels (Pointner et al., 2014). Chemical content of rice husk consists of 50% cellulose, 25-30% lignin, and 15-20% silica [Ismail M. S et al., 1996]. Researchers have found that RHs contain silica (15-28 wt. %) and lignocellulose (LC, 72-85 wt. %), which can be further categorized into cellulose (35-40 wt. %), hemicellulose (15-20 wt. %), and lignin (20-25 wt. %). Estimated rice husk composition to be cellulose, hemicellulose, lignin, protein and ash content of 33–43%, 20–34.5%, 8–14.1%, 5% and 4%, respectively [Lim et al. 2012].

The ethanol productivity of rice husk varies with the variety. The difference in their cellulosic content causing variations in ethanol produced. The choice of pretreatment method plays an important role in increasing the efficiency of enzymatic saccharification, thereby making the whole process economically viable [Binod et al., 2010].

2.7 Geographical Distribution of Rice husk in Ethiopia

Rice production in Ethiopia has started a few decades ago and currently the researches shows that the country has realistic potential to grow different species of rice for rain fed lowland, upland, and irrigated ecosystems. Now a day's rice is considered as one of the strategic food security crops and also uses as an income source, employment opportunity and animal feed has been well recognized in Ethiopia [Teshome and Dawit, 2011]. As shown in the below figure the trend of rice production is increasing in area coverage, participant farmers, and production [MoARD, 2011].



	2006	2007	2008	2009	2010	2011	2012	2013
No of farmers	32250	61862	80500	126432	92232	93286	115832	119497
Size (ha)	6421	6100	13000	47739	29866	30649	41811	58806
production (ton)	11244	11244	71394	103128	90412	88619	121042	184210
Yield (t/ha)	1.8	1.5	5.5	2.2	3.0	2.9	2.9	3.1

Figure 2.4: Rice productive trend in Ethiopia [Dawit, 2015]

EUCORD 2012 reported that Ethiopia has about 17 million hectares of land suitable for rice production. FAO (2009) reported that four rice ecosystems were identified in the country. These are; upland rice, which is grown on naturally drained soils and where the water table always remains below the roots and is entirely rain fed ; Hydro orphic (rain fed lowland) rice, which is grown on soils where the roots are periodically saturated by fluctuating water table in addition to the rainfall; Irrigated lowland ecosystem, whereby crop water requirement is entirely satisfied from irrigation, and rainfall is not a limiting factor, and Paddy rice (with or without irrigation) which is grown under water-logged or submerged condition.

Table 2.3: Size of potential area by level of production potential from rain-feed Rice (ha) [NRRDSE 2010]

Region	Highly	Suitable	Moderately Suitable	Total
Tigray	-	1,278,254	935,565	2,213,810
Afar	2318	147,084	124,158	273,560
Amhara	483,839	5,289,945	2,229,180	8,002,964
Benishangul Gumuz	2,053,332	2,817,944	53,235	4,924,511
Somali		69,893	375,222	445,115
Oromiya	2,051,787	8,082,388	3,993,068	14,127,243
Dire Dawa		601	38,553	39,154
Gambela	373,848	2,752,345	38,037	3,164,230
SNNPR	625,771	4,472,184	1,035,648	6,163,603
Total	5,590,895	24,910,629	8,852,666	39,354,190

Table 2.4: Irrigation potentials in Ethiopia by basin [Awulachew et al., 2007]

River Basin	Irrigable land (ha)	Region (s)
Tekeze	83,368	Tigray and Amhara
Abay	815,581	Amhara, Oromiya, Benishangul Gumuz
Baro-Akobo	1,019,523	Benishangul Gumuz, Gamebella, Oromiya and SNNPR
Omo-Gibe	67,928	SNNPR and Oromiya
Rift Valley (lake)	139,300	Oromiya and SNNPR
Mereb	67,560	Tigray
Afar/Danakil	158,776	Afar and Tigray
Awash	134,121	Amhara, Oromiya, Afar and Somali
Wabi-Shebelle	237,905	Oromiya, Harrari and Somali
Genale-Dawa	1,074,720	Oromiya, SNNPR and Somali

2.8 Biochemical conversion of rice husk to ethanol

Due to the complex composition of lignocellulosic biomass its conversion to bio-ethanol are involved three processes. First step is pretreatment to remove lignin and disrupt/loosen-up the crystalline structure of cellulose and increase the porosity of the biomass. The Second step is hydrolysis of cellulose to produce reducing sugar by chemical and enzymatic process and the third step is Fermentation of the sugar to ethanol by yeast. [Yang B, Wyman C E (2008)].

2.8.1 Pretreatment

The purpose of pretreatment is to remove lignin, reduce cellulose crystallinity and increase the porosity of the materials. Pretreatment must improves the formation of sugar, avoid loss of carbohydrate, avoid formation of by product inhibitors and must be cost effective. There are different types of pretreatment methods like physical (milling, chemical, physic-chemical), chemical (acidic, alkaline, etc), biological or combination methods (alkaline and dilute acids, microwave assisted alkaline, microwave assisted acid pretreatment etc.) pretreatments.

Physical pretreatment - aims at reducing the particle size and crystallinity, increasing the surface area, reducing the degree of polymerization and shearing the biomass. These factors increase the total hydrolysis yield of lignocellulose and reduce the digestion time. This treatment method cannot remove lignin and require high energy.

Chemical pretreatment - is performed by adding one or more chemicals (acid, alkaline, oxidized agents and other chemicals) to treat the lignocellulose biomass. This method results in increasing accessible area of cellulose and hemicellulose, partial or nearly complete delignification, decreasing cellulose crystallinity and partial or complete hydrolysis of hemicellulose.

Biological pretreatment - is performed by introducing one or more of microorganism species like fungi, actinomycetes and others. This results in delignification, reduction in the rate of cellulose polymerization and hemicellulose hydrolysis partly. It has an advantage of zero chemical requirement, lesser energy desires and mild eco system condition. Disadvantage is very low from other pretreatments.

Microwave pretreatment - the effect of any pretreatment on the lignocellulose biomass depend upon mainly on three factors these are temperature, retention time and chemical concentration. Studies have shown that pretreatment performed at high temperature have given successful results in increasing the hydrolysis rate and product yields. This temperature increment may be performed by conventional heating or by using microwave radiation. MW has some advantage over conventional heating like, quick heat penetration, avoids degradation of product strength and surface properties caused by excessive and long heating, superficial heat transfer, no direct contact of heating source and material, providing a volumetric and rapid heat and selective heating of more polar part and creating a hot spot with inhomogeneous materials [Huang et al 2009]. MWs are the electromagnetic waves between the frequency ranges of 300MHz to 300GHz. The MW energy is directly transferred to the molecules of the material. These molecules become stimulated and rotate millions of times a second in response to the electromagnetic field. This rotation quickly generates heat within the material in a manner similar to friction.

Ultrasound pretreatment - is based on the principle of cavitation, which is described as the spontaneous formation, growth and subsequent collapse of micro size cavities/bubbles caused by the propagation of ultrasonic waves in liquid medium. The collapse of these cavities produces high temperature and pressure gradients locally for microsecond durations, creating the hot spot effect in the liquid, thus the overall conditions remain ambient. The high energy density in the vicinity of the collapsing cavity can give enhancing surface areas.

Many researchers' studies on the effect of different pretreatments methods as follow:

S.M Laghari et al. , 2018 studied microwave assisted acid and alkaline pretreatment methods to enhance the potential of energy recovery from rice husk and the study shows optimum result were achieved with MW assisted alkali pretreatment using 3.5% NaOH solution the lignin reduces from 17.8 to 10.2, cellulose increases from 38.6 to 59.3%.

Table 2.5: Chemical Composition of rice husk before and after pretreatment [S.M. Laghari et. al., 2018]

Sample	Cellulose %	Hemicellulose %	Lignin %	Ash %	Moisture %
Untreated RH	38.6	19.7	17.8	12.5	7.9
NaOH MW Treated					
2%	51.6	13.7	11.3	13.6	6.3
3.5%	59.3	10.5	10.2	9.4	6.9
5%	57.4	9.3	12.4	11.2	6.4
Na_2CO_3 MW Treated					
2%	40.3	18.9	16.4	14.9	6.1
3.5%	42.6	18.4	15.7	13.3	6.7
5%	44.5	17.6	14.5	13.7	6.2
H_2O_2 MW Treated					
2%	44.3	17.8	15.2	12.8	7.2
3.5%	47.2	16.1	14.6	11.2	7.4
5%	48.3	13.6	14.1	13.3	7.2
H_2SO_4 MW Treated					
2%	44.7	15.9	16.5	12.9	6.5
3.5%	54.2	11.4	14.8	10.3	6.4
5%	49.3	13.8	14.2	12.8	6.2

Kim S. et al., 2006 studied Alkaline pretreatments to acid pretreatment of lignocelluloses, with alkaline solutions minimize the loss of carbohydrate due hydrolysis, helps to remove acetyl groups, promoting the late hydrolysis and inhibit furfural. NaOH is the most popular alkali to treat lignocelluloses due to its low cost and high efficiency.

M. Nikzad, et al., 2013 studied using three different pretreatment methods for rice husk. In order to determine how each method affects the composition of the rice husk, the digestibility of the rice husk in enzymatic hydrolysis and ethanol production, dilute-H2SO4 (1%v/v, 121°C, 30 min), dilute-NaOH (3% w/v, 121°C, 30 min) and heat treatment (121°C, 30 min) were employed. Among them, the best results were obtained when the pretreatment of rice husk was carried out with 3% NaOH solution. Pretreatment of rice husk with NaOH substantially increased the lignin removal, enzymatic digestibility of cellulose, accessibility of cellulose and fermentable sugar production. The highest glucose concentration, glucose yield and ethanol concentration were 14.54 g/L, 59.6% and 6.22 g/L, which were 5.44, 3.77 and 6.15 times higher than the untreated control samples, respectively. Moreover, the SEM analysis of the pretreated sample illustrated significant physical changes of the rice husk after NaOH pretreatment.

Obiora S. et al., 2017 studied the effects of microwave-assisted alkali pre-treatment on pellets' characteristics and enzymatic saccharification for bio-ethanol production using lignocellulosic biomass of canola straw and oat hull and the results showed that the chemical composition analysis of microwave-assisted alkali pre-treatment was able to disrupt and break down the lignocellulosic structure of the samples, creating an area of cellulose accessible to cellulase reactivity than microwave or chemical only. The effects of pre-treatment results were supported by SEM analysis and its was found that microwave-assisted alkali pre-treatment of canola straw and oat hull at a short residence time enhanced glucose yield.

Microwave irradiations could increase the chemical reaction rate through their synergic effect or in fact, leads to an explosion effect among the biomass particles. Microwave irradiation is a process which has been widely used because of its high heating efficiency and easy operation. The residence time in microwave irradiation ranges from 5 to 20 min. It could change the ultrastructure of cellulose by degrading lignin and hemicelluloses and by increasing the enzymatic susceptibility of lignocellulosic materials [Maurya et al. 2013].

According to the literatures reviewed, microwave assisted chemical pretreatments are by far the most successful technique and results in increasing the saccharification of rice straw and rice husk [Singh A. et.al; 2011]. In this study, this pretreatment is going to be applying to evaluate a raw rice husk potential for ethanol production in Ethiopia.

2.8.2 Hydrolysis

Hydrolysis of cellulosic materials converts the carbohydrate polymers to monomer sugars (cellulose and hemicellulose into fermentable sugars). These polymers can be catalyzed enzymatically by cellulase or chemically by acids such as sulfuric acid (Kumar et al., 2009). Utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions: pH 4.8 and temperature 45−50ºC and does not have corrosion problem. Dilute acid hydrolysis is an easy and productive process and the amount of alcohol produced in case of acid hydrolysis is more than that of alkaline hydrolysis [Duff and Murray, 1996].

Dilute Acid Hydrolysis

Dilute or weak acid (DA) hydrolysis is one of the most effective pretreatment method lignocellulosic biomass. DA hydrolysis is a more established method and provides less probability of sugar degradation to inhibitors but requires more time and higher temperature to increase the reaction rate for sugar release [C. Luo, et al., 2002]. Different acids like sulfuric acid, phosphoric acid, nitric acid, hydrochloric acid and so forth have been evaluated, but sulfuric acid has been commonly used for biomass pretreatment. A typical DA hydrolysis process follows the acid concentration range 1-6%, temperature range from 100-200^{o}C and reaction time of up to 300min.

Table 2.6 acid hydrolysis conditions reported in the literature for various lignocellulosic materials.

Raw material	Hydrolysis onditions	Remarks	References
Corn Stover, poplar and switch grass	1.2% H_2SO_4 at 180 °C for 1min	80% sugar recovery.	Esteghlalian et al., 1997
Sugar cane bagasse	2% H_2SO_4 at 122 °C for 24min	Release of 21.6g xylose/L, 3g glucose/L, 0.5g furfural/L and 3.65g acetic acid/L was reported. 90% hemicellulose hydrolysis was reported.	Aguilar et al., 2002
Sugar cane bagasse	2% H_2SO_4 at 128 °C for 51.1min	Release of 22.6g xylose/L, 3.31g arabinose/L, 3.77g glucose/L, 1.54g furfural/L and 3.59g acetic acid/L was reported. Xylose with low concentration of inhibitors was observed.	Bustos et al., 2003
Wheat bran	1% H_2SO_4 at 130 °C for 40min	52.1g of sugar per 100g starch free residue was reported and the result showed very low content of furfural.	Choteborska et al., 2004
Sugar cane bagasse	0.5% H_2SO_4 at 121 °C for 60min	Release of 11g glucose/L, 11.29g xylose/L, 2.22g arabinose/L, 2.48g acetic acid/L and 0.12g furfural/L.	Pattra et al., 2008
Wheat straw	4% H_2SO_4 at 120 °C for 30min	36.2g/L production of ethanol yield reported.	Tomas-Pejo et al., 2009
Wheat straw	2% H_2SO_4 at 130 °C for 29min	99% of hemicellulose and 11% of glucan hydrolysis were observed.	Guerra-Rodriguez et al., 2012
Rice husk	1.5% H_2SO_4 at 131 °C for 24min	25.52g/l of fermentable sugar concentration was reported.	Mustafa Germec et al., 2015.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Materials and chemicals used

Rice husk sample was collected from Fogera district in Amhara region part of Debub Gonder Zone. In the Fogera wetlands the rice production had the highest position compared to the other crops which contributes 84.8% of the total income. The raw material was washed with tap water, dried in sunlight for 7 days, grinded to a size of 1-1.5 mm and stored with plastic bag at room temperature until used.

Chemicals:

NaOH is used as a pretreatment chemical for the removal of lignin and preparation of DNS reagent. Ethanol 97% is used as a solvent for extraction of untreated RH. Dilute sulfuric acid is used for hydrolysis process. Sodium sulfite, sodium hydroxide and distilled water used for preparation of 3, 5- dinitrosalicylic acid (DNS) reagent solution. A 40% potassium sodium tartrate aqueous solution used to prevent the dissolution of oxygen in the reagent. Na_2CO3 is used for neutralizing. All the chemicals was obtained from Ethiopian conformity Assessment Agency chemical laboratory.

Equipment's:

Digital balance (sensitivity offset of 0.2mg) is used for measuring mass, oven (CABOLITE Aston Lane, Hope Sheffield, S30 2RR) to dry, Domestic microwave with glass mirror, model MG402MADXBB output power of 950W and input power of 1500W was used for assisting alkali pretreatment, furnace (THERMOCENCEPT, KLS 45/13) for determination of ash content, coffee Grinder to reduce the sample size, sieve to evaluate particle size distribution, Soxhlet extraction unit to extract extractives using ethanol volatile matters, fast filter paper to separate the solid residue, autoclave (model MaXterile 60, design pressure 2.5bar, operation pressure 1.2~2 bar and operation temperature 121~132^{o}C) for hydrolysis, centrifuge (HERMLE Z323 and operating maxi speed of 15000 rpm) to separate the hydrolysate and un hydrolyzed residue, desiccators to keep the sample out of moisture, magnetic stirrer hot plate for alkali pretreatment, UV-Vis Spectrometer (model PerkinElmer UV/VIS Lambda, wavelength range and accuracy 190-1100nm; 0.5nm and scan speed 1-4000nm/min) to determine reducing sugar and acid soluble lignin based on the absorbance and digital pH meter to check the solution pH value.

3.2 Experimental Methods

3.2.1 Preparation of samples and chemical solutions

Sample preparation

The raw material (rice husk) collected from Fogera area of Amhara region was washed with tap water, dry in sunlight for seven days, grind to a size of 1-1.5 mm (the smaller the particle size the bigger the potential for cellulose degradation to reduce the cellulose degradation these was used) and oven dried at 105^{o}C for 24hr then store at room temperature until used.

(a) (b)

Figure 3.1 Rice husk sample (a) Raw sample; (b) Size reduced sample

Chemical preparation

A concentration of 3.5% sodium hydroxide was prepared using solid pellets. DNS reagent solution was prepared by dissolving 2g 3 and 5 a dinitrosalicylic acid, 400mg phenol, 100mg sodium sulfite and 2g sodium hydroxide in distilled water in a 200ml volumetric flask. Then, the solution heated in hot plates for 30 min. A 40% potassium sodium tartrate aqueous solution was prepared in another 200-ml volumetric flask.

3.2.2 Experimental setup and description

The dried rice husk samples were placed in the flask for alkaline pretreatment (with and without Microwave assisted) at different process condition. After pretreatment, the slurry is subjected to filtration with filter paper. The solid residue was washed out gently with the distilled water several times to neutralize and dried in the oven until constant weight. The pretreated powder is used for acid hydrolysis process by using autoclave. After hydrolysis the solid residue is removed from liquid filtrate, the hydrolysate and un hydrolyzed residue was separated by using centrifuge at 4000 rpm for 15 min. Finally, the hydrolysate is kept in deep freezer at 4^oC until it is used for analysis of reducing sugars.

The reducing sugar content following acid hydrolysis of rice husk was determined using DNS method and the sample measured at 540nm using UV-VIS spectrometer. The reducing sugar content of unknown sample was then read off a calibration curve (standard curve) created using known glucose concentration.

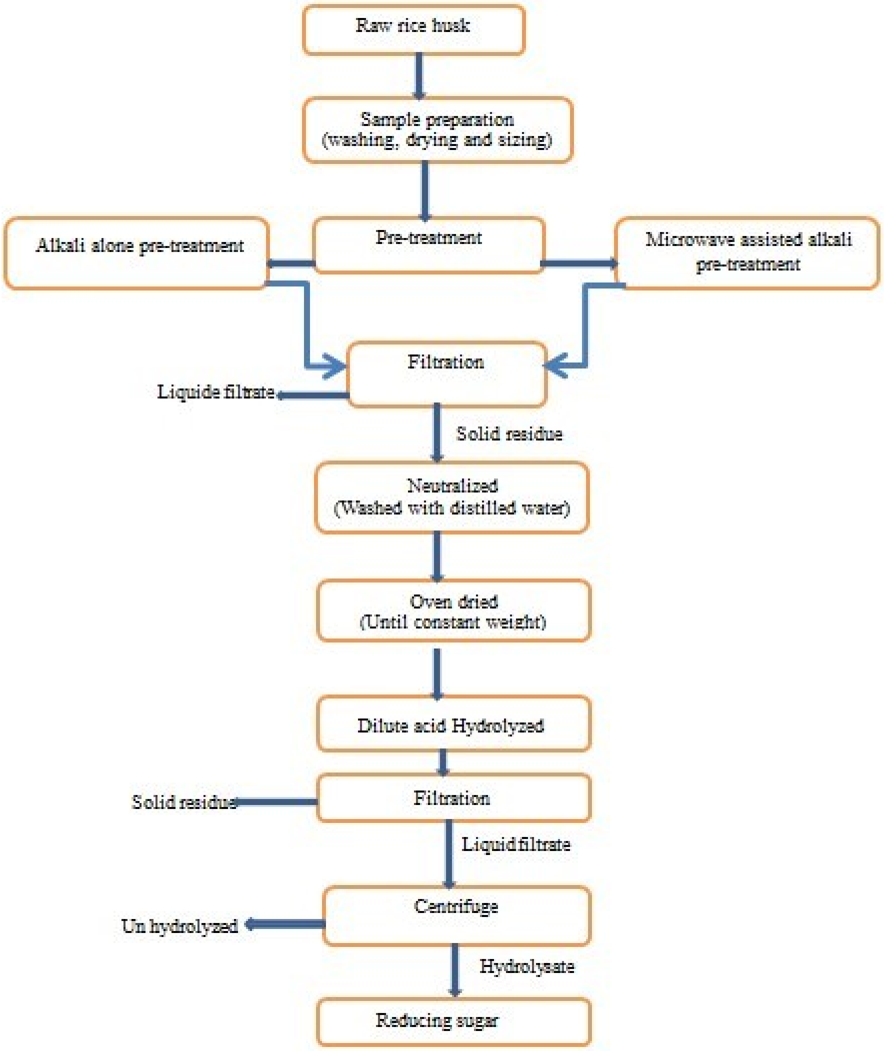


Figure 3.2 Experimental process flow schematic chart

3.3 Pretreatment of rice husk

3.3.1 Alkaline treatment

15g of extractive free dried RH samples with the size of (1-1.5) mm was measured into 500 ml beaker, and then 150 ml of alkali (3.5%NaOH) solution was added with solid to liquid ratio of 1-10w/v [L. $\mathrm{T_{AO}}$ et al. 2013]. The hot plate with magnetic stirrer was used to control the temperature at 40ºC, 60ºC and 80ºC [Zhixiam Wang et al 2016]and reaction time of 15min, 30min and 60min

[Shengdong Zhu et al. 2005]. After completion of reaction, the solution was filtered on fast filter paper, and the solids were continually washed with distilled water until the pH of filtrate was almost to 7. Filtrate was carefully scraped off the paper filter and the filtrates were dried in oven at 105 ºC until the weight remaining constant. For mass balance, the dry weight of de-lignified solids was recorded. The weight difference was calculated and considered as weight loss. Then alkali pretreated samples was stored in a plastic bag until it was used for analysis of chemical composition, and reducing sugar.

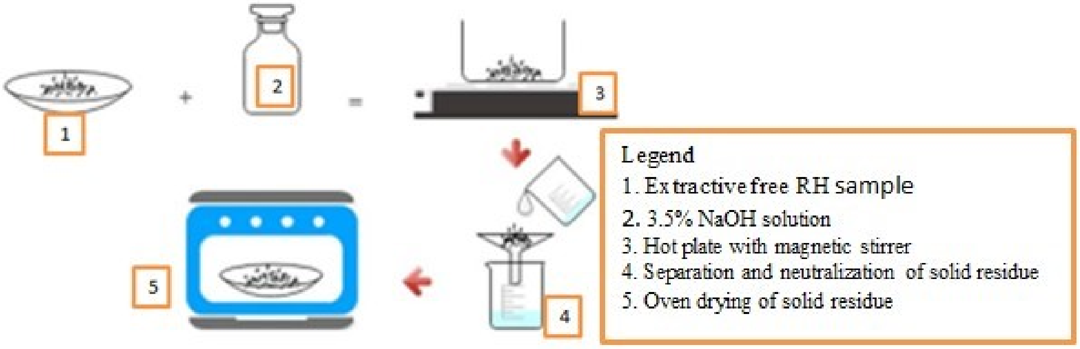


Figure 3.3 Process flow diagram for alkali pre-treatment of rice husk

3.3.2. Microwave treatment

Domestic microwave with glass mirror, model MG402MADXBB output power of 950W and input power of 1500W (source 220V/50~60Hz) was used to assist an alkali pretreated rice husk sample. 10g of extractive free dried RH samples with a size of (1-1.5) mm is socked in 3.5% concentration of NaOH solution with 1:10 w/v of solid-liquid ratio. Then the mixture was subjected to irradiation in MW oven at three different levels of output power (300, 450 and 600 W) and contact time of (15 min, 30 min and 60 min) [Shengdong Zhu et al. 2005]. At the end of each experiment, the MW assisted alkali pretreated substrate and spent liquor was separate by filter paper. Then the solid residue washed out gently with the distilled water several times to neutralize its pH (6.8-7.3) and dried in oven at 105^{o}C until the weight remaining constant. For mass balance, the dry weight of de-lignified solids was recorded. The weight difference was calculated and considered as weight loss. Then pretreated samples stored in plastic bag until it was used for further analysis (composition, lignin removal and reducing sugar).

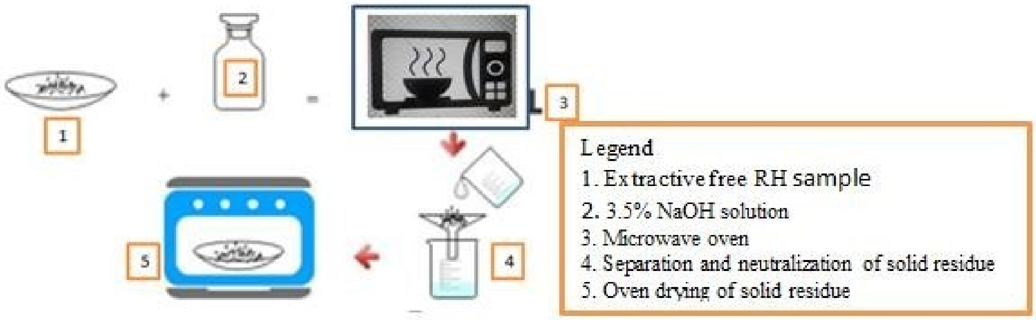


Figure 3.4 Process flow diagram for microwave assisted alkali pretreatment of rice husk

3.4 Acid hydrolysis experiment

H_2SO_4 was used for hydrolysis experiment. Acid hydrolysis of pretreated sample of rice husk was performed in an autoclave (model MaXterile 60, design pressure 2.5bar, operation pressure 1.2~2 bar and operation temperature 121~132°C) at a temperature of 131.04 °C, dilute acid concentrations of 1.5% (w/v), reaction time of 24 min. the reaction mixture was adjusted to Solid: liquid ratio of 1:10 (w/v). After hydrolysis, solid materials remove from flasks by filter paper and neutralize with Na_2CO_3 solution by adjusting the pH between 6.8 and 7.3, separation of the un hydrolyze and glucose was performed by centrifuge at 4000 rpm for 15 min. Then Hydrolysates filtrate was kept in a refrigerator at 4ºC until used for determination of fermentable sugar by using DNS methods.

3.5 Characterization of treated and untreated rice husk

3.5.1 Proximate analysis

A. Determination of Moisture content (MC)

A sample was weighed in clean preheated pan of known weight by using digital balance (initial weight). Then the sample and pan was kept inside a hot air oven at a temperature of 105°C. The sample is weighed at a regular interval until constant weight obtained (oven dried weight). The percentage of moisture content were determined as follow

$$\textbf{Moisture content } (\%)= \frac{[(\text{ initialweight-ovendriedweight})\ ]}{\text{initial weight}} *100 \ldots\ldots.\ (3.1)$$

B. Determination of Volatile Matter Content (VMC)

The volatile matter was determined using a muffle furnace (ASTM, E-872, 2006). 5g dried sample was taken in a closed crucible and kept inside the muffle furnace at 650°C for six minutes and again at 750°C for another six minutes. The crucible was removed from furnace and placed in desiccators to cool, then reweighed. The loss of weight was determined and the volatile matter was calculated as percentage of weight loss of the sample as follow

$$\textbf{Volatile matter content } (\%)= \frac{\text{weightlossofasample}}{\text{weight of moisture free sample}} *100 \ldots\ldots.\ (3.2)$$

C. Determination of Ash Content (AC)

The ash content of the sample is performed using the ASTM D1102. A 5 g of oven dried sample was placed into preheated crucible of known weight and the sample is heated in a muffle furnace at 575°C for 4 h. Then the crucible was removed from the furnace and directly placed in a desiccator and cooled. Then the ash with the desiccator is weighed (weight of ash plus crucible). The percentage of Ash was calculated as below

$$\textbf{Ash content } \%= \frac{\text{Weightofashpluscrucible-weightofcrucible}}{\text{weight of sample}} *100 \ldots\ldots..\ (3.3)$$

D. Determination of Fixed Carbon Content (FC)

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

$$\mathbf{FC\ (\%)=100-[MC(\%)+AC\ (\%)+VM\ (\%)]\ldots\ldots\ldots.\ (3.4)}$$

3.5.2 Chemical composition analysis of untreated and treated RH

The amount of lignin, hemicelluloses and cellulose of the raw and treated samples of rice husk was determined according to the national renewable energy laboratory analytical methods and the ASTMs for biomass

A. Determination of extractives by Soxhlet extraction

The Soxhlet extraction has been used for extraction of inorganic material, nonstructural material, chlorophyll wax, and other minor components. The extraction method is adopted from National Renewable Energy Laboratory (NREL) protocol. The solvent was 95% ethanol. The 8g biomass sample was added in thimble (initial weight), and a 200ml solvent was added in the extraction flasks. The extraction was performed for 24 hours in ethanol for continues extraction process. When the extraction time completed the flask was placed in a water bath temperature of 45°C to remove the solvent. After all the visible solvent was removed the flask plus extractives were dried in an oven and allow to cool to room temperature in a desiccator. The flask was weighted and record as a total weighed.

$$\mathbf{\%\ extractives=\frac{wightofflluskplusextractives-weightofflusk}{weight\ of\ initial\ sample}*100\ldots\ldots\ldots\ (3.5)}$$

D. Determination of Hemicellulose in biomass

For determining the amount of hemicellulose present in the biomass, 75ml of sodium hydroxide (NaOH) solution (0.5M) was added to 500mg of extractive free rice husk (initial weight of RH sample). Then the solution was kept in a water bath at 80°C for 3:30h. Then the solution was filtered through filter paper and the residue was washed with distilled water until it neutralized. Placed the residue in a pan and dry it in an oven at 105°C until constant weight observed and the difference in weight of RH residue plus pan and weight of pan is recorded as final weight [Mansor A.M. et al 2019]. By using Eq.(3.6) the percentage of hemicellulose content was estimated.

$$\textbf{\% Hemicellulose} = \frac{\textbf{initialweight-finalweight}}{\textbf{initial weight}} \; \textbf{*100………..(3.6)}$$

E. Determination of acid soluble and acid insoluble lignin

The total lignin content is determine as the sum of acid insoluble lignin and acid soluble lignin contents and where determined by NREL procedure. Inclusion of acid soluble lignin concentration in the total lignin value is necessary, as acid soluble lignin can be a significant fraction of the lignin. Acid insoluble lignin (AIL) is considered high molecular weight lignin while, acid soluble lignin (ASL) is low molecular weight lignin that is solubilized in the acidic hydrolysis solution. A sample of 150mg was added to 1.5 ml of 72% of H2SO4, the solution was mixed well and incubated in the water bath for 60min at 30°C. Then it was diluted with 42ml of distilled water to 4% H2SO4 concentration and autoclaved at 121°C for 1 hr. Then solution was filtered and the liquid filtrate obtained was considered as the acid soluble lignin and carbohydrates. The residue contains the acid insoluble lignin, and it was oven dried until constant weight, then it was placed in a muffle furnace in a silica crucible at 575°C for 4hr. The ash obtained was weighted and the lignin was calculated based on the following expressions.

$$\%AIR = \frac{\text{WeightofcrucibleplusAIR-Weightofcrucible}}{\text{oven dried sample}} * 100 \ldots\ldots\ldots (3.7)$$

$$\%AIL = \%AIR - \%Ash$$

UV-VIS Spectroscopy analysis

UV spectra was recorded on a ultraviolet/visible spectrophotometer, The hydrolysate (filtrate) from the Acid Hydrolysis Stage was quantitatively transferred using a pipette to a 1cm path-length (3 mL volume) quartz cuvette following the national renewable energy laboratory method (NREL). The UV Visible (240nm) transmission spectrum of the sample was obtained using the spectrophotometer. This dilution and UV-Visible analysis were carried out twice for each hydrolysate, meaning that each sample has done four spectra collected (two spectra for each of the duplicate hydrolysates). The acid soluble lignin content is then calculated based on the absorbance value at 240nm, the dilution factor, and a given absorptivity constant (A. Sluiter, 2012). The calculations of percentage of Acid soluble lignin (%ASL) can be expressed in the following formula are given as below (A. Sluiter, 2012).

$$\%ASL = \frac{\text{UVabs*volumefiltrate*Dilution}}{\text{€*ODW sample*path length}} * 100 \ldots\ldots\ldots\ldots (3.8)$$

Where:
UVabs = average UV-Vis absorbance of the sample at the appropriate wavelength
Volume hydrolysis liquor = volume of filtrate
Dilution = [volume sample + volume diluting solvent]/ [Volume sample]
€ = Absorptivity of biomass at specific wavelength
ODW sample = weight of sample in milligrams
Path length = path length of UV-Vis cell in cm
The total amount of lignin on an extractives free basis is given by:

$$\%\text{ of lignin extractive free} = \%\ AIL + \%ASL \ldots\ldots\ldots\ldots (3.9)$$

The general formula of lignin removal could be expressed in the following

$$\text{\% of Lignin removal}=\frac{\text{untreatedweightoflignin-treatedweightoflignin}}{\text{untreated weight lignin}}*100\ldots\ldots\ldots\ (3.10)$$

F. Determination of cellulose in biomass:

The cellulose (T.K, 1987) in the biomass was calculated by using the values obtained from their corresponding lignin, hemicelluloses, and ash contents. The cellulose content is determined by using ASTM and NREL methods. The cellulose was calculated using the following expression in:

$$\text{\% Cellulose}=100-\text{\% (Lignin+ Hemicellulose+ Ash)}\ldots\ldots\ldots\ (3.11)$$

G. Determination of weight loss of a biomass after pretreatments

An oven dried extractive free samples of rice husk was weighed in clean preheated pan of known weight by using digital balance. The sample is pretreated with MW assisted alkali and alkali pretreatment at different pretreatment time, temperature and microwave power. The treated sample is filtered and the solid residue washed with distilled water until its pH becomes neutral. Then the pretreated samples and pan was kept inside a hot air oven at a temperature of 105°C. The sample is weighed at a regular interval until constant weight obtained. The difference in weight is considered as weight loss and the percentage of weight loss was determined as follow:

$$\text{Weight loss (\%)}=\frac{\text{[Weightdifference]}}{\text{oven dried initial weight}}*100\ldots\ (3.12)$$

3.6 Determination of reducing sugar by DNS

For estimation of reducing sugar Dinitrosaclicylic Acid Method (DNS Method) is one of the alternatives. This method tests the presence of free carbonyl group (C=O), present in the reducing sugars. This involves the conversion of reducing sugar furfural under alkaline conditions, which reduces one of the nitro group (-NO_2) of DNS to amino group (-NH_2) to produce orange brown color 3-amino-5dinitrosalicylic acid, with absorbance maximum at 540nm.

Standard calibration curve is a graphical tool method for determining the concentration of a substance in an unknown sample by comparing it with the set of standard sample of known concentration. In this study glucose calibration curve is used to determine the glucose concentration in an unknown sample by demonstrating the relationship between optical densities and know value of glucose concentrations then comparing with unknown sample. To avoid errors, the standard samples should run in the same matrix as the unknown sample (Robert K. Delong, 2015). The calibration curves were measured using the 3,5dinitrosalicylic acid (DNS) method with glucose.

Standard curve of total reducing sugar is prepared using concentration of glucose (0-1mg/ml). For this, 3 ml of each concentration is filled into test tube and added with 3 ml of dinitrosalicylic acid (DNS) solution and subsequently boiled for 5-10 min, and 1.0 ml of 40% potassium sodium tartrate is added when the content of the tubes were warm, and then cooled. After the homogenization of reaction mixture, the absorbance at 540 nm was measured by using UV- VIS spectroscopic. Plot (standard glucose calibration curve) glucose concentration Vs absorbance at 540nm to determine the total reducing sugar in the unknown solution.

A 3 ml of DNS reagent was added to 0.2ml of hydrolysate sample and 2.8 ml water was added in a test tube. To avoid the loss of liquid due to evaporation, the test tube is covered with a piece of paraffin film. The mixture is boiled for 5-10 min to develop a red-brown color in the water bath and 1 mL of 40% potassium sodium tartrate (Rochelle salt) solution was added to stabilize the color; then it is allowed to cool to room temperature in a water bath. The absorbance was recorded with the spectrophotometer at 540 nm.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Raw material characterization

4.1.1 Proximate analysis

The proximate analysis was performed on the RH sample to find the actual moisture, volatile, ash and fixed carbon contents. It gives an idea to the chemical make-up of the raw material which is a fundamental importance for biomass energy use.

Proximate analysis of the raw rice husk was performed following ASTM methods (ASTM E871, ASTM D1102 and ASTM E872 for moisture, Ash and volatile mater contents respectively) and the result is presented in Table 4.1.

Table 4.1 proximate analysis of Rice husk

Proximate Analysis	Moisture content	Total Solid content	Ash content	Volatile matter	Fixed Carbon content
Results wt/wt (%)	5.41	94.59	11.46	76.59	11.95

The moisture content is a measure of the amount of water in a rice husk. The analysis of total moisture is used to determine other properties such as volatile matter, ash content and fixed carbon. Ash is none combustible residue left over after the water and organic matter have been driven off during combustion. It is a measure of inorganic impurities in the rice husk. As it can be seen in Table 4.1, the amount of moisture and ash content is found to be 5.41 % and 11.46%, respectively. A similar report has been reported by S.M. Laghari et al., 2018 was found moisture and ash content of 7.9% and 12.5% respectively. Guofeng Wu et al., 2015 also reported the moisture content of 9.8% and Roshanak Khandanlou et al., 2016 reported that the ash content of 13.9%. These indicate that the results in this study are in a comparable range with other studies. It was found that the high ash content, which may consist of large amount of SiO_2 in rice husk act as another physical barrier, protecting the cellulose from acid hydrolysis. Removal of ash/silicon can benefit in the increase of accessibility of the cellulose.

The volatile matter is a gaseous phase formed from the thermal degradation of the material and the value is found to be 76.59%. This implies that rice husk biomass has high number of organic fractions as compared with other biomasses, which suggests the suitability of rice husk for total reducing sugar productions. Finally, fixed carbon (FC), it is essentially carbon, but contains minor quantity of hydrogen, oxygen, nitrogen and sulfur found in a material which is left after volatile matters are driven off and the value is found to be 11.95 %. This result is in comparable rage with the values reported by Riying Qi et al 2019, was found the FC content of 7.06%, Jianfeng shen et al, 2012 reported FC of 15.06% and Mohamad Yusof et at, 2008 reported the FC of rice husk as 13.4%. This FC (essentially carbon) in a biomass is stored in the form of carbohydrate through photosynthesis which is important for the production of bioethanol.

4.1.2 Chemical compositional analysis

The second generation bioethanol processes use lingo-cellulose biomass that ultimately produces simple sugar; therefore chemical compositional analysis is performed in order to know the biomass composition. Characterization of RH was carried out to determine cellulose, hemicelluloses, and lignin content of the raw rice husk (extractive free) material. Table 4.2 shows that the cellulose, hemicellulose, and lignin contents of the rice husk used in this research has 33.97%, 30.48, and 24.13% (2.83% ASL and 21.30% of AIL) respectively. These results are found to be in a comparable range of the previous work reported by Zhixuan Wang et al., 2016 found 31.13% of cellulose, and 28.25% of lignin contents, Kalita et al., 2015 reported 37.1% of cellulose, 29.4% of hemicellulose and 24.1% of lignin contents, M. Nikzad et al., 2013 found 37.55% of cellulose, 15.24% of hemicellulose and 19.22% of lignin contents, Pajaro et al., 2004 found 36.7% of cellulose, 20.05% of hemicellulose and 21.3% of lignin contents and S.M. Laghari et al., 2018 found 38.6% of cellulose, 19.7 hemicellulose and 17.8% lignin contents. Some of the compositional variations could be due to environmental factors, growing conditions, season, and maturity of the plant. This is summarized in the table 4.3.

Table 4.2 Chemical composition analysis result of raw rice husk

Cellulose	Hemicellulose	Lignin	Extractives	Ash	Reference
33.97	30.48	24.13	5.45	11.5	Present study
31.13	19.11	28.25	16.5	16.5	Zhixuan Wang et al., 2016
37.1	29.4	24.1	-	-	Kalita et al., 2015
37.55	15.24	19.22	-	-	M. Nikzad et al., 2013
36.7	20.05	21.3	-	-	Pajaro et al., 2004
38.6	19.7	17.8	-	12.5	S.M. Laghari et al., 2018

Lignocellulose biomass, which has cellulose (30-35%), hemicellulose (20-30%) and lignin (10 − 20 %) can be used as an alternative feedstock for bioethanol production [Achinas and Euverink, 2016]. This analysis found evidence for rice husk which is sufficient to extract fermentable sugars with various pretreatments.

4.2 Effect of alkaline (NaOH) pretreatment

The main challenge of utilizing of lignocellulosic feedstock for bioenergy production such as bioethanol production is hydrolyzing the feedstock to monosaccharide due to the reluctant lignin as the main component. Therefore, the main purpose of pretreatment is mainly to remove the lignin and decrease the degree of crystallinity of cellulose structure (O'Connor et al., 2007). The crystallinity of the cellulose affects its accessibility (difficult to digest).

Alkaline pretreatment of biomass has been found to cause swelling, leading to an increase in internal surface area , decreased degree of polymerization and crystallinity, separation of structural linkages between lignin and carbohydrates through saponification reaction and disruption of the lignin structure [J. Xu et al., 2010]. Adequate pretreatment time and temperature is required to improve the digestibility of lignocellulosic biomass for the production of bioethanol (Xin Meng, 2014). The effect of alkaline pretreatment on lignin removal and the total sugar yield of pretreated rice husk after acid hydrolysis are presented in this section.

4.2.1 Effect of pretreatment time and temperature on lignin removal

The effects of pre-treatment time and temperature on the lignin removal were investigated at various pre-treatment times of 15, 30 and 60 min. The ratio of solid to liquid was 1:10, and at pre-treatment temperature of 40, 60 and 80°C. The recovery of cellulose-enriched residues and the compositions of cellulose, hemicellulose, and lignin content are displayed in Appendix 3 and appendix 1, respectively. It can be observed in the appendixes that the recovery rate of the cellulose-enriched residues weight gradually decreases with the increase in the pretreatment time, reducing from 100% (untreated RH) to 58.28% at a pretreatment time of 60min and at pretreatment temperature of 80°C.

Figure 4.1 shows the influence of treatment time and temperature on the percentage of lignin removal. The amount of delignification was found to be in the range of 10.64% - 25.15% for the range of pretreatment temperature from 40°C – 80°C at reaction time of 60min and alkali concentration of 3.5% NaOH. At higher treatment time and lower temperature, the alkali action is not very fast. So, the lignin decomposition is very low. At longer treatment time and higher treatment temperature better results are obtained than at low temperature and higher treatment time and vice versa. Maximum lignin removal was found at treatment time of 60min, treatment temperature of 80°C and at concentration of 3.5%NaOH, it was found that the lignin content decreased from 24.13% to 18.06% which resulted in 25.15% lignin removal efficiency. Clearly, as the treated reaction time prolonged (15-60min), the removal rate of lignin gradually increased from 11.04% (15 min) to 25.15% (60min). Meanwhile, the rate of hemicellulose loss gradually increased from 24.31% (15min) to 33.49 % (60min). However, the cellulose recovery was increased from 22.99% (15min) to 64.20% (60min).

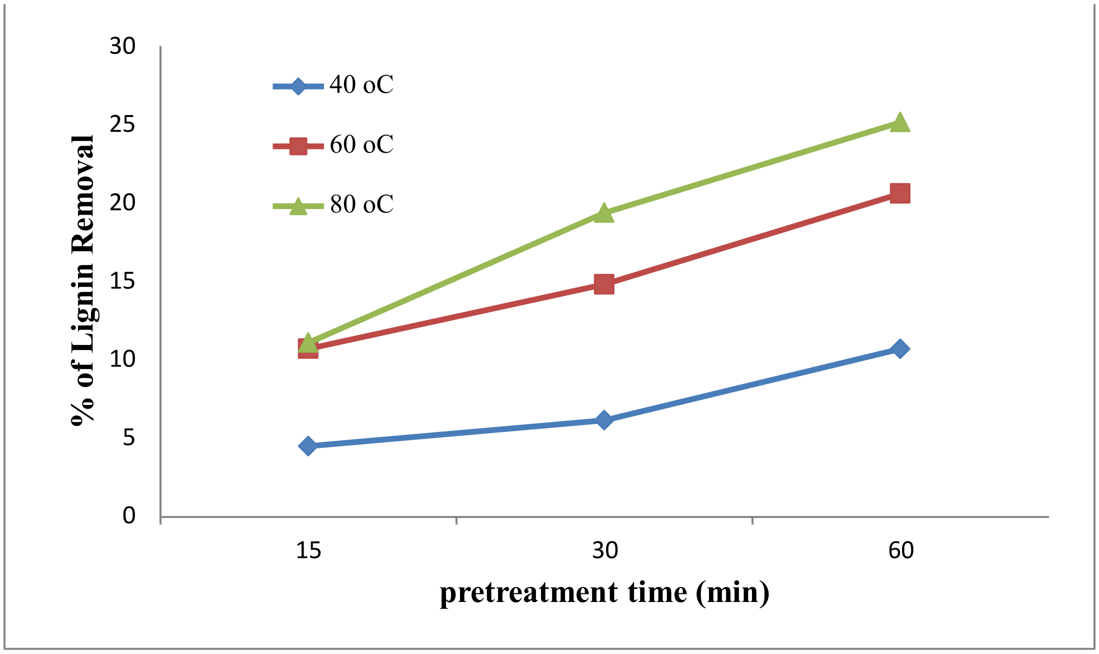


Figure: 4.1 Effect of pretreatment time and temperature with alkali treatment on lignin removal of rice husk.

The presences of saponification reaction in the process lead to disruption of the intermolecular ester linkages between hemicelluloses and lignin. As the temperature increase, the internal surface area, porosity increase, and the bond breakage increase due to swelling of substrate. This improves the accessibility of cellulose for hydrolysis reaction (Julie Baruah B. K., 2018).

From Figure 4.1 it can be observed that temperature and contact time have directly proportional to lignin removal with the operating range of 40-80°C and 15-60min, respectively. The higher the temperature and contact time the higher the lignin removal. Both pretreatment temperature and contact time have significant ($P<0.5$) effect on the removal of lignin and cellulose recovery (Appendix 6).

4.2.2 Effect of pretreatment time and temperature on reducing sugar yield

The decrease in the rate of hydrolysis is due to the lignin that bounds cellulose and hemicellulose which act as a physical barrier during hydrolysis process. In case of alkaline pretreatment contact time and temperature are the main factors that influence the yield of cellulose during hydrolysis. The effect of pretreatment time and temperature on the cellulose-enriched residues acid hydrolysis after alkali pre-treatment was investigated (Appendix 4).

Figure 4.2 show that the effect of pretreatment time and temperature on the total reducing sugar yield. As can be seen in Figure 4.2; an increase in pretreatment time (15-60min) and temperature (40-80°C) results in an increased of total reducing sugar yield. This phenomenon may be due to the increase in temperature, which facilitates the removal of lignin and hemicellulose, thereby increasing the accessibility and accelerating the hydrolysis of cellulose. It can be also observed that the increment of reducing sugar yield more pronounced in the lower temperature ranges (40°C to 60°C) than higher temperature ranges (60°C to 80°C) for all pretreatment time.

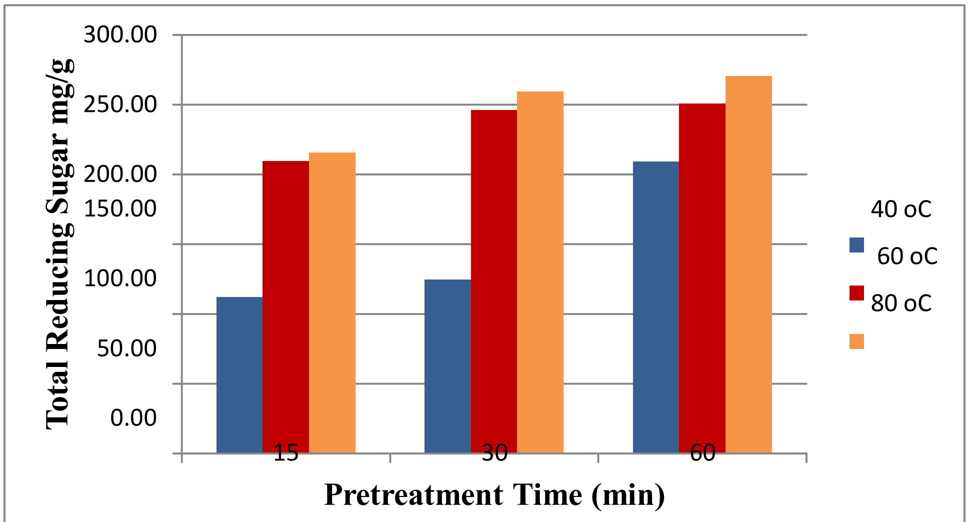


Figure 4.2: Effect of pretreatment time and temperature on total reducing sugar

It can be observed in Figure 4.2, that the range of total reducing sugar of alkali (NaOH) pretreated rice husk is (111.97 – 270.30) mg/g and the maximum total reducing sugar yield of 270.30mg/g were achieved for pretreatment conditions of 60 min, 80°C and 3.5 % w/v NaOH concentration. Both pretreatment temperature and contact time have significant ($P<0.5$) effect on the total reducing sugar (Appendix 7)

4.3 Effect of MW assisted alkaline (NaOH) pretreatment

Microwave assisted alkali pre-treatment separates the lignocellulosic biomass components by disrupting the biomass structure, reducing the crystallinity of cellulose, increasing the formation of fermentable sugar and reduce the degradation of carbohydrate. MW heating is directly from the inside material-wave interactions, leading to heat transfer and basically has a higher energy yield in comparison with conventional oven techniques which transmit heat by conduction-conventional mechanism [Obiora S. Aug et al 2018].

4.3.1 Effect of pretreatment time and MW power on lignin removal

Effect of microwave irradiation power and contact time on removal of lignin content in the biomass (RH) had been performed. MW- assisted alkali pre-treatment can penetrate the biomass and vibrate the molecule. The rapid oscillation of the molecules causes continuous heat generation and disruption of lignocellulosic structure. Figure 4.3 shows that, as the pretreatment time increased from 15 to 30 min, it has a great consequence on the lignin removal rate (34.92%, 26.38%, 27.85% at microwave power of 300W, 450W and 600W respectively) and contact time rising from 30 to 60 min the removal rate of lignin decreased 22.72% at microwave power of 300W and there is no effect observed of contact time rising from 30 to 60 min at microwave power of 600W on the loss of lignin.

In the MW assisted alkali pretreatment the percentage of lignin removal was found to be 47.17%, 47.15% and 47.57 % at a pretreatment time of 60 min, 42min and 30 min and MW irradiation power of 300, 450 and 600W respectively which is nearly the same. This implies the lower MW power needs longer pretreatment time and the higher MW power needs shorter pretreatment time to achieve the same amount of lignin removal. Both parameters (MW power and contact time) has no significant ($p>0.05$) effect on the lignin removal (Appendix 8). Rather their interaction does have a significant effect, hence the energy consumed that matters the removal of lignin rather than the irradiation power level. The maximum value of lignin removal was found to be 47.57 %, at 600W MW power, 3.5 % w/v alkali concentration, and 30-minute contact time. Whereas the maximum lignin removal of 25.15% was found in alkali pretreatment at pretreatment temperature of 80^oC and contact time of 60min and this shows that the lignin removal efficiency of microwave assisted alkali pretreatment is 89.29% greater than alkali pretreatment and also the pretreatment time reduced by half.

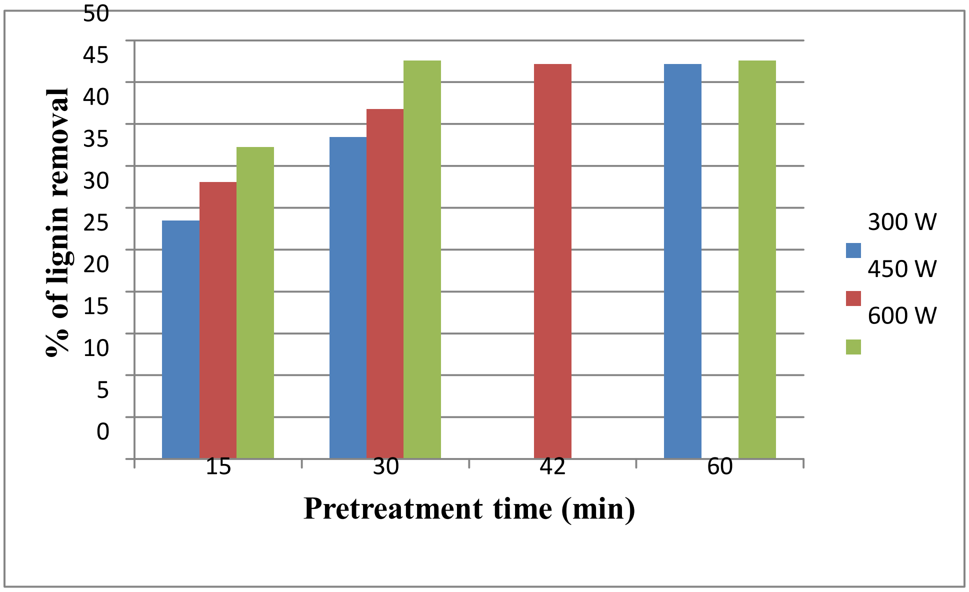


Figure: 4.3: Effect of pretreatment time and MW power on lignin removal of rice husk

4.3.2 Effect of pretreatment time and MW power on total reducing sugar yield

The effectiveness of pretreatment is measured by its success to increase the accessible surface area and de-crystallizes cellulose, solubilize hemicellulose and lignin, maximize digestibility of the pretreated material and minimize loss of sugar. In this study cellulose-enrich residue is broken down in to monomeric sugar via dilute sulfuric acid hydrolysis process. The effect of pretreatment time and microwave power on the cellulose-enriched residues through dilute acid hydrolysis after microwave assisted alkali pre-treatment was investigated (Appendix 4). Figure 4.4 shows that the effect of pretreatment time and MW power on the total reducing sugar yield. As can be seen in Figure 4.4; an increase in pretreatment time from 15 to 30 min and microwave power from 300-600 results an increase in total reducing sugar yield. However, further increase in the pretreatment time to 60 min at a microwave power of 600W results in a reduction of the total reducing sugar yield. This result may be explained by Biond et al. (2012), for every microwave irradiation power there was an optimal treatment time. Longer exposure time results in decrease sugar content due to the assumption that; when the energy consumption was higher than a certain value the substrate were probably carbonized with degradation of sugar. Therefore the phenomenon that the reduction in reducing sugar when the time further increased to 60 min at microwave power of 600W might be explained by these.

The range of total reducing sugar of microwave assisted alkali (NaOH) pretreated rice husk varies from (208.80 – 380.20) mg/g and the maximum total reducing sugar yield at microwave power of 300, 450 and 600w and/with pretreatment time of 60, 42 and 30 min was 379.96, 380.2 and 380.2 mg/g respectively. Nearly the same total reducing sugar yield is observed at different pretreatment time and microwave power. The higher the MW power is the lower the pretreatment time required and vice versa.

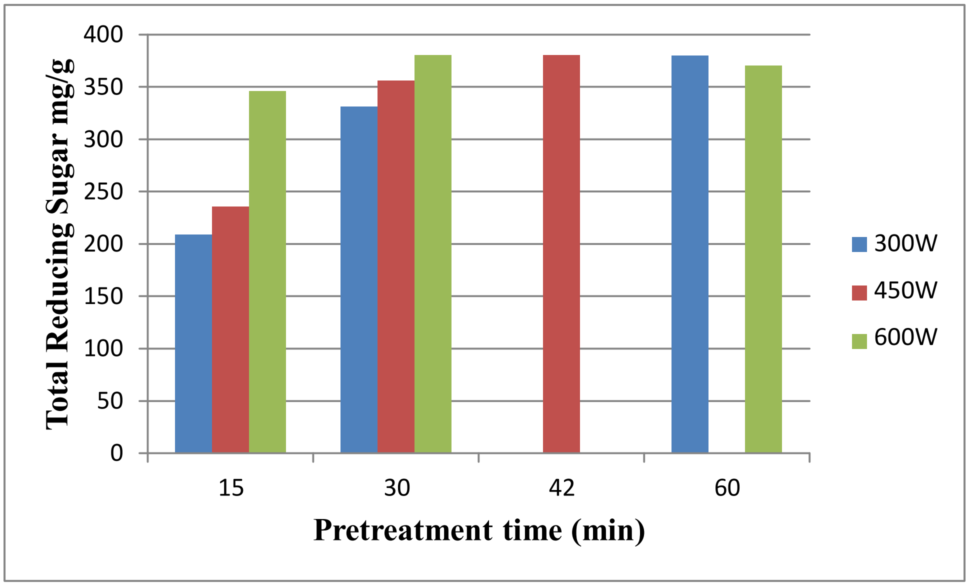


Figure 4.4: Effect of pretreatment time and MW power on total reducing sugar yield

In this study both parameters, time and MW power has shown no significant ($p>0.05$) effect on the total reducing sugar yield (Appendix 9). Rather their interaction does have a significant effect, therefor it can be concluded that the energy consumption that matters on the total reducing sugar yield of the pretreated RH.

4.4 Effect of pre-treatment methods of rice husk on its weight loss

The weight loss of a MW assisted alkali and alkali pretreated rice husk samples were investigated in this study. The weight loss of a biomass is an important index for the effectiveness of the pre-treatment. Figure 4.5 shows that, the effect of pretreatment time and temperature on the weight loss of the RH when it was treated by alkali pretreatment. It shows that there is a direct relationship between pretreatment time and temperature on the weight loss of alkali pre-treatment. As the contact time increase, the pretreated RH weight loss increase. This is due to an increase in removal of lignin and hemicellulose.

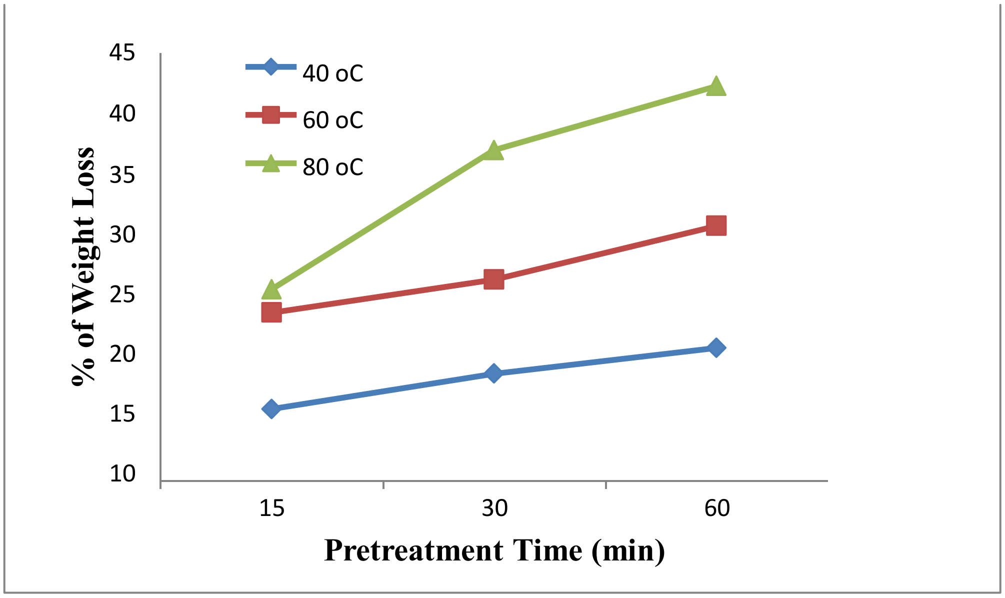


Figure 4.5: Effect of time and temperature on the percentage of weight loss

The mass loss of pretreated sample has 41.72%, at maximum pretreatment temperature of 80^{o}C and contact time of 60min, whereas in the case of MW assisted alkali pre-treatment the maximum weight loss was 49.75% (Figure 4.6).

For the MW assisted alkali pretreatment the weight loss nearly the same at 60, 42 and 30 min pretreated at 300, 450 and 600 W, respectively. This implies that the lower MW power needs longer treatment time to achieve the same final weight loss, thus it is the energy which has an effect on the final weight loss rather than MW power and/or contact time.

The rice husk pretreated with MW assisted alkali pretreatment has higher cellulose content, lower hemicellulose, lignin, ash content and had more weight loss than when it was pretreated by alkali alone pretreatment.

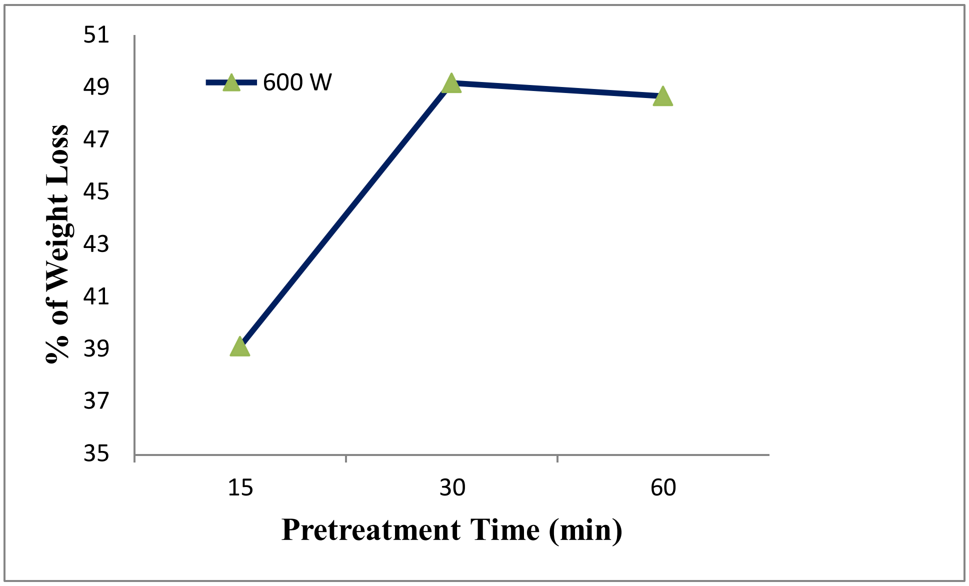


Figure 4.6: Effect of time and MW power on the percentage of weight loss at 600W

4.5 Compositional analysis of rice husk biomass based on treatments

The chemical composition of untreated rice husk has been found to be 33.97% cellulose, 30.48% hemicelluloses, 24.13% lignin (21.3% acid insoluble lignin, 2.83% acid soluble lignin), and 11.5% ash on a dry weight. Table 4.3 shows the summary of chemical compositional analysis comparison between untreated rice husk and treated rice husk at their optimum conditions.

Table 4.3: Results of component analysis of the untreated and pretreated rice husk

Composition	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Extractives (%)
Untreated rice husk	33.97	30.48	24.13	5.45
Alkali Treated Rice husk	55.78	20.27	18.06	
MW Assisted Alkali treated Rice husk	66.92	15.48	12.65	

It shows that the pretreated rice husk results increased of the cellulose recovery due to solubilize of the lignin and hemicellulose. Rice husk pretreated with the MW assisted alkali pretreatment results an increase in the composition of cellulose content. The range of cellulose content is found to be in between 33.97% to 66.62%; it was increased by 96.11% at operating conditions of contact time of 60 min, MW power of 300W and 3.5% w/v of NaOH concentration, for alkali alone pretreated RH the cellulose content ranges 33.97% to 55.78%; it was increased by 64.20% when RH pretreated with NaOH concentration of 3.5% w/v, contact time of 60min and pretreatment temperature of 80 ^{o}C as compared with the result obtained with untreated rice husk.

Similarly, on the same pretreatment conditions it had been observed that the range of hemicellulose and lignin content varied from 30.48% to 15.58%; it decreases by 48.88% and from 24.13% to 12.75%, it decreased by around 47.16% respectively for MW assisted alkali pretreatment and from 30.48% to 20.27%; it decreases by 33.49% and from 24.13% to 18.06%; it decreased by 25.16% for alkali alone pretreatment as compared to the raw rice husk. From these results it can be observed that MW assisted alkali pretreatment removes more lignin and hemicellulose and recover more cellulose than alkali pretreatment. This is due to in MW assisted alkali pretreatment; the microwaves penetrate the material and directly vibrate the molecules. The vibration of polar molecules and movement of ions results in the generation of heat and extensive collusion. These effects can accelerate chemical process/reaction and causes eventually rapture of encrusted lignocellulose structure and may be avoid superficial overheating and carbohydrates degradation [Alejandra Aguilar et al 2017].

4.6 Comparison of alkali pretreatment with and without MW assisted on total reducing sugar production

The effectiveness of pretreatment has measured by its success in increasing the susceptibility of the biomass to acid hydrolysis. The combination of microwave pretreatment with alkali increase the rate of hydrolysis of rice husk by removing lignin and hemicellulose in large quantity and increase cellulose accessibility than alkali alone pretreatment. As a result the maximum total reducing sugar yield was achieved with microwave assisted alkaline pretreatment.

The effects of pretreatment methods on the total reducing sugar yield during acid hydrolysis are shown in Figure 4.7, it show that there was a difference in reducing sugar yield between alkali and MW assisted alkali pretreatments at the same pretreatment time, it indicates that MW assisted alkali pretreatment was good for cellulose hydrolysis. The maximum yield of total reducing sugar of alkali (NaOH) and MW assisted alkali (NaOH) pretreated rice husk are found to be 270.30 mg/g (at pretreatment time of 60min and temperature of 80^{o}C) and 380.20 mg/g (at pretreatment time of 30min and microwave power of 600W) respectively which indicates the total reducing sugar yield of MW assisted alkali (NaOH) pretreated rice husk is 1.41 times higher than that of alkali (NaOH) pretreated with less pretreatment time.

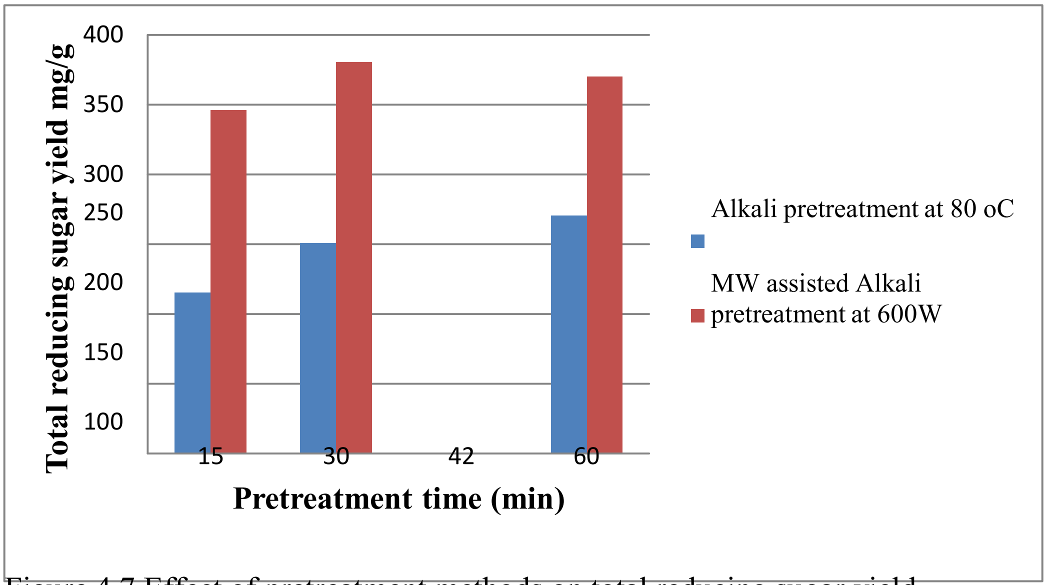


Figure 4.7 Effect of pretreatment methods on total reducing sugar yield

In this study the total reducing sugar yield of the two pretreatment methods indicates that the evidence is not strong enough (P>0.5) to suggest a significant effect exist in the methods (Appendix 10). This was may be the sample size is too small (lab scale). But, we can conclude that the MW assisted alkaline pretreatment is preferable than alkaline alone pretreatment considering the shorter pretreatment time required. The highest yield of total reducing sugar was found to be 380.20 mg/g of pretreated rice husk at specific condition in 600W, 30 min, and 3.5 % w/v NaOH.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The effect of microwave assisted alkali pretreatment and alkali pretreatment alone at different pretreatment conditions on percentage of lignin removal and reducing sugar yield of rice husk was addressed in this study. The raw rice husk was characterized and the result was found to be 33.97% cellulose, 30.48 % hemicellulose, and 24.13% lignin content.

The maximum lignin removal and cellulose recovered of rice husk pretreated with alkali (3.5%NaOH) was found to be 25.13% and 55.78% respectively at a pretreatment temperature of 80°C and pretreatment time of 60min and at this pretreatment condition 270.3 mg/g reducing sugar yield from dilute acid hydrolysis were obtained. From MW Assisted alkali pretreated rice husk, the maximum lignin removal, cellulose recovery and total reducing sugar yield nearly the same at MW power of 300W, 450W and 600W and treatment time of 60, 42 and 30min was found to be 47 %, 66.62% and 380.2 mg/g respectively.

From the result it can be seen that the microwave assisted alkali pretreatment improved the lignin removal, cellulose recovery and reducing sugar yield 1.89 times, 1.19 times and 1.41 times that of alkali pretreated respectively. this indicates a MW Assisted alkali pretreatment had a higher cellulose recovery and lignin removal effect that led to release higher quantity of total reducing sugar through dilute sulfuric acid hydrolysis process than alkali pretreatment, hence, it can be concluded that MW assisted alkaline pretreatment through dilute sulfuric acid hydrolysis is a potential alternatives of rice husk pretreatment mothed for production of bioethanol from rice husk lignocellulosic materials.

5.2 Recommendation

Bioethanol offers a great benefit for safeguarding the environment, improving the rural economy and ensuring fuel security. Based on the current study of this work the following recommendations are forwarded:

Further characterizations has to be conducted on pretreated rice husk samples for its morphological changes using XRD and SEM to know the structural and composition modification due to microwave assisted alkali pretreatment.

Additional studies should include the effect of alkali concentration, particle size and rice husk species on the yield of bioethanol products.

Investigate effective pretreatment method for removal of ash content of rice husk.

Investigate the effect of retention time of alkali immersed rice husk before exposing to conventional and microwave heating on the delignification of lignin, cellulose recovery and reducing sugar yield.

In addition an economic feasibility analysis of the overall conversion process should be beneficial for the purpose of commercialization.

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7. APPENDIX

Appendix 1: Effect of Alkali (3.5%NaOH) pretreatment on the composition of Rice husk

Treatment method	Temperature (oC)	Time (min)	Composition %							
			Cellulose	mean	Hemicellulose	mean	Lignin	mean	Ash	mean
Untreated Rice Husk			32.55	33.97	31.46	30.48	23.86	24.13	12.13	11.42
			35.39		29.5		24.4		10.71	
Alkali (NaOH) treated	40	15	35.85	36.08	27.92	29.27	24.15	23.06	12.08	11.59
	40	15	36.31		30.62		21.97		11.1	
	40	30	39.01	40.48	29.73	28.17	22.03	22.66	9.23	8.69
	40	30	41.95		26.61		23.29		8.15	
	40	60	42.98	43.88	25.96	27.27	22.9	21.56	8.16	7.29
	40	60	44.78		28.58		20.22		6.42	
	60	15	44.76	42.28	25.17	26.17	21.13	21.56	8.94	7.99
	60	15	39.8		31.17		21.99		7.04	
	60	30	45.01	45.58	25.94	25.87	20.03	20.56	9.02	7.99
	60	30	46.15		25.8		21.09		6.96	
	60	60	51.05	50.68	23.57	24.27	18.26	19.16	7.12	5.89
	60	60	50.31		24.97		20.06		4.66	
	80	15	40.54	41.78	24.11	23.07	22.18	21.46	13.17	13.69
	80	15	43.02		22.03		20.74		14.21	
	80	30	48.78	49.08	22.99	22.47	18.78	19.46	9.45	8.99
	80	30	49.38		21.95		20.14		8.53	
	80	60	55.06	55.78	19.87	20.27	18.23	18.06	6.84	5.89
	80	60	56.5		20.67		17.89		4.94	

Appendix 2: Effect of MW Assisted Alkali (3.5%NaOH) pretreatment on the composition of Rice husk

Treatment method	MW power (W)	Time (min)	Composition %							
			Cellulose	mean	Hemicellulose	mean	Lignin	mean	Ash	mean
Untreated Rice Husk			32.55	33.97	31.46	30.48	23.86	24.13	12.13	11.42
			35.39		29.5		24.4		10.71	
MW Assisted Alkali Treated	300	15	47.62	49.82	24.13	23.48	18.14	17.25	10.11	9.45
	300	15	52.02		22.83		16.36		8.79	
	300	30	55.22	57.22	22.24	21.78	15.32	14.85	7.22	6.15
	300	30	59.22		21.32		14.38		5.08	
	300	60	65.4	66.62	16.18	15.58	12.08	12.75	6.34	5.05
	300	60	67.84		14.98		13.42		3.76	
	450	15	48.35	51.32	23.42	22.18	17.14	16.15	11.09	10.35
	450	15	54.29		20.94		15.16		9.61	
	450	30	58.8	61.42	19.78	18.78	15.24	14.05	6.18	5.75
	450	30	64.04		17.78		12.86		5.32	
	450	42	63.26	65.82	16.25	15.38	13.28	12.75	7.21	6.05
	450	42	68.38		14.51		12.22		4.89	
	600	15	50.21	52.62	20.29	19.88	16.21	15.15	13.29	12.35
	600	15	55.03		19.47		14.09		11.41	
	600	30	63.11	66.52	16.94	15.48	13.57	12.65	6.38	5.35
	600	30	69.93		14.02		11.73		4.32	
	600	60	61.88	64.92	15.21	14.08	13.76	12.65	9.15	8.35
	600	60	67.96		12.95		11.54		7.55	

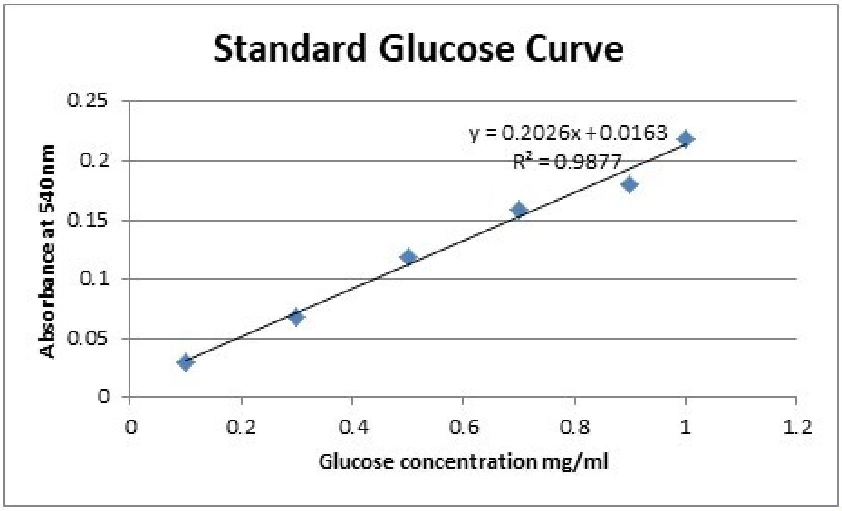
Appendix 3: Effect Of Pretreatments on the percentage weight loss/recovery of Rice husk

Treatment method	Heat source		Time (Min)	Mean weight (%)			
	MW power (W)	Temperature (oC)		initial weight (g)	final weight (g)	Weight loss (%)	Weight recovery (%)
Alkali Treated	-	40	15	15.01	12.717	15.27	84.73
		40	30	15.01	12.281	18.17	81.83
		40	60	15.01	11.966	20.27	79.73
		60	15	15.01	11.526	23.17	76.83
		60	30	15.01	11.131	25.87	74.13
		60	60	15.01	10.465	30.27	69.73
		80	15	15.01	11.246	25.07	74.93
		80	30	15.01	9.535	36.47	63.53
		80	60	15.01	8.747	41.72	58.28
Microwave Assisted Alkali Treated	300	-	15	10.01	7.459	25.48	74.52
	300		30	10.01	6.142	38.64	61.36
	300		60	10.01	5.03	49.75	50.25
	450		15	10.01	6.488	35.18	64.82
	450		30	10.01	5.427	45.78	54.22
	450		42	10.01	5.167	48.38	51.62
	600		15	10.01	6.097	39.09	60.91
	600		30	10.01	5.09	49.15	50.85
	600		60	10.01	5.11	48.95	51.05

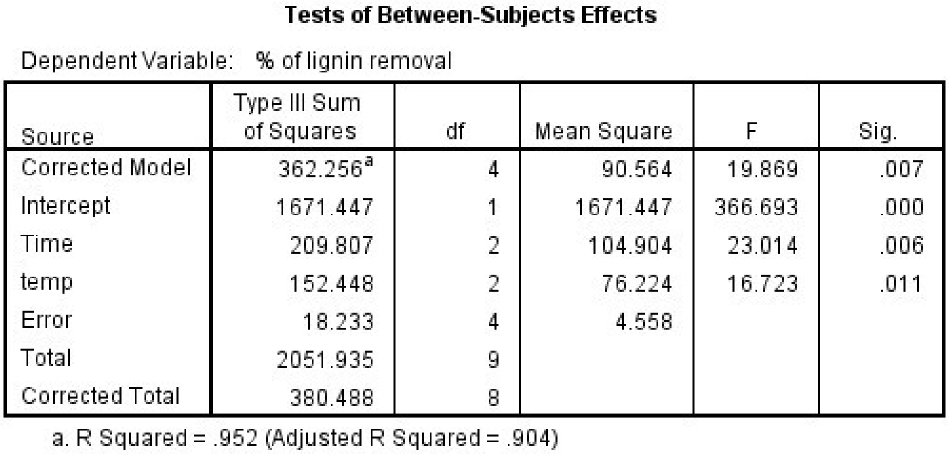
Appendix 4: effect of pretreatments on lignin removal and total reducing sugar concentration

Treatment methods	temperature/ Power	Time (min)	Lignin (%)	% of lignin removal	sugar reducing (mg/g)
Raw	-		24.13		-
Alkali (NaOH) Treated	40 °C	15	23.06	4.43	111.97
	40 °C	30	22.66	6.08	124.55
	40 °C	60	21.56	10.64	209.21
	60 °C	15	21.56	10.65	209.27
	60 °C	30	20.56	14.77	246.07
	60 °C	60	19.16	20.57	259.43
	80 °C	15	21.46	11.04	215.41
	80 °C	30	19.46	19.34	250.55
	80 °C	60	18.06	25.13	270.30
ıcrowave Assisted Alkali Treated	300 W	15	17.25	28.49	208.80
	300 W	30	14.85	38.44	331.35
	300 W	60	12.75	47.17	379.96
	450 W	15	16.15	33.05	235.50
	450 W	30	14.05	41.77	356.00
	450 W	42	12.75	47.15	380.20
	600 W	15	15.15	37.21	346.12
	600 W	30	12.65	47.57	380.20
	600 W	60	12.65	47.57	370.17

Appendix 5: Glucose calibration curve



Appendix 6: % of lignin removal with NaOH treatment by ANOVA



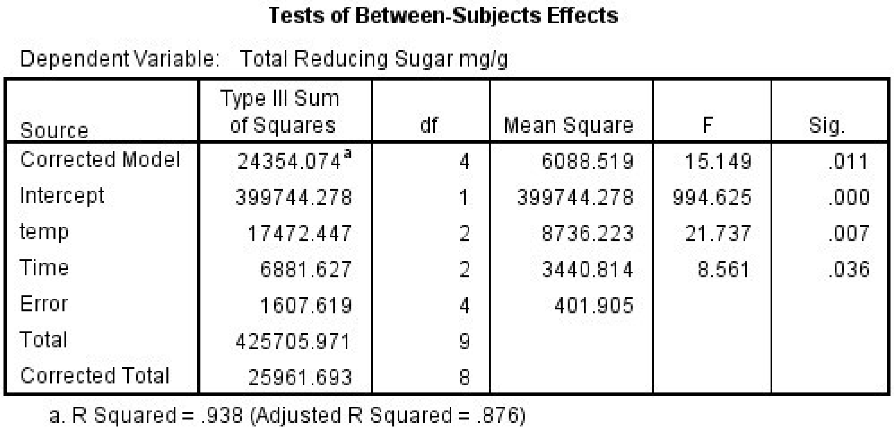
Tests of Between-Subjects Effects

Dependent Variable: % of lignin removal

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	362.256[a]	4	90.564	19.869	.007
Intercept	1671.447	1	1671.447	366.693	.000
Time	209.807	2	104.904	23.014	.006
temp	152.448	2	76.224	16.723	.011
Error	18.233	4	4.558		
Total	2051.935	9			
Corrected Total	380.488	8			

a. R Squared = .952 (Adjusted R Squared = .904)

Appendix 7: Total reducing sugar with NaOH treatment by ANOVA



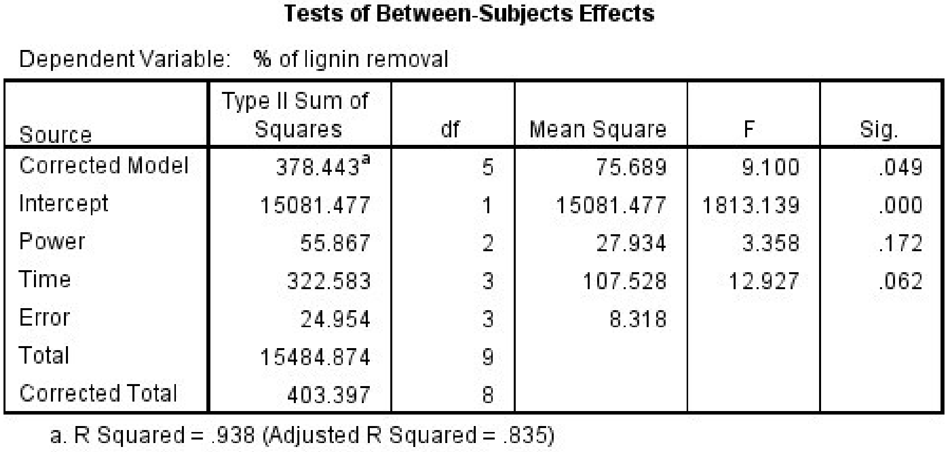
Tests of Between-Subjects Effects

Dependent Variable: Total Reducing Sugar mg/g

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	24354.074[a]	4	6088.519	15.149	.011
Intercept	399744.278	1	399744.278	994.625	.000
temp	17472.447	2	8736.223	21.737	.007
Time	6881.627	2	3440.814	8.561	.036
Error	1607.619	4	401.905		
Total	425705.971	9			
Corrected Total	25961.693	8			

a. R Squared = .938 (Adjusted R Squared = .876)

Appendix 8: % of lignin removal with MW Assisted NaOH treatment by ANOVA



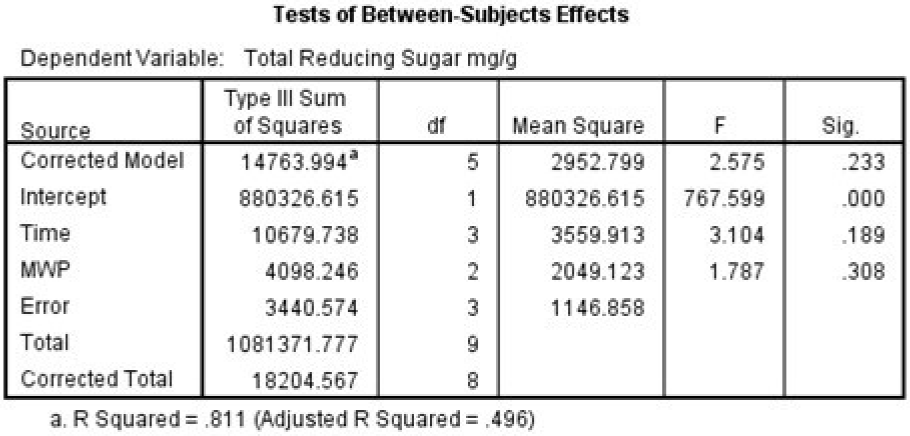
Tests of Between-Subjects Effects

Dependent Variable: % of lignin removal

Source	Type II Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	378.443[a]	5	75.689	9.100	.049
Intercept	15081.477	1	15081.477	1813.139	.000
Power	55.867	2	27.934	3.358	.172
Time	322.583	3	107.528	12.927	.062
Error	24.954	3	8.318		
Total	15484.874	9			
Corrected Total	403.397	8			

a. R Squared = .938 (Adjusted R Squared = .835)

Appendix 9: Total reducing sugar with MW Assisted NaOH treatment by ANOVA



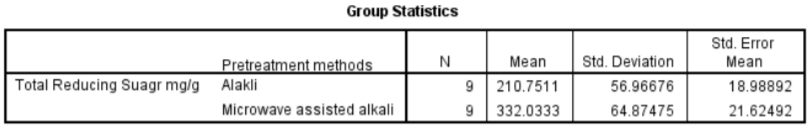
Tests of Between-Subjects Effects

Dependent Variable: Total Reducing Sugar mg/g

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	14763.994[a]	5	2952.799	2.575	.233
Intercept	880326.615	1	880326.615	767.599	.000
Time	10679.738	3	3559.913	3.104	.189
MWP	4098.246	2	2049.123	1.787	.308
Error	3440.574	3	1146.858		
Total	1081371.777	9			
Corrected Total	18204.567	8			

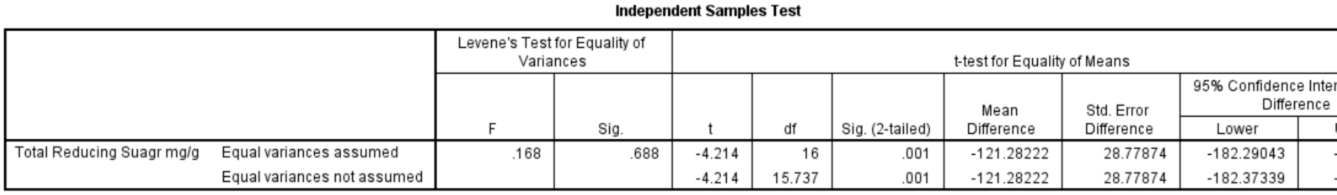
a. R Squared = .811 (Adjusted R Squared = .496)

Appendix 10: comparison of total reducing sugar yield between the two methods (with and without Microwave Assisted Alkali Pretreatments).



Group Statistics

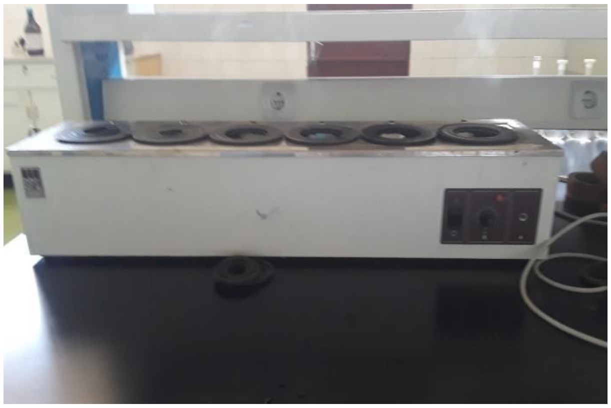
	Pretreatment methods	N	Mean	Std. Deviation	Std. Error Mean
Total Reducing Suagr mg/g	Alakli	9	210.7511	56.96676	18.98892
	Microwave assisted alkali	9	332.0333	64.87475	21.62492



Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means					
									95% Confidence Inter Difference
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower
Total Reducing Suagr mg/g	Equal variances assumed	.168	.688	-4.214	16	.001	-121.28222	28.77874	-182.29043
	Equal variances not assumed			-4.214	15.737	.001	-121.28222	28.77874	-182.37339

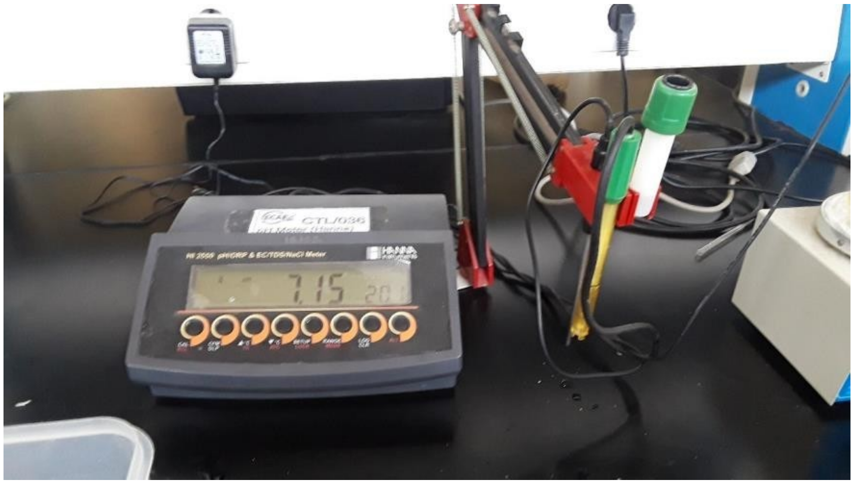
Appendix 11: Laboratory equipment’s



Hot Plate with Magnetic stirrer Water bath

Digital Microwave oven sieve

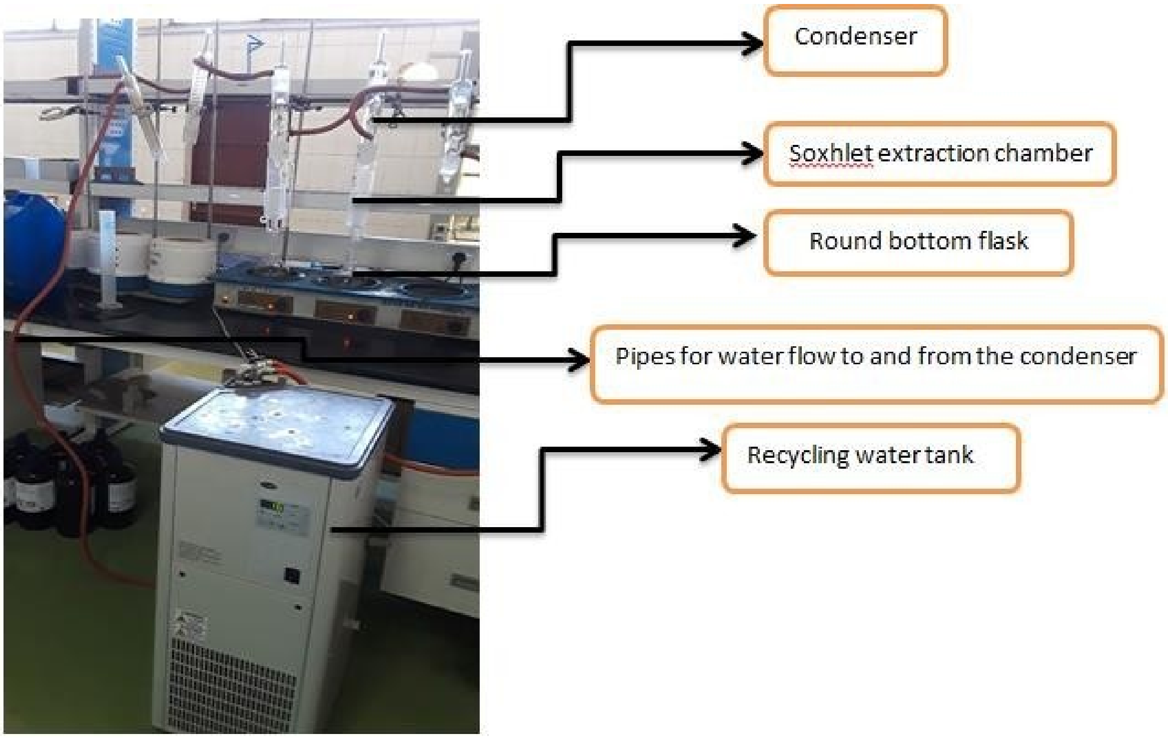

Authoclave



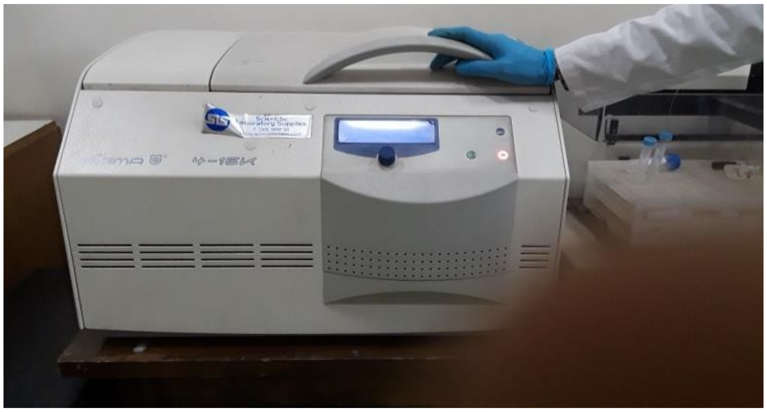
Digital PH meter

Ovene and Furnece

Desiccator and Digital Balance



Soxhlet Extractor



Centrifuge

UV-Vis Spectrometry