



BIOETHANOL PRODUCTION FROM EXCESS FOOD CROPS IN NIGERIA: PROCESS DESIGN, OPTIMIZATION, AND TECHNO-ECONOMIC ANALYSIS

By

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As the candidate's Supervisor, I agree to the submission of this thesis.

.....
Prof. David Lokhat

DECLARATION 1 – PLAGIARISM

I, AWOYALE Adeolu Abiodun, declare that;

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
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DECLARATION 2 - PUBLICATIONS

This section presents the articles that form part and/or include the research presented in this thesis. The following papers have been published or are under review:

ISI/DoHET/Scopus Accredited Journals

- (1) **Awoyale, A.A.** and D. Lokhat, Harnessing the Potential of bioethanol production from lignocellulosic biomass in Nigeria—a review. *Biofuels, Bioproducts and Biorefining*, 2019. **13** (1): p. 192-207. <https://DOI.org/10.1002/bbb.1943>
- (2) **Awoyale, A. A.**, Lokhat, D., & Eloka-Eboka, A. C. (2019). Experimental characterization of selected Nigerian lignocellulosic biomass in bioethanol production. *International Journal of Ambient Energy*, 1-9. DOI: 10.1080/01430750.2019.1594375
- (3) **Awoyale, A.A.** and Lokhat, D., 2021. Experimental determination of the effects of pretreatment on selected Nigerian lignocellulosic biomass in bioethanol production. *Scientific reports*, 11(1), pp.1-16. DOI:10.1038/s41598-020-78105-8
- (4) **Awoyale, A.A.**, Lokhat, D., and Okete P., 2021. Investigation of the effects of pretreatment on the elemental composition of ash derived from selected Nigerian lignocellulosic biomass. *Scientific Reports*, 11(1), 1-14. DOI: [10.1038/s41598-021-00672-1](https://doi.org/10.1038/s41598-021-00672-1).
- (5) **Awoyale, A.A.** and Lokhat, D., 2021. Hybridization of selected Nigerian lignocellulosic biomass feedstocks for bioethanol production: modeling and optimization of pretreatment and fermentation process parameters using response surface methodology. *Journal of Oleo Science*, ess21038. DOI: 10.5650/jos.ess21038

The candidate for all the publications is the main and corresponding author respectively while Prof David Lokhat is the supervisor.

DEDICATION

Dedicated to God Almighty and the loving memories of my parents, Late Engr David Abolaji Awoyale
and Late Mrs Mary Olufunmilayo Awoyale.

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ABSTRACT

The global drive for diversification of energy sources, particularly by focusing less on non-renewable fossil fuels and harnessing renewable energy resources like bioethanol, has motivated this research work. Before the last 10 years, bioethanol meant for use as fuel was produced from carbohydrate-rich crops such as cassava, yam, maize, millet, rice amongst others. Because the production of bioethanol from these food crops has been envisaged to jeopardize food security, the focus has been shifted to the production of bioethanol from the residues left behind after processing the food crops. These residues can be classified as lignocellulosic biomass. The major concentration of this study is the production of bioethanol from residues of food crops, namely, corn cobs, rice husks, sugarcane bagasse, cassava peels, and yam peels. The biomass used in this research were sourced from different locations in Nigeria, where they are found in abundance at certain seasons yearly. In the course of the work, the biomass were sieved into two mesh sizes of 300 and 425 microns, and also some of the biomass as well as all the five biomass were all mixed and firstly characterized to evaluate the effects of particle size as well as hybridized biomass mixtures on the end products and production efficiency of bioethanol. The effects of the adopted pretreatments in this study on the biomass were also investigated, as such, three types of pretreatments were adopted in this study namely; combined hydrothermal and acid pretreatment, combined hydrothermal and alkaline pre-treatment, and hydrothermal only pretreatment. The results of the characterization of the different biomass, including the hybridized biomass after pretreatment showed the pore features for hybridized corn cobs and rice husks biomass have the maximum specific surface area and pore volume of 1837 m²/g and 0.5570 cc/g respectively. Also, the values of the cellulose content improved slightly with the pretreatment and the value of the lignin content decreased considerably. The cellulose values range from 34.2 to 36.5 wt% for the acid, alkali and hot water pretreated hybridized biomass. Releases from the pretreatment process to air, soil, and water were measured with SimaPro. The environmental impact categories accessed include global warming potential (GWP)/climate change, and acidification (AP). With a mean value of 15.82 kg CO₂ (eq), the alkaline pretreatment using sodium hydroxide shows the highest release of GHG emissions, while acid pretreatment employing dilute sulphuric acid generated a mean value of 8.68 kg CO₂.

Hybridized feedstocks of cassava peels plus yam peels, and corn cobs plus rice husks biomass, were optimized using the Response Surface Methodology (RSM) centred on the statistical design of experiments (DOE) of the Box-Behnken design (BBD), in the production of bioethanol. The BBD was harnessed using a 3-level, 3-factor process variables using pH, time, and particle size. The bioethanol yield from the two hybridized biomass feedstocks was predicted by the developed quadratic polynomial models from BBD. The hybridized rice husks plus corn cobs biomass with a maximum bioethanol yield of 160 ml/1500 g biomass gave a better prospect for bioethanol production when compared with hybridized cassava peels

plus yam peels biomass with a maximum bioethanol yield of 125 ml/1500 g biomass. This reinforces the finding that hybridizing the feedstocks enhances the capacity for better bioethanol yield after fermentation.

The economic analysis of the produced bioethanol gave a price of 0.41 USD/l, which is a good deal as it compares favorably well with the 0.45 USD/l price of ethanol in the Nigerian open market.

Chapter ONE

Introduction and Background of Study

Chapter ONE

INTRODUCTION

1.1 Background of Study

Bioethanol is the leading manufactured biofuel around the globe. This is because it has a very mild negative effect on the environment, it is renewable and has proven to be a better alternative to fossil fuels and as such, its use as a transportation fuel can mitigate the prevailing global climate change and ultimately contribute to the global sustainable development (Slathia et al., 2020).

Bioethanol as a transportation fuel presents numerous benefits such as nanoparticle emissions reduction due to the neat burning associated with oxygenated fuels. Also, bioethanol has better octane rating qualities in comparison to the currently marketed gasoline and therefore it comes with better engine thermal efficiency as it enables greater compression ratios. The major drawback of bioethanol use as a transportation fuel include cold start complications at very low temperatures, and to tackle this problem, ethanol flexible fuel vehicles (FFVs) were designed many years ago in the automotive markets of countries like Brazil, the US, and European Union (EU) (Jin et al., 2017).

Nigeria's biofuels policy promotes the use of cassava as raw material for a 10% blend biofuel replacement alternative in the country's automotive energy requirement. The policy decision is envisaged to tackle energy sustainability and the attendant environmental degradation accompanying the utilization of fossil fuels as the only source of automobile fuel in the country (Ogundari et al., 2012).

Nigeria is situated in the western part of Africa and is surrounded by the Gulf of Guinea, the Benin Republic on the west, and Cameroon on the east. It has an area of 923,768 square kilometers (356,376 square miles) and is the most populous black nation in the world (Uche, 1989). Despite the enormous hydrocarbon resources available in Nigeria, the country is still confronted with a myriad of problems in the energy arm of the economy.

Bioethanol is categorized into first-generation bioethanol, second-generation bioethanol, and third-generation bioethanol. First-generation bioethanol production involved the use of carbohydrate-rich food crops to produce bioethanol. The process of obtaining bioethanol from carbohydrate-rich food crops is easier than that of second-generation bioethanol production as second-generation bioethanol production involves the use of waste materials generated from the preparation of carbohydrate-rich food crops, thereby making it a bit difficult to obtain bioethanol from them. Corn cobs (*Zea mays*), cassava peels (*Manihot esculenta*), rice husks (*Oryza sativa*), white yam peels (*Discorea rotundata*), and sugar cane bagasse (*Saccharum officinarum*) are some of the most prominent wastes derived from the processing of harvested crops in Nigeria, and they all belong to the second-generation bioethanol production (Adiotomre, 2015).

Bioethanol production from cassava peels has a great prospect in Nigeria as in several other countries in Africa due to its capability to thrive and produce yields even in less fertile soils. In Nigeria, cassava yield per hectare of land is on the low side, currently approximately 11 ton/h. The country has however continued to be the leading producer of cassava in the world (Onubuogu et al., 2014). Cassava can be cultivated on lands with very little nutrients where other crops cannot thrive. Also, cassava can be planted twice or even thrice each year as it requires little water for growth and development (Hillocks, 2002).

Nigeria has been ranked 10th position among the world's corn producers and in Africa, Nigeria is a leading producer of corn. Corn is cultivated countrywide in Nigeria with the Northcentral state of Kaduna functioning as the principal base for its production. From 2010 to 2015, the mean corn harvest in Nigeria was 8.18 million metric tons. The large production of corn in Nigeria has resulted in the production of large quantities of corn residues mainly in form of cobs. Residue from corn can serve as feedstock for generating energy and the development of new materials. The use of residues from corn as a possible energy source comes with novel problems that require attention. Some of the problems include; reaping, collection, heating rate, storage facilities, and energy transformation procedures (Mohlala et al., 2016).

Sugarcane is a predominantly tropical plant that blossoms well in the hot climatic environment and with enough precipitation. It is cultivated in Nigeria mainly to produce refined sugar. Chewing cane (soft cane) and industrial cane are the two main species of sugarcane plant that are usually grown in Nigeria. The soft cane is usually cultivated by local farmers mainly for chewing while the industrial cane composes of a hard fiber and is typically cultivated commercially by sugar manufacturing industries to produce refined sugar. The industrial cane sugar processing leaves by-products, mainly bagasse, which may be utilized for bioethanol production (Agboire et al., 2002). Sugarcane is planted on about 25 000 – 30 000 hectares of land in Nigeria with industrial sugarcane occupying approximately 12 000 hectares. A typical sugar processing industry in Nigeria produces an average of 82.2 kg of refined sugar and 31 kg of bagasse for every tonne of sugar cane harvested. Also, the full amount of fermentable sugar matter of bagasse is approximately 46 %, which means that 31 kg of bagasse will predictably have a 14.26 kg fermentable sugar content. It can therefore be assumed from stoichiometry that 14.26 kg fermentable sugar will yield 7.29 kg of ethanol (Nasidi et al., 2010).

Rice straw ranks among the most copious lignocellulosic biomass available globally. Rice is the 3rd most prominent grain crop in the world after corn and wheat. According to FAO data, the annual rice production for the 2007 was approximately 650 million tons. For every kilogram of grain processed, 1 – 1.5 kg of straw is generated (Binod et al., 2010). In Nigeria, rice is commonly grown mainly as a grain crop, and after harvesting, its husks are copiously generated as waste and in some areas, they are used to feed ruminant

animals. Hitherto, rice husks were considered to have little or no economic value until it was discovered to be a good feedstock for producing bioethanol (Madu and Agboola, 2018).

Yam is a major food crop found in Africa, the Americas, the Caribbean, South Pacific, and Asia. Nigeria is the foremost yam grower globally and accounts for more than 65% (approximately 38 million metric tonnes) of the total world's production in 2012 and it is more commonly supplied and copiously obtainable than cereals. The white yam, *Dioscorea rotundata* has been reported to be the principally planted yam and it is processed into a broad variety of foodstuffs in Africa and other parts of the world (Aruna et al., 2017).

It is noteworthy that the lignocellulosic biomass such as corn cobs, rice husks, yam peels, cassava peels, sugarcane bagasse among other biomass are always copiously available during certain seasons of the year as wastes from food crops processing. The cobs are generated after the removal of corn seed from the cobs, the rice husks are generated from the de-husking of the rice grains mainly in rice mills across the country, peels from cassava and yam tubers are generated mainly from local food processing factories.

Ethanol produced from lignocellulosic biomass is gradually gaining recognition and acceptance as a substitute to fuel gasoline derivable from fossil fuels. Conversely, the synthesis of bioethanol from carbohydrate-rich foodstuffs such as grains (first-generation bioethanol production) has led to an unfavorable effect on the food supply chain. The use of a more copious nonfood component of the carbohydrate-rich foodstuffs will in no doubt lessen the pressure on the food crops. A very high fraction of the wastes from carbohydrate-rich foodstuffs is composed of complicated carbohydrates such as cellulose, and hemicellulose which can be transformed into fermentable sugars. Microorganisms, such as enzymes, can act on these sugars, thereby converting them to ethanol (Binod et al., 2010).

Currently, bioenergy resources supply approximately 50 exajoules of the world's energy. It also has the capacity to guaranty energy sufficiency while also lessening the emission of greenhouse gases (GHG) normally generated from fossil fuels (Singh et al., 2014). It has an edge over other sources of renewable energy, since it can easily be kept and is freely obtainable all year long.

Bioethanol is the most synthesized liquid biofuel globally. As an automobile fuel, bioethanol can be employed either in the blended form alongside gasoline or even as unblended ethanol.

Presently, the USA is the foremost producer of bioethanol from corn globally, Brazil follows with production from sugarcane. To lessen the envisaged world's food crunch, lignocellulosic biomass is forecasted as a practically unending feedstock in the production of bioethanol (Sarkar et al., 2012).

The annual global output of gasoline ethanol is around 26 billion liters, with Brazil accounting for roughly 60% of that, followed by the United States and China. Sugar crops, primarily sugarcane and sugar beet, provide for roughly 60% of global bioethanol production; grain, primarily maize, accounts for the

remaining 40%. In many developed and developing countries, such as Japan and India, ethanol is being considered as the best fuel for octane enhancement and as a gasoline substitute in various quantities. Bioethanol supply on a global scale is becoming increasingly dependent on additional initiatives to encourage its low-cost synthesis utilizing already accessible raw materials. To replace the current global gasoline usage, more than 2 trillion liters of ethanol would be required. As an example, to generate this massive amount of ethanol using current best practices, Brazil would need up to 400 million hectares of land dedicated to energy crops. (Cortez et al., 2003).

1.2 Research Motivation

Currently, all the four refineries in Nigeria are not producing any petroleum products, as such, all the petroleum products are being imported into the country to meet the domestic energy demand, a situation which has consistently drained the foreign reserve of the country. Apart from the large hydrocarbon deposit, Nigeria is also blessed with abundant arable land on which food crops are grown year in year out, it is imperative to take advantage of this natural resource by developing the renewable energy segment of the country's energy sector. To guaranty food security, it is imperative to ensure that only the non-food parts of the carbohydrate-rich foodstuff produced in large quantities are used for biofuel production. The non-food parts of the crops produced in the country are usually obtained after processing the crops for consumption and are generally known as lignocellulosic biomass. Examples of lignocellulosic biomass include; cassava peels, yam peels, corn cobs, sugar cane bagasse, rice husks, potato peels, and a host of others. A relatively high amount of biomass is being produced year in year out, which had been wasted before now and even in some instances have been a source of environmental pollution owing to their indiscriminate disposal.

Nigeria currently lacks any industrial processing plant for large-scale bioethanol production from lignocellulosic biomass. It is, therefore, necessary to critically study some of the lignocellulosic biomass generated from Nigerian food processing industries to obtain process parameters data for their conversion into bioethanol to serve as a guide for future investors in this all-important field.

1.3 Statement of Research Problems

The wastes generated from the processing of carbohydrate-rich foodstuff is very high and poses a challenge of processing and disposal in Nigeria. Lignocellulosic biomass wastes are generated domestically and industrially from food and commodity processing industries such as sugar industries using sugar cane, animal feed mills using corn, flour mill industry using cassava tubers, and rice mill industries among others. Discovering other purposeful use for the generated lignocellulosic biomass wastes in Nigeria such as their use as feedstocks to produce bioethanol is a venture that should be vigorously pursued. This work, therefore,

seeks to probe further into the production of bioethanol from five (5) most generated lignocellulosic biomass obtained during different seasons of the year in Nigeria. To ensure the availability of feedstock for a bioethanol processing plant, this study also seeks to study and generate data on process design for hybridized (mixed) lignocellulosic biomass feedstocks especially for some biomass with little or no information on their potential for bioethanol production in the literature, e.g. yam peels biomass. Arising from the research subject, the following research questions were addressed in this study:

- What is the effect of biomass hybridization in bioethanol production from multiple feedstocks?
- What are the effects of pretreatment on different biomass feedstocks singularly and hybridized, before fermentation?
- Which biomass feedstocks give the highest bioethanol yield as individual feedstock and as hybridized feedstock?
- What is the optimum condition for bioethanol production parameters from hybridized biomass feedstocks?

1.4 Research Hypothesis

The study posed the following hypothesis:

- Hybridization which is the mixture of two or more lignocellulosic biomass enhances their properties and potentials of bioethanol production in terms of yield(s) and quality.
- Hydrothermal plus alkali pretreatment is more effective than hydrothermal plus acid pretreatment and hydrothermal only pretreatment for singular and hybridized biomass feedstocks.

1.5 Aims and objectives of the study

The overall aim was to evaluate the use of hybridized feedstocks for bioethanol production, with a critical look into the process design, optimization, and techno-economic analysis. To achieve this aim, the following objectives were drawn:

- (a) To carry out the study of the bioethanol production from five (5) different lignocellulosic biomass feedstocks which are produced in large quantities at certain seasons of the year locally in Nigeria.
- (b) To optimize the production process parameters of hybridized (mixed) biomass feedstocks in the production of bioethanol to ensure all-year-round availability of feedstocks for bioethanol production plants.
- (c) To provide data on the effects of different pretreatment methods on the individual and hybridized biomass in terms of costs, yield, and environmental impact.

- (d) To provide data for the fuel properties of the bioethanol produced, viz a viz that of gasoline from the individual as well as hybridized feedstocks.

1.6 Layout of the thesis

This thesis comprises seven (7) chapters and appendices.

Chapter 1 focused on the background of the study as well as motivation for the study. The basis and significance of the production of bioethanol from selected Nigerian lignocellulosic biomass feedstock were also highlighted. The overall aim and objectives were also presented.

Chapter 2 is the first paper contribution and it presents a detailed literature review of harnessing the potential of bioethanol production from lignocellulosic biomass in Nigeria. The paper presented the background information on some of the lignocellulosic biomass generated at certain seasons of the year, their history and origin were also highlighted. The capacity of Nigeria for bioethanol production from lignocellulosic biomass was also presented. It was published in one of the leading journal publications, *Biofuels, Bioproducts and Biorefining* published by Wiley Online Library.

Chapter 3 is the second paper contribution on the characterization of five commonly generated Nigerian lignocellulosic biomass at certain seasons of the year. Experimental characterization of five biomass wastes namely corn cobs, cassava peels, rice husks, white yam peels, and sugar cane bagasse based on proximate analysis and x-ray diffraction (XRD) analysis has been presented in this study due to their vast intrinsic capability for bioethanol production. This chapter is published in the *International Journal of Ambient Energy* by Taylor and Francis Series.

Chapter 4 is the third paper contribution and presents the experimental determination of the effects of pretreatment on selected Nigerian lignocellulosic biomass in Bioethanol production. The five lignocellulosic biomass were subjected to three types of pretreatment and the products were then characterized using proximate analysis and other spectroscopic analysis such as Xray diffraction (XRD), Fourier transforms infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and Brunauer–Emmett–Teller (BET) Analysis and published in *Scientific Reports* by Nature Series.

Chapter 5 is the fourth paper contribution which was on the Investigation of the effects of pretreatment on the elemental composition of ash derived from selected Nigerian lignocellulosic biomass.

Chapter 6 is the fifth paper contribution on the hybridization of selected Nigerian lignocellulosic biomass feedstocks for bioethanol production: modeling and optimization of pretreatment and fermentation process parameters using response surface methodology. In this study, hybridized feedstocks (mixtures of biomass) of cassava peels plus yam peels, as well as corn cobs plus rice husks biomass, were optimized using the response surface methodology centered on the statistical design of experiments (DOE) of the Box-Behnken design (BBD), to produce bioethanol.

Chapter 7 is the Conclusion and Recommendations for future work.

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Chapter TWO

LITERATURE REVIEW

Paper 1, HPBPLB, Accepted and Published

Journal Paper of Biofuels, Bioproducts and Biorefining, John Wiley & Sons, Ltd

Awoyale Adeolu A. and Lokhat, David (2019)

Harnessing the Potential of bioethanol production from lignocellulosic biomass in Nigeria – a review

This chapter represents the literature review of this study which has been peer-reviewed, accepted in the form of a journal paper, and published: DOI: [10.1002/bbb.1943](https://doi.org/10.1002/bbb.1943); ISSN: 1932104X. The paper was awarded recognition for one of the most-read publications in Biofuels, Bioproducts, and Biorefining journal as shown in appendix A.

Awoyale, A.A. and D. Lokhat, Harnessing the Potential of bioethanol production from lignocellulosic biomass in Nigeria—a review. *Biofuels, Bioproducts and Biorefining*, 2019. **13**(1): p. 192-207.

PAPER ONE

Harnessing the potential of Bioethanol production from Lignocellulosic biomass in Nigeria – A Review

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Abstract

The need to diversify energy sources to renewable energy sources from the present non – renewable hydrocarbon sources has prompted huge investments in research activities to produce bioethanol from carbohydrate-rich foodstuffs such as cassava tuber, sugarcane molasses, yam tubers, rice grains, corn grains, and a host of others. In recent times, however, more attention is being given to producing bioethanol from the non-edible part of these carbohydrate-rich foodstuffs, which are obtained mainly as waste products from the food crops processing. Such non-edible parts of these carbohydrate-rich foodstuffs include the peels, bagasse, straw, stalk, cobs, etc. The most important component of the biomass is lignocellulose, which will be broken down into carbohydrates and then fermented. The major challenge in using biomass as a feedstock for bioethanol production is the fact that lignocellulosic biomass is highly recalcitrant to fermentation and therefore requires more vigorous pretreatment before saccharification and fermentation. The main techniques used for pretreating lignocellulosic materials are physical and thermal methods as well as chemical and biological methods. In Nigeria, lignocellulosic bioethanol production potential from agricultural residues amounts to about 7.556×10^9 liters per annum with more than 62 % of this being generated from process residues. Cassava biomass alone can produce more than 114 liters of bioethanol for every ton of cassava peeling after processing. Nigeria is blessed with more than enough agricultural residues for bioethanol production to meet its bioethanol blending demand.

Keywords: Bioethanol; Biomass; Lignocellulose; Renewable energy

1 Introduction

The quest for diversification from conventional fossil fuels to renewable sources of energy for industrial and domestic use has been receiving unprecedented attention in recent years. This has become necessary owing to the high rate at which the crude oil is being depleted, instability in the price of crude oil, environmental degradation arising from the production, processing, and use of crude oil and crude oil derivatives (Onoji et al. 2016). Sources of renewable energy include biomass, hydropower, solar, wind, tidal, geothermal among others.

From biomass, biofuels and biomaterials such as bio alcohols (mainly bioethanol and biomethanol), biohydrogen, biodiesel, biogas, can be produced (Kurian et al.2013). Nigeria is blessed with a very large expanse of fertile arable land for crops cultivation, (about 71.2 million hectares) (Ohimain 2013) and as such, there is the possibility of cultivating different varieties of carbohydrate-rich foodstuffs such as yam, cassava, sorghum, cocoyam, and rice from which bioethanol can be conveniently produced. To forestall a situation where the production of bioethanol from the crops disrupts the normal foodstuff supply for human consumption, lignocellulosic residues obtained as wastes from processing the food crops can be used for the bioethanol production. In this way, waste can be turned into wealth and this will constitute a safer and neater means of disposal of the wastes from these food crops processing.

Lignocellulosic biomass can be obtained from the cellulose of woody plant material and consists of cellulose, lignin, and hemicellulose polymers that form a complex heterogeneous structure. Different plant species have a varying combination of masses of cellulose and hemicelluloses in the plant material which range typically from 50 – 70 % of the dry mass with lignin taking up the remaining part. The cellulose and hemicelluloses can be converted to sugars through a series of Physico-chemical and biological processes and can then be easily fermented to bioethanol (Ullah et al. 2015). Typical examples of lignocellulosic residues that can be used for bioethanol production include; sorghum stalks, wheat chaff, sugar cane bagasse, nutshells, rice husks, corn cobs, corn stover, forest harvest, wood process residues, banana peels, coconut fibers, groundnut shell, bamboo (Elemike, Oseghale, and Okoye 2015). The use of any of these sources of biomass for producing bioethanol is an example of second-generation bioethanol production. Bioethanol production on a large scale from these lignocellulosic residues will in no doubt impact positively on the economy of the rural communities in Nigeria as well as create more jobs for the teeming unemployed youths in the country. This work therefore aims to critically review the processes of bioethanol production from selected cellulosic residues.

2 Potential lignocellulosic biomass feedstocks in Nigeria

2.1 Cassava biomass (residue) (Manihot Spp.)

In Nigeria, cassava is a much sought-after food crop owing to its vast and varied use (Elemike, Oseghale, and Okoye 2015). From the rural areas to urban areas in the country, different species of cassava are being cultivated and significant research is ongoing by different agricultural research institutions, notably, the International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State, National Root Crops Research Institute, Umudike, to develop hybrid species of cassava. Nigeria produces cassava on a very large scale annually and has been classified among the largest producers of cassava in the world. Research has shown Nigeria is a producer of over 53 million tonnes of cassava annually (Adekunle, Orsat, and Raghavan 2016). In processing cassava, the residue (biomass) that is left behind includes one or more of the peels, leaves, and roots. Often, this biomass is not used for anything tangible. The cassava peels are especially disposed of

haphazardly, thereby causing serious degradation to the environment as they decay. The cassava peels can thus be processed into bioethanol to transform these wastes into useful and useable bioethanol. A typical average proximal composition of cassava peel is shown in Table 1.1.

Table 1. 1: Typical average proximal composition of cassava, sweet cassava variety ADP3

Parameters	Percentage
Dry matter	30.13
Ash	7.00
Crude Protein	3.50
Crude fiber	10.0
Ether extract	12.00
Neutral detergent fiber	52.00
Acid detergent fiber	25.00
Acid detergent lignin	11.00
Cellulose	14.00
Hemicellulose	27.00

Source: Nasidi et al. (2015)

Also, the cassava peel contains 73 % (w/w) carbohydrate (Adekunle, Orsat, and Raghavan 2016). The cassava peel has a density of about 0.2511 g/cm³ (Sangodoyin and Amori 2013) which corresponds to approximately above 114 liters of bioethanol for every tonne of cassava peelings after processing. Bioethanol for use as fuel is better produced from cassava peels rather than the cassava pulps itself because the food supply balance will not be stressed. Figure 1.1 shows a summary of experimental procedures for transforming cassava peels into bioethanol. In Nigeria generally, quite many dishes are made from peeled cassava tubers, therefore, the cassava tubers are peeled, and the peelings are then sun-dried preferably or in a ventilated oven at a temperature, not more than 45 °C. The dried peels will then be taken to a grinding mill for grinding into powdery form and then sieved with a mesh of 0.5 mesh size or less. The cassava powder will then be characterized using proximate analysis in determining the proximate composition of the powder for the lignin, cellulose, and hemicellulose content before hydrolysis and further pretreatment to make the cellulose available for fermentation. The pretreatment can be done by using biological means, i.e. use of enzymes such as accellerase 1500. Fermentation and subsequent distillation then follow. Generally, the lignocellulosic biomass can be processed to produce fuel ethanol biologically by the following processes: (1) pretreatment to liberate cellulose and to remove either lignin or hemicellulose; (2) The use of cellulase to depolymerize the carbohydrate polymers to obtain free sugars; (3) fermentation of the obtained free sugar, consisting of pentose and/or hexose to produce ethanol; (4) distillation of the produced ethanol. The use of bioethanol fuel reduces air pollution to a large extent and helps to reduce greenhouse gas emissions, thereby mitigating climate change (Canilha et al.

2012).

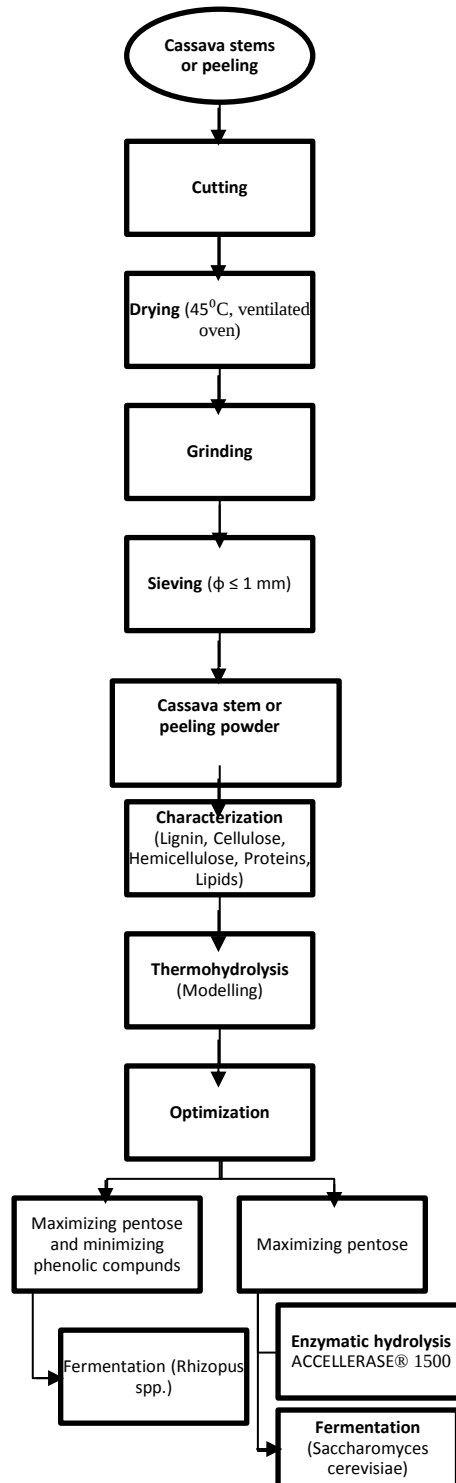


Figure 1. 1: Experimental Approach for bioethanol production from cassava peels.

Adapted and modified from Nanssou, Nono, and Kapseu (2016).

2.2 Sugarcane bagasse (*Saccharin spp*)

Bagasse and molasses are wastes generated from the sugar industry. They are used in electricity generation, paper production, bioethanol production, and animal feed (Mohlala et al.2016). Bagasse is a fibrous residue produced from sugarcane processing after the juice must have been extracted from the sugar cane stalks (Mbohwa 2013). The bagasse, like most other biomass, is composed mainly of lignin, cellulose, and hemicelluloses and as such fits in as a perfect source of raw material for second-generation bioethanol production (Nsafu 2012). Sugarcane bagasse has a high calorific value and as such, it is very attractive for energy production (Mohlala et al.2016). About 5% of the entire sugar cane produced globally is from Africa and countries in sub-Sahara Africa generate about 30% of the sugarcane produced in Africa. In Africa, South Africa is the largest producer of sugar cane followed by Sudan, Kenya, and Swaziland. Annually, South Africa alone generates close to 7 million tons of bagasse (Davidson et al.2006).

The European sailors brought sugarcane to Nigeria around the 15th century through the eastern and western coasts. Unfortunately, Nigeria still imports sugarcane from other countries to meet up the sugarcane products demand, which is estimated at 1.5 billion tonnes (Galadima et al.2011). Sugarcane production is expected to be improved upon in Nigeria as the government set up the first sugarcane bio-refinery in Zaria in 2015. One ton of sugarcane stems can generate close to 270 kg of bagasse, which is a reasonable quantity for processing to produce bioethanol (Ogwo et al. 2012). The typical composition of sugarcane biomass is as shown in Table 1. 2.

Table 1. 2: Typical Constituents of the pretreated sugar cane bagasse (dried basis)

Pretreatment	Cellulose (%)	Hemicellulose (%)	Total lignin (%)	Organic solvent extractives (%)	Hot water extractives (%)	Ashes (%)
None (raw material)	38.59 ± 3.45	27.89 ± 2.68	17.79	± 0.62 1.61 ± 0.16	1.11 ± 1.23	8.80 ± 0.02
Combined acid and alkaline	65.03 ± 2.34	10.95 ± 0.19	8.12	± 0.31 0.98 ± 0.61	10.97 ± 0.98	4.60 ± 0.76
Combined hydrothermal and alkaline	62.14 ± 4.12	11.52 ± 0.73	7.87	± 1.88 0.77 ± 0.09	10.08 ± 0.58	8.10 ± 0.06
Alkaline	47.21 ± 3.23	29.29 ± 2.32	4.31	± 1.78 1.22 ± 0.47	12.52 ± 0.53	6.05 ± 1.22
Peroxide	53.85 ± 2.76	22.02 ± 3.27	7.99	± 3.84 0.78 ± 1.33	10.65 ± 1.55	4.22 ± 1.27

Source: (Chinnadurai, Muthukumarappan, and Juson 2008)

The bulk density of sugar cane bagasse is low and this makes it difficult to handle, store, transport, and processed. Pelletization of the bagasse makes it easier to transport and process as well. Bioethanol produced

from sugarcane biomass is one of the most suitable alternatives and probably a future replacement for hydrocarbon fuels as it provides a renewable source of energy and has a lesser number of carbons than gasoline. The use of bioethanol fuel reduces air pollution to a large extent and contributes to mitigating climate change by reducing greenhouse gas emissions (Canilha et al. 2012).

2.2.1 Bioethanol production from sugar cane bagasse

Antunes et al. (2014) worked on ethanol production from sugar cane bagasse using novel xylose-fermenting yeast, named '*Scheffersomyces shehatae* UFMG-HM 52.2.' The pretreatment of the sugar cane bagasse was carried out with the use of dilute acid to hydrolyze the sugar cane bagasse to obtain sugarcane bagasse hemicellulosic hydrolysate (SBHH) which was subsequently concentrated, detoxified, and supplemented with nutrients in different formulations to prepare the fermentation medium to the yeast evaluation performance. The *Scheffersomyces shehatae* UFMG-HM 52.2 used in the fermentation process was obtained from the Atlantic rain forest ecosystem in Brazil. The fermentation was carried out in Erlenmeyer flasks maintained in a rotator shaker at 30 °C for 72 hours at 200 rpm. The use of a fermentation medium composed of SBHH supplemented with 5 g/L ammonium sulfate, 3 g/L malt extract, and 3 g/L yeast extract resulted in 0.38 g/g of bioethanol yield.

Martín et al. (2002) worked on the production of bioethanol from enzymatic hydrolysates of sugarcane bagasse with recombinant xylose-utilizing *Saccharomyces cerevisiae*. In the work, steam explosion at 205 and 215 °C was used to pretreat the sugarcane bagasse, after which it was hydrolyzed with cellulolytic enzymes. The hydrolyzed bagasse was then subjected to enzymatic detoxification using the phenoloxidase laccase to treat them and then to chemical detoxification using liming process. Thereafter, the hydrolysates were then subjected to fermentation with the recombinant xylose-utilizing *Saccharomyces cerevisiae* laboratory strain TMB 3001, which is a CEN.PK derivative having over-expressed xylulokinase activity. The xylose reductase and xylitol dehydrogenase of *Pichia stipitis*, and the *S.cerevisiae* strain ATCC 96581, isolated from a spent sulphite liquor fermentation plant were also expressed. A yield of 0.18 g/g dry bagasse was recorded for the fermentation of the hydrolysate detoxified by over-liming, whereas it gave only 0.13 g/g dry bagasse in the undetoxified hydrolysate. Figure 1.2 shows the processes involved in transforming sugar cane bagasse into bioethanol.

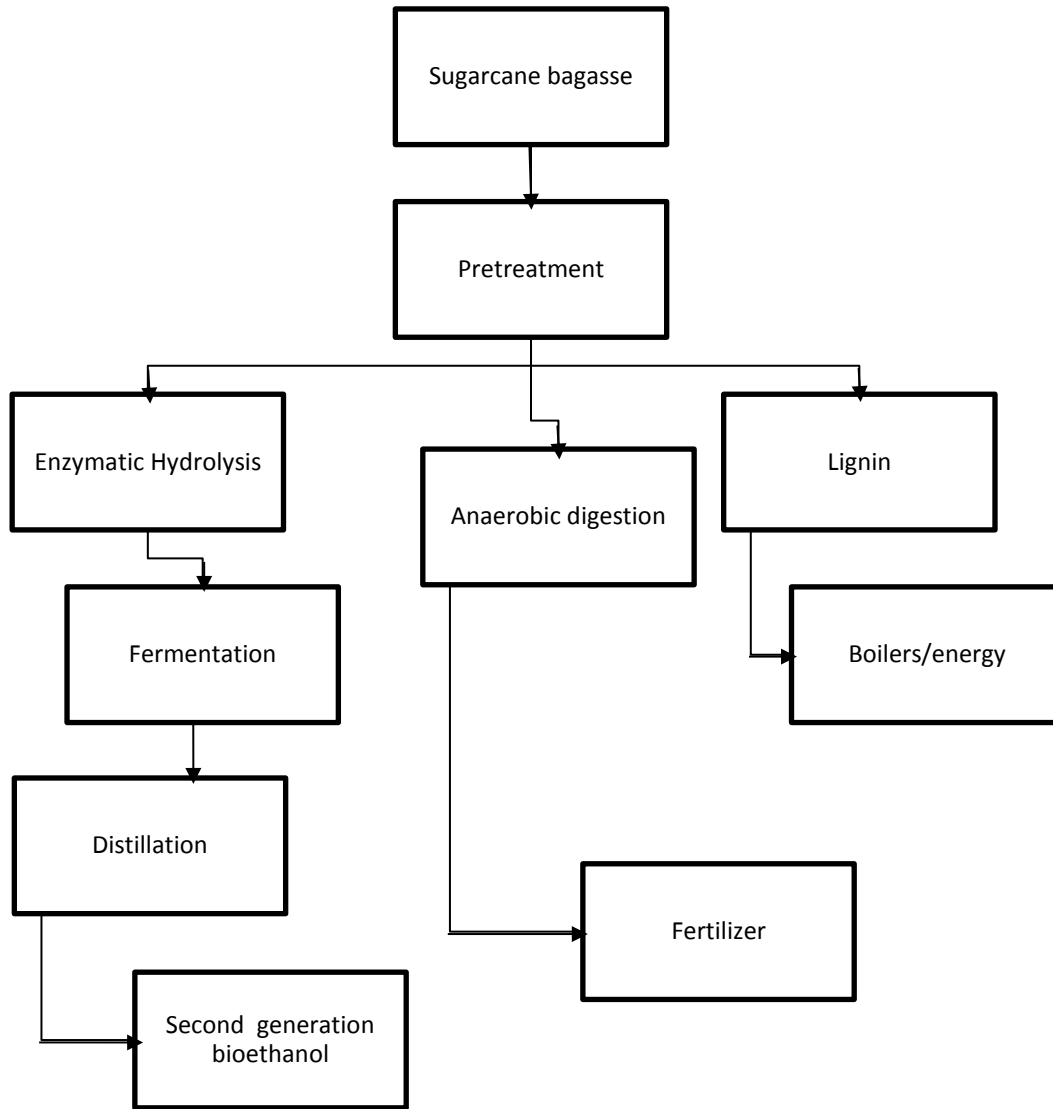


Figure 1. 2: Integrated process for the production of bioethanol from sugar cane bagasse

Adapted and modified from Rabelo et al. (2011).

2.3 Corn cobs (*Zea mays*)

Corn was brought to Africa in the 1500s and has become one of Africa's most planted food crops (McCann 2001). Africa's largest corn producer is South Africa, with corn production reaching about 14.9 MMT in 2013 and 11.3 MMT in 2014. Nigeria is recognized as the 10th largest corn producer in the world and a major producer of the crop in Africa. An estimated 60 % of the corn produced in Nigeria is processed into malt drink, flour, starch, beer, corn flakes, syrup, animal feeds, and dextrose. Disposing of the residues (cobs) of corn can pose serious environmental issues in countries like South Africa and Nigeria where the

corn is produced in large quantities. The corn residues consisting of stalks and cobs serve as raw materials for energy generation in some countries. The use of corn cobs to produce bioethanol comes with some challenges and to surmount these challenges, some factors including but not limited to the mode of harvesting, crop handling, storage/preservation methods, and conversion methods should be developed further (Zych 2008). The heating value of corncob is about 19.14 MJ/Kg, it can therefore be used as a potential thermo-chemical feedstock (Davies et al. 2014). The large quantities of corn cobs generated annually make it a perfect potential feedstock for bioethanol production. Table 1.3 shows the proximate composition of the corn cob.

Table 1. 3: Proximate Composition of Corn Cob biomass

	Fractions	Weight %
	Holocellulose	73.04
(a)	α -Cellulose	34.45
(b)	β -Cellulose	18.73
(c)	γ - Cellulose	19.84
	Pentosans (Xylan)	28.23
	Total lignin	16.03
(a)	Klason Lignin (Acid insoluble)	14.01
(b)	Acid soluble lignin	2.02
(c)	Ethanol-Benzene soluble	4.33
	Others (Difference)	6.64

*The results are expressed as weight percent, oven-dry basis of raw materials.

Source: (Sun and Chen 2008).

2.3.1 Bioethanol production from corn cobs

Ado et al. (2009) worked on the production of bioethanol from corn cobs by using co-cultures of *S. cerevisiae* and *Aspergillus niger*. The work involved the simultaneous saccharification and fermentation (SSF) of 1% and 10% (w/v) dry pre-treated corncobs. A control fermentation of the same concentration in a synthetic medium was also carried out. The maximum bioethanol yields at 1 % and 10 % were 4.17 % and 6.17 % respectively for the medium containing the corn cobs and 3.45 % and 6.23 % respectively for the control fermentation.

Yah et al. (2010) worked on optimizing the temperature for producing bioethanol from corn cobs using mixed yeast strains. Dilute sulphuric acid and enzymatic hydrolysis methods were used to extract xylose and glucose sugars from corncobs. From their work, acid hydrolysis of corn cobs gave higher sugars than when enzymatic hydrolysis was used. The work also gave the optimal temperature and time for sugar

fermentation to be approximately 25 °C and 50 hours by the two yeast strains (*S. cerevisiae* and *P. stipitis*) used respectively. Conclusively, they could establish that varying the temperature of the fermentation process improves the bioethanol production from corn cobs sugars.

2.4 Mango Peels (*Mangifera indica*)

The mango tree is erect, branched, and small to large-sized plant with leaves that are evergreen and alternatively arranged. It has a wide crown and inflorescences with numerous flowers (Ugese, Iyango, and Swem 2012) Asia, eastern India particularly is home to the mango of the species *Anacardiaceae*. The Persians brought it to East Africa around the 10th century, while the Portuguese brought it to West Africa in the 16th century. Itinerant merchant missionaries and colonialists introduced Mango to Nigeria in the 20th century and over time, it has become an integral part of indigenous cropping systems. Among the ten leading nations where mangos are being produced, Nigeria ranks 9th position. Many species of mango trees grow in Nigeria and the mango fruits produced from them vary in terms of form, shape, size, color, quality, and taste. The mango fruit can be used for different purposes, more especially as juice, which is produced from the ripe fruit. The mango peel is a major waste obtained during mango processing, and it constitutes about 15-20 % of total weight (Henrique et al. 2013). The chemical compositions of mango peel have cellulose as the main component. Thus, the utilization of mango peels could be creating a new cellulose source. Table 1.4 shows the chemical composition of untreated and treated mango fibers.

Table 1. 4: Chemical composition of untreated and treated mango fibers in the different processing stage

Samples	Chemical composition (%)		
	α -cellulose	Hemicellulose	Lignin
Untreated mango peel fibers	38.35	13.90	27.90
Steam exploded fibers	51.28	8.41	21.84
Bleached fibers	89.21	0.42	7.42

Source: Yingkamhaeng and Sukyai (2014)

2.4.1 Bioethanol production from mango peels

Reddy, Reddy, and Wee (2011) worked on the production of bioethanol from dried mango (*Mangifera indica*) peel by *S. cerevisiae*. Their work entailed direct fermentation of mango peel extract, from which only 5.13 % (w/v) ethanol was obtained and the fermentation was very slow. The ethanol production was significantly increased up to 7.14 % (w/v) when nutrients such as yeast extract, peptone, and wheat bran extract were supplemented with the mango peel.

Fernando et al. (2014) worked on bioethanol production from agro-industrial wastes with mango, *M. indica* as a case study. The purpose of the study was to evaluate the fermentation performance and behavior of yeast, *S. cerevisiae* in the pulp and peel of mango. Pulp and peel of 75% and 25% respectively and seeds were used as raw material for the bioethanol production. The result obtained from the study reveals that the maximum yeast growth was observed in the treatment of peel and pulp with 150 g/L of initial reducing sugar.

Walia, Bedi [47] worked on the production of bioethanol from mango peel. The mango peels were dried and grounded to powdery form and then dissolved in distilled water. The sugar concentration was adjusted to 15% and with baker's yeast, *S. cerevisiae*, the substrate solution was fermented, and the bioethanol was produced after five days of incubation. The produced ethanol was subjected to a Gas Chromatography test, which showed that the mango peel extract after fermentation contains 95% of bioethanol.

2.5. Sorghum straw (*Sorghum bicolor*)

Sorghum is the fifth vital cereal crop after maize, rice, wheat, and barley. It is the main food grain for more than 750 million people living in the semi-arid tropics of Asia, Africa, and Latin America. Sorghum is predominantly produced by small-scale subsistence farmers who do not have access to farming-assisted inputs such as fertilizers, pesticides, and improved/hybrid seeds. Sorghum has a structure broadly similar to that of other cereal crops. The major constituents of the sorghum grain are the pericarp, which is the outer covering, the testa between pericarp and endosperm (which may or may not be present, depending on the species), the embryo, and the endosperm. There are many species of sorghum with varying colors ranging from white through red to brown. Grain sorghums are normally grown in regions that are very dry or very hot as compared to maize which is grown in areas that are moderately wet and cool for a successful production. Table 1.5 shows the composition of lignocellulosic sorghum straw. Sorghum can successfully thrive in the drier climates due to several factors viz;

- The inherent ability to stay dormant during the dry season and then continue growth during the wet season;
- They have leaves that can roll up as they wither, thereby reducing the area of leaf open for transpiration
- Sorghum leaves and stalks are coated with a waxy substance that protects them from drying easily.

Sorghum is a crop grown worldwide and has good prospects in South American countries such as Brazil, Argentina, and Mexico and some African countries such as Nigeria and Ethiopia (Ullah et al. 2015).

Table 1. 5: Lignocellulosic sorghum straw composition

Component	% dry basis
Cellulose	35.87
Hemicellulose	26.04
Lignin	7.52

Source: Cardoso et al. (2013).

2.5.1 Bioethanol production from sorghum straw

McIntosh and Vancov (2010) worked on the use of dilute alkali pretreatment for enhanced enzyme saccharification of *Sorghum bicolor* straw. The work involved the investigation of the effects of changing important pre-treatment parameters namely temperature, alkalinity, and time on the enzymatic hydrolysis of sorghum straw. They were able to come up with the fact that total sugar release climaxed when the sorghum straw was pre-treated in 2% sodium hydroxide at 121 °C for 60 min which represented a 5.6-fold higher yield as compared to samples that were pre-treated at 60 °C without the use of alkali.

Mehmood et al. (2009) worked on ethanol production from *Sorghum bicolor* by separate and simultaneous saccharification and subsequent fermentation in batch and fed-batch systems. The work involved finding a good combination of different experimental conditions in the process of sorghum bicolor straw pretreatment, enzymatic saccharification, detoxification of inhibitors, and the fermentation process. The pre-treatment process was optimized by using varying concentrations of dilute sulphuric acid, different temperatures, and residence times. The optimum condition was achieved at 121°C, 1 % acid concentration, 60 min residence time, and enzyme saccharification using cellulase and glucosidase at 50 °C and pH 4.8 for 48 h. 98.5 % of theoretical ethanol yield was achieved in separate hydrolysis and fermentation.

2.6 Rice husks or hulls (*Oryza sativa*)

Rice is a cereal crop that is the world's second most popular cereal after maize. Rice needs no introduction in every Nigerian home as it is one of the most important staple foods found in the country. It is consumed in homes the well-to-do and the poor alike. Rice has become a major staple food in Nigeria which has placed the demand to be on the increase. Nigeria occupies the first position in rice production in West Africa and the third position in Africa after countries like Egypt and Madagascar, producing approximately 3 million metric tons annually. This is grossly below the country's local demand of about 5 million tons annually (agronigeria.com.ng/rice-farming). Rice husks are the hard-protecting layers of rice grains. The husks help to protect the rice grains while growing, after harvesting the rice and the rice has been dehusked, the husks can then be put into other uses such as a source of building materials, fertilizer, or fuel. The rice husk is mostly indigestible to humans and hence, the rice grains must be dehusked before the rice can become edible. The modern rice hulling machine was invented in Brazil in 1885. The hulls are removed from the raw rice grain during the milling processes to reveal whole brown rice grains, which can sometimes

be milled further to remove the bran layer, resulting in white rice. Rice husks have been proven to be able to produce bioethanol from the reported works.

Table 1. 6: Proximate composition of rice husk

Composition	Percentage
Cellulose	31.12
Hemicellulose	22.48
Lignin	22.34
Mineral ash	13.87
Water	7.86
Extractives	2.33

Source: Kumar et al. (2010).

2.6.1 Bioethanol production from rice husks

Saha and Cotta (2008) used rice husks as a feedstock for bioethanol production through sequential lime pretreatment and simultaneous saccharification and fermentation (SFF) for 53 hours. The results obtained showed that 11g/l bioethanol was obtained.

Singh, Bajar, and Bishnoi (2014) treated rice husk with alkali and the result obtained from the work showed 0.36 g bioethanol/g substrate by *Scheffersomyces stipites* enzyme fermentation. Also, 0.4 g bioethanol/g substrate by *S. cerevisiae* enzyme fermentation; and 0.42 g bioethanol/g substrate by the co-culture fermentation combining the two enzymes.

Roslan et al. (2011) worked on the conversion of rice straw into bioethanol using cellulase obtained from local *Aspergillus sp*, and the results obtained from the work showed 62.61 % bioethanol yield or 0.102 g ethanol/g rice straw.

3 Cellulosic biomass capacity for bioethanol production in Nigeria

In Nigeria, lignocellulosic bioethanol production capacity from agricultural biomass has been estimated at 7.556×10^9 liters per annum, and close to 62 % of this amount is obtained from process residues. This is amount is quite close to the volume of premium motor spirit consumed annually for the period 2001 to 2006 and is greater than the 10 % mandate for renewable fuel content as stipulated in the biofuel policy of the Nigerian government. It is therefore noteworthy that Nigeria is blessed with enough agricultural biomass to conveniently cater to its bioethanol blending demand. Tables 1.7 and 1.8 showed the approximated mean annual bioethanol production from agricultural biomass in Nigeria from 2001 to 2006 and commercially available lignocellulosic bioethanol processing plants in different geographical locations in Nigeria respectively. Table 1.8 is centered on mean production (2001 – 06) from agricultural biomass annually and

an output of 2.50×10^9 liters annually. Millet, sorghum, rice, and groundnut as seen in the table, are grown almost solely in the northern part of Nigeria while the other crops seen on the table, i.e., maize, cassava, and yam, are grown equally distributed between the northern and southern parts of Nigeria. The north-central zone has the highest capacity for producing lignocellulosic bioethanol with 1.403×10^9 liters per annum. Each of the other zones in the country has a capacity of about 5.0×10^9 liters per annum. Yam and Cassava peelings biomass have the highest potential (approximately 80 %) for bioethanol production. From Table 1.8, the North-West zone has the greatest capacity for bioethanol production of about 1.071×10^9 liters per annum from field biomass and is closely followed by the North East and North Central zones. Maize stalks, millet straw, and sorghum straw represented about 75 % of the potential resource available for producing bioethanol from field biomass. The southern zones show very little capacity for bioethanol production from field residues with each zone having the capacity for not more than 1.50×10^{10} liters per annum. The North Central zone had the uppermost capacity of about 2.062×10^9 liters per annum for lignocellulosic bioethanol production from a combined (hybrid) process and field biomass of agricultural feedstocks, this was followed by the North West and North East (Iye and Bilsborrow 2013).

Table 1. 7: Projected mean annual bioethanol production (liters annum⁻¹) from field biomass availability (Cg) in Nigeria from 2001 to 2006. (Iye and Bilsborrow 2013).

Feedstock	Northwest		Northeast		Northcentral	
	Residue (Cg)	Bioethanol yield (10 ⁶ liters a ⁻¹)	Residue (Cg)	Bioethanol yield (10 ⁶ liters a ⁻¹)	Residue (Cg)	Bioethanol yield (10 ⁶ liters a ⁻¹)
Rice straw	425.41	114.86	363.78	98.22	486.19	131.27
Sorghum straw	1260.74	340.40	761.12	205.50	602.22	162.60
Millet straw	1315.35	355.14	722.98	195.20	247.2	66.74
Maize stalks	687.59	185.65	701.71	189.46	708.55	191.31
Cassava stalks	33.6	9.07	36.29	9.80	135.76	36.66
Groundnut straw	244.61	66.04	166.63	44.99	260.85	70.43
Feedstock	Southwest		Southeast		South-south	
	Residue (Cg)	Bioethanol yield (10 ⁶ liters a ⁻¹)	Residue (Cg)	Bioethanol yield (10 ⁶ liters a ⁻¹)	Residue (Cg)	Bioethanol yield (10 ⁶ liters a ⁻¹)
Rice straw	55.43	14.97	119.59	32.29	8.86	2.39
Sorghum straw	28.35	7.65	0	0	0	0

Millet straw	0.18	0.05	0	0	0	0
Maize stalks	365.96	98.81	238.82	64.48	266.37	71.92
Cassava stalks	96	25.92	112.09	30.26	112.54	30.39
Groundnut straw	7.85	2.12	1.7	0.46	2.98	0.80

Table 1. 8: Commercially available lignocellulosic bioethanol processing plants in different geographical locations in Nigeria (Iye and Bilsborrow 2013)

Feedstock	South west		South east		South south		North west		North east		North central	
	Bioethanol yield (10 ⁶ liters a ⁻¹)	Number of commercial facilities	Bioethanol yield (10 ⁶ liters a ⁻¹)	Number of commercial facilities	Bioethanol yield (10 ⁶ liters a ⁻¹)	Number of commercial facilities	Bioethanol yield (10 ⁶ liters a ⁻¹)	Number of commercial facilities	Bioethanol yield (10 ⁶ liters a ⁻¹)	Number of commercial facilities	Bioethanol yield (10 ⁶ liters a ⁻¹)	Number of commercial facilities
Rice husk	8.27	0.03	17.84	0.07	1.32	0.01	63.47	0.25	54.32	0.22	72.54	0.29
Maize cob	49.05	0.20	32.01	0.13	35.70	0.14	92.15	0.37	94.04	0.38	94.96	0.38
Cassava peelings	380.05	1.52	443.75	1.77	445.52	1.78	133.01	0.53	143.68	0.57	537.47	2.15
Ground nut husk	3.21	0.01	0.70	0	1.22	0	100.15	0	68.22	0.27	106.80	0.43
Yam peelings	237.28	0.95	316.12	1.26	247.83	0.99	125.19	0.50	202.84	0.81	591.40	2.37

Feedstock	South west		South east		South south		North west		North east		North central	
	Bioethanol yield (10 ⁶ liters a ⁻¹)	Number of commercial facilities	Bioethanol yield (10 ⁶ liters a ⁻¹)	Number of commercial facilities	Bioethanol yield (10 ⁶ liters a ⁻¹)	Number of commercial facilities	Bioethanol yield (10 ⁶ liters a ⁻¹)	Number of commercial facilities	Bioethanol yield (10 ⁶ liters a ⁻¹)	Number of commercial facilities	Bioethanol yield (km ³ a ⁻¹)	Number of commercial facilities
Rice straw	14.97	0.06	32.29	0.13	2.39	0	114.86	0.46	98.22	0.39	131.27	0.53

Sorghu m straw	7.65	0.03	33.29	0.13	0	0	340.40	1.36	205.50	0.82	162.60	0.65
Millet straw	0.05	0	34.29	0.14	0	0	355.14	1.42	195.20	0.78	66.74	0.27
Maize stalks	98.81	0.40	35.29	0.14	71.92	0.29	185.65	0.74	189.46	0.76	191.31	0.76
Cassav a stalks	25.92	0.10	36.29	0.15	30.39	0.12	9.07	0.03	9.80	0.04	36.66	0.15
Ground nut straw	2.12	0	37.29	0.15	0.80	0	66.04	0.26	44.99	0.18	70.43	0.28

3.1 Pathways to bioethanol production

3.1.1 Thermochemical Pathways

Thermochemical pathways deal with the use of thermal methods to pretreat the lignocellulosic biomass before further processing. The methods involved in this process include; combustion, pyrolysis, and gasification.

- (a) The lignocellulosic biomass combustion method deals with the burning of the biomass in the presence of oxygen. It is an old method of biomass conversion used mainly to generate heat for power plants and power generation. This method is rarely used for 1biofuel production.
- (b) Gasification is a fast-evolving method for producing biofuels. This method involves the biomass undergoing partial oxidation to convert it to a mixture of gases comprising mainly carbon (iv) oxide and hydrogen also called syngas. The syngas can be processed further by a variety of industrial processes or chemical reactions involving catalysis to yield a variety of gaseous and liquid biofuels notably, ethanol, methanol, fisher Tropsh (FT) liquids (gasoline/jet fuel/diesel), and dimethyl ether (DME), etc.

3.1.2 Biochemical biofuels pathways

The biochemical path for biofuel production makes use of the fermentation process which involves the conversion of sugars, such as glucose, fructose, and sucrose in the biomass into alcohols (bioethanol) and finally separation and purification to obtain bioethanol of high purity.

Pretreatment: Lignocellulosic biomass is highly recalcitrant and therefore bioethanol production from them, unlike their starch feedstock entails more vigorous pre-treatment before saccharification and fermentation to make the accessibility of cellulose to enzymes during enzymatic hydrolysis easier. Dissolved catalysts, mainly acids, and bases are frequently used during the pretreatment of lignocellulosic biomass. The effectiveness of the catalyst used coupled with pretreatment conditions determines the yield of bioethanol as well as the economics of the process. Other technologies such as hot water, steam pretreatment, etc. are currently being vigorously employed to breakdown the structure of the biomass.

An effective pretreatment must exhibit the following features:

- (i) Disintegrate the structure of the biomass which is usually a crystalline matrix composed of cellulose, hemicelluloses, and lignin to allow the enzymes to act on each of them.
- (ii) Non- production of noxious wastes or enzyme inhibitors or microbes from the fermentation process.

- (iii) Reduction in the wiping out or loss of the cellulose and hemicelluloses which are the main components for fermentation.
- (iv) Reduction in the production costs to make it economical by reducing the cost of raw materials, energy inputs, reactors, chemicals used, etc.
- (v) Be friendly to the environment.

Some of the techniques utilized in the pretreatment of lignocellulosic materials are as described below:

Physical and Thermal methods

(i) Mechanical Methods:

Lignocellulosic biomass feedstock requires to be mechanically crushed or torn up before further processing. Comminution and extrusion are the most commonly used mechanical methods. Comminuting the lignocellulosic biomass through disintegration, flaking, crushing, or grinding is an important step in the pretreatment process. The extrusion process involves making the feedstock pass through the course of heating, mixing, and shearing, leading to physical and chemical changes (Chinnadurai, Muthukumarappan, and Julson 2008, Kumar 2013). The main purpose of the mechanical method is the reduction in the particle size and crystallinity of the lignocellulosic material to increase the surface area and the degree of polymerization to be reduced as well. However, this process is not economically viable on an industrial scale and particularly as the only pretreatment method owing to the high-energy requirement.

(ii) Steam Explosion Method:

This is a Physico-chemical lignocellulosic biomass pretreatment method that is gradually gaining appreciable recognition in recent times.

This technique for pretreatment is highly efficient and is done by compressing the lignocellulosic biomass with steam and then followed by explosive decompression. It involves the lignocellulosic material being fused in high-pressure steam of about 709.3 – 5066 kPa at a temperature of 160 – 190°C, followed by a sharp reduction of pressure, resulting in the rupture of the lignocellulosic material (Kumar 2013). This results in a marshy solid material with a disordered lignocellulosic compound (cellulignin) and a liquid phase that can be removed by an explosion. The resulting material is composed mainly of xylose, xylooligosaccharides, uronic, and acetic acid. Partial hydrolysis of hemicelluloses takes place, particularly of highly acetylated xylanase owing to the acid characteristics of the mixture. The process is therefore also called ‘autohydrolysis’ (Mosier et al. 2005).

(iii) Liquid Hot Water (LHW) Pretreatment

This process makes use of hot water that has been pressurized at a pressure of about 5 Mpa and temperature range of 170 – 230 °C for a couple of minutes and then proceeded by decompression to atmospheric pressure. This process will reduce the concentration of solubilized hemicellulose and lignin, however, the major drawback of this method is that the water and energy demand is high (Bobleter and Concin 1979). In another work, the liquid hot water pretreatment process, also called thermohydrolysis, involved the showering of the lignocellulosic biomass with hot water at high pressure, with a temperature of about 220 °C for close to 2 minutes. It should however be noted that as compared to the steam – explosion process, the efficiencies of this method of biomass pretreatment are low (Kumar 2013).

Chemical Methods

Several chemical pretreatment methods aimed at the separation of the hemicelluloses have been studied. They include;

(i) Alkaline Pretreatment:

This process is commonly used to improve the digestibility of lignocellulosic biomass. This process was initially used in the paper and pulp industry for the pulping processes to produce a paper that has long fiber owing to its high lignin content. Conditions usually used in the alkaline pretreatment method are; The concentration of sodium hydroxide amounting to 8 – 12 % of the dry lignocellulosic biomass to be pretreated, duration of treatment amounting to 30 – 60 minutes, and temperature of the pretreatment around 80 – 120 °C (Wyman et al. 2005).

(ii) Alkaline Peroxide Pretreatment:

This pretreatment method is carried out with the use of hydrogen peroxide (H_2O_2). The lignin isolation (delignification) from the closely knitted structure of the lignocellulosic biomass using hydrogen peroxide is dependent on the pH of the feedstock and the dissociation occurs at a pH of about 11.5. Highly reactive radicals are formed from the dissociation and these radicals solubilize the lignin and the lignin is then oxidized. In some instances, the alkaline peroxide pretreatment is carried out in two phases namely: phase one makes use of sodium hydroxide and phase two make makes use of a mixture of sodium hydroxide and hydrogen peroxide. Lignin isolation through oxidation with hydrogen peroxide takes place at temperatures ranging from 25 to 40 °C. A major advantage of this pretreatment method is that the wastes (residues) generated does not constitute majorly to environmental pollution (Sun and Cheng 2002)

(iii) Acid Pretreatment methods:

The lignocellulosic biomass acidity is one of the reasons for originating a proficient pretreatment method. The acid pretreatment method has the advantages of speedy rate of reaction and the minimal acid being consumed during the reaction coupled with its low cost as against the alkali pretreatment processes. The major drawbacks of this pretreatment method are; corrosivity and inhibitor formation. The concentration of the acid is 0.1 – 5 %, the temperature is 110 – 220 °C, and the time of exposition 10 – 180 min. Results from different investigations show that multiple stages of acid pretreatment give greater efficiency and the consumption of cellulases is greatly reduced during the enzymatic hydrolysis phase (Hamelinck, Van Hooijdonk, and Faaij 2005). The lignin structure is selectively broken with the aid of peracetic acid, which is a highly oxidizing agent. The peracetic acid acts on the aromatic ring of the lignin, opening it up to form dicarboxylic acids and their lactones (Sun and Cheng 2002, Ogier et al. 1999).

(iv) Organosolv pretreatment

The organosolv pretreatment method involves the use of organic solvents like propanone, methanol, ethane-1, 2-diol, etc. for the pretreatment process. This process can be carried out in presence of a catalyst or without the use of a catalyst (Mesa et al. 2010). Organic acids, inorganic acids, and bases can be used as a catalyst (Zhao, Cheng, and Liu 2009).

For lignocellulosic biomass with high lignin content, this pretreatment method is very efficient because it can easily break the core lignin and hemicellulose bonds and pure lignin can be obtained as one of the by products of the process. Some of the disadvantages of this method include; low boiling point of organic solvent, combustibility, and volatility of the solvents (Sun and Chen 2008). A major attraction of this method is that solvents used can be recycled to reduce operating costs.

Biological methods

The biological or microbial pre-treatment method, unlike the chemical and physical pre-treatment methods, requires no chemical. It is an environmentally friendly method of using microorganisms like fungi, to break down lignocellulosic biomass into compounds that become easily accessible for hydrolysis and subsequent fermentation into bioethanol (Singh et al. 2008). In biological pre-treatment, the kind of fungi used includes white-, brown-, and soft-rot fungi. All these fungi isolate lignin and boost the enzymatic hydrolysis of lignocellulosic biomass (Shi et al. 2009).

Phanerochaete chrysosporium has the highest efficiency among all the species of white-rot fungi known to date due to its fast rate of growth and lignin biodegradation abilities (Chen et al. 1995). Despite the advantages, biological pre-treatment also has some disadvantages, which affects its well-known use as a

biomass pre-treatment method. These disadvantages include extended process time, huge space requirement, and microorganism growth monitoring (Wyman et al. 2005).

Hydrolysis and Fermentation

The cellulose and hemicellulose constituents of the pretreated lignocellulosic biomass will be hydrolyzed to simple sugars, namely, monosaccharides using acids or enzymes.

Ethanol is then produced by fermentation using yeast or bacteria as a catalyst. The produced ethanol will then be made to go through the process of normal atmospheric distillation and other complex purification systems like pervaporation and reverse osmosis which are novel separation processes developed from intensive research (Kumar 2013).

3.2 Challenges of lignocellulosic biomass conversion to bioethanol

Lignocellulosic biomass has three key components namely: cellulose, hemicellulose, and lignin.

(i) Cellulose (15 – 55 %, w/w)

Cellulose is one of the most prominent biological polymers on the surface of the earth. It is a homopolymer containing glucose of the type β -1,4 linking and is the main component of the plant cell wall in budding cells. Once the cellulose section is detached, it can then easily be hydrolyzed by treating it with enzymes or by using microorganisms.

(ii) Hemicelluloses (25 - 85 %, w/w).

Hemicelluloses constitute a category of heteropolymers including various hexoses like D-glucose, D-galactose, and D-mannose and pentoses like L-arabinose and D-xylose. Its main constituent includes xylose linking compounds such as arabinose, glucose, mannose as well as other sugars with the aid of an acetyl chain (Chandel and Singh 2011).

It is noteworthy that Xylose comes after D-glucose in order of sugar abundance in nature. Only a few known microorganisms can, however, work on pentoses to enable them to undergo the fermentation process.

(iii) Lignin (10 – 35 % w/w):

Lignin is a large cross-linked mix-up of organic molecules that holds cellulose and hemicellulose together in a matrix form. They are aggregate, amorphous and a three- dimensional polymers with a phenyl propane structure (Nasidi et al. 2015). As a polymer, lignin also possesses three phenol alcohols namely; P-

coumaryl, sinapryl and coniferyl alcohols. The lignin through adequate pretreatment can be isolated from the lignocellulosic biomass but they are not easily broken down by microorganisms.

The main challenges encountered during the conversion of lignocellulosic biomass into ethanol are;

- (i) Naturally, lignocellulosic biomass is highly recalcitrant to breakdown to its constituent sugars as the lignin and hemicelluloses part of the molecule hinder the activity of cellulose enzymes by joining themselves to the structure of the enzyme and thus behaving like an inhibitor. This inhibiting property of the lignin and hemicellulose is usually achieved by the reduction in pore size of the molecule because cellulose, hemicellulose, and lignin are usually associated closely in a molecule. In most cases, cellulose is not found alone in a free ropy chain, as can be seen in other substrates, but is commonly present in a pack of fibrillar parts with a supramolecular structure consisting of crystalline and amorphous sections (Mansfield, Mooney, and Saddler 1999). The pretreatment step is therefore required to open up the structural makeup of the biomass to make it open for enzymes to hydrolyze the cellulose component effectively for significant yields at appreciable rates (Wyman 1994).
- (ii) The available surface area of the lignocellulosic material is small which results in hindering enzyme activity. Lignocellulosic materials are characterized by exterior and interior surface area. The amount and shape of the particles contained in the material are related to the exterior surface areas, while the capillary make-up of cellulose strands is associated with the interior surface area. Dry lignocellulosic fibers usually have a tiny size of about 15 to 40 μm which confers on them an appreciable exterior specific surface area. The interior surface area of dried lignocellulosic fibers is however lesser than the exterior surface area. Lignocellulosic materials can be made to have larger internal surface areas with the addition of water or other polar solvents. When these fibers are dried, the drying can cause an irreversible breakdown and lessening of the capillary, thereby causing a reduction in the available surface area (Taherzadeh and Karimi 2008).
- (iii) The excessive cost of the enzymes required to degrade the polysaccharides.

3.3. Fuel properties of bioethanol

The fuel properties of bioethanol as compared to that of unleaded regular gasoline are as shown in Table 1.9. From the data shown in the table, the properties of bioethanol are closely related to that of gasoline and so can be used as a suitable alternative and as a good blending component of gasoline.

Table 1. 9: Fuel properties of ethanol and gasoline

Property	Ethanol	Unleaded regular gasoline
Density, Kg/m ³ @298K	790	720 - 780
Air/fuel stoichiometric ratio		
Mole basis	14.29	57.28
Mass basis	9.02	14.6
Higher heating value, KJ/Kg	26 780	41 800 – 44 000
Lower heating value, KJ/Kg	21 156	31 350 – 33 000
Research octane number (RON)	106	91 - 93
Motor octane number (MON)	89	82 - 84
(RON + MON)/2	98	88
Blending RON	114 – 141	-
Blending MON	86 -97	-
(Blending RON + MON)/2	115	-
Atmospheric boiling point, K	351.6	300 - 498
Heat of vaporization, KJ/Kg	839	377 - 502
Flash point., K	285	< 233
Ignition pt.,	697	553 - 702
Reid vapor pressure, kPa		
Pure component	15.85	48.3 + 96.5
Blending	82.7 – 186	55.1 – 103.4
Water solubility, weight %		
Fuel in water	100	negligible
Water in fuel	100	negligible
Water azeotrope (atm b.p.), K	351.4	-
Water in azeotrope, wt %	4.4	-

4.0 Global perspectives in bioethanol production

The hydrolysis process, which has not been fully established, is the method for converting lignocellulosic materials to ethanol. The utilization of lignocellulosic materials as a sugar source for ethanol production particularly in the United States and Brazil, is quickly developing. Bioethanol has been used in Brazil since the 1930s, when ethanol was initially blended into gasoline to provide a "sink" for excess sugar production.

The Brazilian government established Proalcool in 1975, an innovative attempt to promote ethanol as a large-scale gasoline alternative. In the late 1980s, ethanol was used in nearly all new passenger cars. The country invested heavily in R&D to convert the Otto engine to run on ethanol, gaining expertise and technical know-how in its manufacture, delivery, and use. From 3,900 liters per hectare in 1980 to roughly 5,600 liters per hectare in 2001, productivity has improved significantly.

Bioethanol has been used as a fuel in the United States around since 1908. Since the oil supply disruptions in the 1980s, production has skyrocketed. With the help of federal and state tax breaks, ethanol production increased from 665 million liters in 1980 to 7 billion liters in 2000.

If the oxygenating ingredient methyl tertiary butyl ether (MTBE) is outlawed, demand for ethanol may rise even more. About a fourth of global MTBE output is destined for California, which has already declared steps to phase it out.

The Clean Air Act Amendments (CAAA) were signed by the President of the United States in 1990, restricting the use of reformulated, oxygenated gasoline, primarily in highly polluted areas and especially during the winter months. According to this document on the worldwide use of bioethanol 513, a set percentage of oxygenated fuels should come from sustainable sources, with ethanol being the best option (Cortez et al., 2003).

5.0 Conclusion

Bioethanol has proven to be a suitable alternative to petrol because it can be readily and cheaply produced from carbohydrate-rich food crops and recently from the wastes generated from processing the food crops. Bioethanol for use as fuel is better produced from biomass because the food supply balance will not be stressed. The following biomass are available for processing to bioethanol in Nigeria; cassava peels, sugarcane bagasse, corn cobs, mango peels, sorghum straw, and rice husks. Conversion of these biomass to ethanol apart from helping to dispose of the wastes from processing the food crops, from which they are obtained, will also create wealth from such wastes. The main components of biomass are cellulose, hemicellulose, and lignin. As the lignocellulosic biomass is highly recalcitrant, ethanol production from them requires more vigorous pretreatment before fermentation to increase the accessibility of cellulose to enzymes during enzymatic hydrolysis. This is a major challenge facing bioethanol production from biomass, but with further efforts through extensive research, all the challenges can be surmounted.

Lignocellulosic bioethanol production potential from agricultural residues in Nigeria was 7.556×10^9 litres per annum with about 62 % of this coming from process residues. It can therefore be concluded that Nigeria has enough agricultural residues to comfortably meet its bioethanol-fuel blending demand.

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Chapter THREE

Paper 2, ECSNLBBP

Experimental characterization of selected Nigerian lignocellulosic biomass in Bioethanol production

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PAPER 2

Experimental characterization of selected Nigerian lignocellulosic biomass in Bioethanol production

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ABSTRACT

Experimental characterization of five biomass wastes: corn cobs, cassava peels, rice husks, white yam peels, and sugar cane bagasse based on proximate analysis and x-ray diffraction (XRD) analysis has been reported in this study due to their huge inherent potential for bio-ethanol production. X-ray diffraction indicated the presence of cellulose and carbon in the biomasses. The thermo-gravimetric analysis showed that the cellulose composition of the five biomass are quite close, with sugar cane bagasse having the highest content of 39.8 weight % and cassava peels biomass presented the lowest content of 25.8 weight %. For each of the biomasses presented in this study, their cellulose content conforms with reported values from previous work on them. The results demonstrate that the collected lignocellulosic biomasses are a potential substrate for energy and bioethanol production which can constitute a huge market as the biomasses are sustainably produced as wastes in Nigeria.

Keywords: Characterization, lignocellulosic biomasses, bioethanol, proximate analysis

1.0 INTRODUCTION

Nigeria is well known for its massive agricultural food crop production at certain seasons of the year. The agricultural food crops produced on a large scale in Nigeria annually include corn, rice, yam, cassava, sugar cane, millet, potatoes (sweet and Irish) among others. The processing of these food crops leaves a quantified large amount of wastes (biomasses) which can constitute a nuisance to the environment if not well managed (Limayem and Ricke 2012). Some examples of biomass residues left behind from the processing of agricultural food crops include the husk from rice de-husking, the cobs from corn, the peels from cassava, yam, potato, pawpaw, pineapple, mango, bagasse from sugar cane, and a host of others. Further processing of the agricultural wastes (biomasses) to produce bioethanol will not only save the environment from pollution but also turns the wastes into useful products that can generate huge revenue to the farmers if properly harnessed (Awoyale and Lokhat 2019).

In the past, lignocellulosic wastes were often disposed of by burning, which causes environmental pollution arising mainly from the particulates and CO₂ gas produced during the combustion processes (Levine 1996).

In addition to environmental pollution, burning these wastes contributes to soil nutrients such as nitrogen, phosphorus, and potassium depletion (Dobermann and Fairhurst 2002).

The biomasses, also known as lignocellulosic wastes, represent an important source of sugars, which can be fermented to produce bioethanol (Sánchez 2009). In recent times, lignocellulosic wastes are being considered as important feedstock in the production of competitive bioethanol in the open market due to their abundance and renewability (Mtui 2009). It is thus of great interest that these lignocellulosic wastes could be investigated for energy potential and converted to important end products such as bioethanol, biobutanol, and possibly as briquettes for fuel.

The major composition of lignocellulosic feedstock includes; cellulose, hemicellulose, and a small amount of lignin (Oberoi et al. 2010). The plant cell wall structure is composed mainly of cellulose which gives it mechanical vigor and chemical stability. Cellulose is the form in which the solar energy absorbed by the process of photosynthesis is stored in the plant. Hemicellulose is a copolymer of various C5 and C6 sugars existing in the plant cell wall. Lignin is a polymeric compound comprising aromatics and is formed through a biosynthetic process. It constitutes the protective covering for the cell wall of plants. Aside from these three chemical compounds contained in the lignocellulosic biomass, other compounds contained therein include water, a small number of proteins, ash, organic acids, and minerals. Cellulose has been reported to be the organic compound of the highest abundance on the earth's surface (Harmsen et al. 2010). Cellulose and hemicellulose provide the sugar needed for the bioconversion to bioethanol (Malherbe and Cloete 2002). Lignin is a phenolic polymer, which is a major feedstock for industrial processes, for example, adhesive resin and lignin gels (Pizzi and Salvadó 2007). The composition of different biomass differs from one variety to another, also the production location and process conditions affect biomass composition, and ultimately affect the processing approach (Singh et al. 2009).

The characterization of the different lignocellulosic biomass for biofuel production involves a range of methods and analytical techniques to obtain the important parameters which can be used to express the composition of the solid fraction of the biomass and the product obtained from them. The comprehensive and correct characterization of lignocellulosic biomass feedstock, intermediate products, and final products is a fundamental requirement for any process involving the conversion of biomass to biofuel.

Precise results from the proximate analysis of the lignocellulosic biomass feedstock make the techno-economic analysis of the process easier to carry out (Sluiter et al. 2010). Aside from three main components of biomasses namely cellulose, hemicellulose, and lignin content, other parameters measured in the proximate analysis include; moisture content, ash content, dry matter, crude protein, crude fat content, pH among others. The three main components of the biomasses can be determined using the thermogravimetric

analyzer (TGA), while the other parameters can be determined using the standard methods prescribed by the Association of Official Analytical Chemists (AOAC), Analytical analysis that can be carried out on the biomass include X-ray diffraction analysis (XRD), which is used to identify any crystallographic structures present in the biomass.

Pointner et al. (2014) worked on the composition of corncobs as a substrate for the fermentation of biofuels. Their work involved taking ten species of corncobs and characterizing them to see if the different variety of corn has varying compositions. It was observed from the work that no significant differences exist in the composition of different varieties of corn and the result obtained shows cellulose to be $38.8\% \pm 2.5\%$, hemicellulose: $44.4\% \pm 5.2\%$, lignin: $11.9\% \pm 2.3\%$ in the dry matter. Larsen et al. (2008) reported the integrated biomass utilization system (IBUS), which is a process developed by Inbicon A/S for the conversion of lignocellulosic waste biomass to bioethanol on a large scale. The IBUS plant features new continuous and energy-efficient technology developed for pretreatment and liquefaction of lignocellulosic biomass. The process has been used for years with promising results.

In the present study, five agricultural biomass wastes commonly generated annually in Nigeria have been selected for characterization in bioethanol production to provide the desired information on their potential bioethanol production capabilities. The results obtained from the characterization of each of the biomasses analyzed were compared and critically analyzed to give information for future researchers, as no previous single research work has been able to give comprehensive information on the comparison of the characterization of the selected biomasses in Nigeria.

In the light of the foregoing, proximate analysis using the standard methods of Association of Official Analytical Chemists (AOAC), chemo-physical properties, and thermo-gravimetric analysis (TGA), as well as XRD analysis, has been carried out on the five selected biomasses with prospective attraction for lignocellulosic bioethanol production to establish if there are any considerable dissimilarities in morphological and chemical properties in terms of composition between the selected biomasses. The lignocellulosic biomass being considered in this work include corn cobs, cassava peels, rice husks, sugar cane bagasse, and yam peels.

2.0 Materials and Methods

2.1 Biomass sampling

In the present study, corn cobs (*Zea mays*), cassava peels (*Manihot esculenta*), rice husks (*Oryza sativa*), white yam peels (*Dioscorea rotundata*), and sugar cane bagasse (*Saccharum officinarum*) as residue from crops processing, are used as biomass sources. Figure 2.1 shows the processed waste biomass presented in this study.



Figure 2.1: Biomass samples (sugar cane bagasse, rice husks, yam peels biomass, corn cobs biomass and cassava peels biomass)

In Nigeria, corn is produced on a large scale during the rainy season between April and October. It is therefore common to see women roasting and boiling the corn along the roadside in most Nigerian communities thereby generating a huge mass of left-over corn cobs. All the biomasses used in this work except rice husks have been collected from different local communities in Delta State of Nigeria. The rice husks were collected from a rice mill in Abredang-Abbayong, Cross River State of Nigeria. The collected corn cobs were sun-dried for fourteen days and then mechanically crushed in a grinding mill to powdery form before being sieved in a sieve No 350 with an aperture size of 0.045 mm. The cassava peels which were obtained from a local ‘garri’ (cassava flakes) processing factory were washed to remove sand and then sun-dried for ten days before crushing to powdery form and sieved using sieve No. 350. The rice husks, sugarcane bagasse, and yam peels collected were also sun-dried for three days before milling and sieving to uniform particle size in sieve No. 350. The five milled and sieved biomass samples were then stored in different plastic jars at ambient temperature.

2.2. Proximate analysis

2.2.1. Moisture content determination

The moisture content of the biomass samples was determined using the standard procedure described in AOAC 930.15. Two grams of each of the biomass samples were weighed into different aluminum dishes with a cover and then dried in an oven at 135 ± 2 °C for 2 hours. The dish was covered before removal from the oven and then transported to a desiccator and then cooled to ambient temperature before taking the final mass.

2.2.2. Crude Protein determination

The crude protein content of the biomass samples was determined using the standard procedure described in AOAC 997.09. The crude protein content in the samples was determined through the determination of the total nitrogen composition using the combustion method. Through the burning process conducted at approximately 900 °C, the nitrogen content of the samples was converted to NO_3 , which is further reduced by the copper to form nitrogen gas. The nitrogen gas was then measured by passing the gas via a column that has a thermal conductivity detector (TCD) at the end. The TCD was earlier calibrated by analyzing a pure material with a known nitrogen concentration, and ethylenediaminetetraacetic acid (EDTA) which equals 9.59 % nitrogen was used for this purpose. Hence, the signal from the thermal conductivity detector can be converted into a nitrogen content. The concentration of nitrogen in the sample was then converted to protein content. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) was used for the conversion.

2.2.3 Ash content determination

The ash content of the biomass samples was determined using the standard procedure described in AOAC 942.05. A clean and empty crucible was positioned in a muffle furnace at 600 °C for one hour after which it is then cooled in a desiccator. It is then weighed empty and the weight assigned 'W'. Five grams of each of the samples were taken in the crucible and assigned 'W2'. The sample was then ignited with the aid of a burner and a blowpipe, and allowed to char. The crucible was then placed in a muffle furnace at 550 °C for about two to four hours. Ash of gray-white color appears, and this suggests that all the organic matter in the sample has been completely oxidized. The crucible is then allowed to cool and then reweighed and assigned 'W3'. The percentage ash was then calculated.

$$\text{Difference in weight of Ash} = W_3 - W_2 \quad (1)$$

$$\% \text{ Ash} = \frac{\text{difference in weight of Ash}}{\text{weight of sample}} \times 100\% \quad (2)$$

2.2.4 Fat content determination

The fat content of the biomass samples was determined using the standard procedure described in AOAC 983.23 (chloroform-methanol extraction method).

About 10 g of the air-dried corncobs were ground in a mill. 5 g of the groundmass was then weighed and transferred into a round-bottomed flask. Chloroform-methanol was then used to extract the fat and gravimetric analysis was used to measure the extracted fat.

2.2.5 Dry matter determination

Biomass is normally defined based on its dry matter, which is usually the weight of plant material after extraction of the moisture content. Different plant species have varying moisture content during the year depending on the stage of growth, the form of growth (herbaceous, woody, or succulent), the moisture level of the soil, and humidity.

Dry matter substance is determined by drying the sample in an oven at about 60 °C until the weight is constant.

2.2.6 Lignin hemicellulose and Cellulose content determination

The lignin, hemicellulose, and cellulose content of the biomasses were determined using thermogravimetric analysis (TGA). The thermogravimetric analysis uses thermal energy to effect changes in the physical and chemical composition of materials and thereby measuring a change in mass concerning temperature and time (Singh et al. 2009). The standard procedure for thermogravimetric analysis was followed in this work to determine the celluloses, hemicelluloses, and lignin content of the biomasses. The nitrogen was turned on and the flow was verified with the flowmeter. The ball valves behind the TGA were then opened. The pressure gauge was checked to ensure its functionality. The pressure gauge was then set to 50 psi before

the TGA instrument and computer were turned on. The TGA gas switching accessory device was also turned on and the operation switch was set to auto position. Thereafter, the TGA instrument was then run. The thermogram was then opened and analyzed for weight changes in the biomasses.

2.2.7 XRD analysis

X-ray powder diffraction (XRD) is a quick analytical technique mainly used to identify phases of biomass and can also supply data on cell components of the biomass. The biomass to be analyzed must be finely grounded, homogenized, and the mean bulk constitution is determined. This analysis is based on the practical intrusion of monochromatic X-rays and a biomass sample. Cathode ray tube generates the X-rays, which have been filtered for monochromatic radiation production. The X-rays are also collimated to converge and pointed at the biomass sample. Interaction of the incident rays with the biomass sample enables the production of positive interference and a diffracted ray when specifications satisfy Bragg's Law ($n\lambda = 2d \sin \theta$). The diffracted X-rays are then identified, processed, and calculated. The biomass samples were scanned through a range of 2θ angles for all possible diffraction routes of the lattice to be achieved, as the powdered biomass is randomly oriented. As each mineral has a set of unique d-spacings, diffraction peaks had to be converted to d-spacing to identify the minerals present in the biomass. The d-spacings are then compared with a standard reference pattern.

3.0 Results and Discussion

Using standard analytical procedures, the proximate composition of corn cobs, cassava peels, rice husks, sugar cane bagasse, and yam peels were determined and presented in Table 2.1 and represented graphically with a bar chart as shown in Figure 2.2.

Table 2. 1: Proximate analysis of selected lignocellulosic biomasses

Biomass Samples	Moisture Content (wt %)	Cellulose Content (wt %)	Hemi Cellulose Content (wt %)	Lignin Content (wt %)	Dry Matter (wt %)	Crude Protein (wt %)	Ash Content (wt %)	Crude Fat Content (wt %)
Corn cobs	3.98	38.60	40.5	8.36	5.46	4.26	0.58	-
Cassava peels	15.13	25.8	11.6	4.29	30.5	1.86	0.33	0.67
Rice husks	8.19	38.6	24.9	18.6	5.46	1.96	0.36	0.30

Sugar cane bagasse	7.32	39.8	24.9	13.5	6.26	1.79	1.35	2.00
Yam peels	28.63	36.8	14.7	8.64	4.11	0.31	4.26	0.43

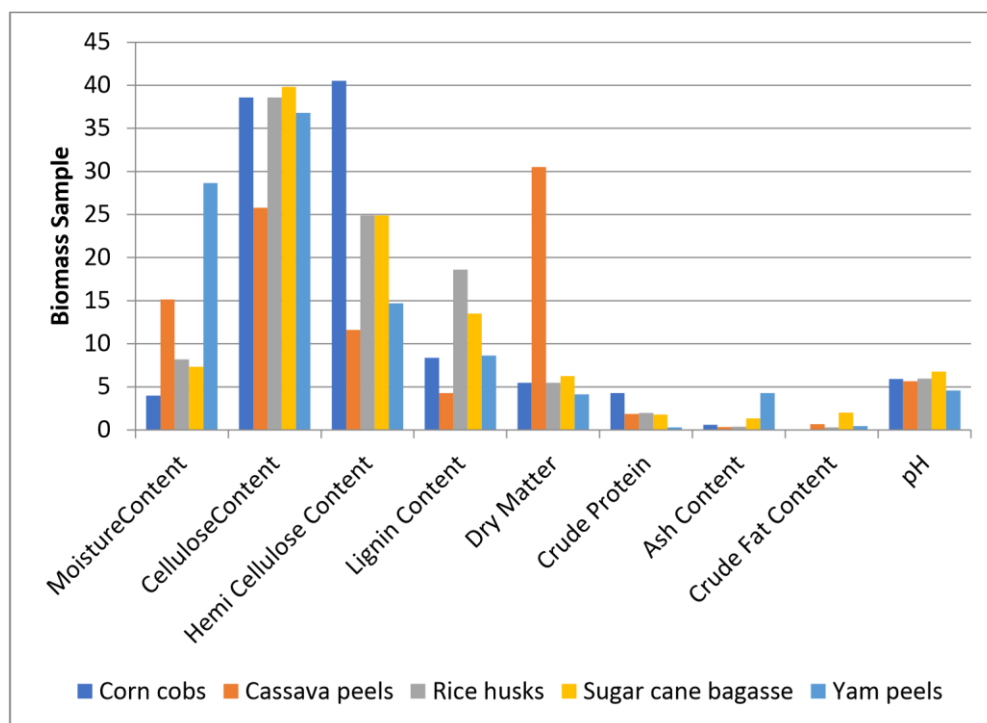


Figure 2. 2: Comparison of proximate analysis of the biomass samples

3.1 Proximate Analysis

The cellulose content of the biomass samples analyzed fell within the range of 36.8 to 38.60 wt % with exception of cassava peels that have a cellulose content of 25.8 wt % which might be due to the species of cassava used in this study. This result shows that the selected lignocellulosic biomasses are rich in cellulose, which is the basis for their conversion into bioethanol. The fact that they have high cellulose content, buttresses the fact that they are a good and ready source of raw material for bioethanol production. The result also conforms with results from previous works by other researchers. The reported values of cellulose content in literature are compared with the present outcome in Table 2.2

Table 2. 2: Comparison of cellulose content of biomasses with previous research

Lignocellulosic biomass	Present study cellulose content	Reported values	References
Corn cobs	38.60	50.50, 38.80, 34.45	[72], [73], [74]
Cassava peels	25.8	9.71, 14.00, 37.9	[26], [22], [75]
Rice husks	38.6	26.45, 28.60, 38.6	[76], [72], [11]
Sugar cane bagasse	39.8	34.80, 38.59, 35.2	[76], [77], [78]
Yam peels	36.8	18.02	[79]

Hemicelluloses are a class of heterogeneous polymers that occupies about 15-35 % of the entire plant material. It is next to cellulose in order of complexity in the structure of the components embedded in the plant's cell wall (Avanthi et al. 2017). Except for cassava peels biomass, all other biomass analyzed in this study falls within the range of the reported values. Corn cobs biomass has the highest hemicellulose content of 40.5 wt % while cassava peels biomass has the lowest hemicellulose content of 11.6 wt %. The value of hemicellulose content obtained in this study shows a significant variation from reported values. This could be due to the species of cassava used in this study. Table 2.3 shows the comparison of hemicellulose content with values obtained from previous studies.

Table 2. 3: Comparison of hemicellulose content with previous research

Lignocellulosic biomass	Present study Hemicellulose result content (wt %)	Reported values (wt %)	References
Corn cobs	40.5	31.0, 44.4	(Wannapeera et al. 2008), (Pointner et al. 2014)
Cassava peels	11.6	32.36, 27.00, 37.00	(Nanssou et al. 2016), (Adekunle et al. 2016), (Aripin et al. 2013)
Rice husks	24.9	27.29, 28.6, 19.7	(Quintero et al. 2013), (Wannapeera et al. 2008), (Binod et al. 2010)
Sugar cane bagasse	24.9	28.96, 27.89, 24.5	Quintero et al. 2013), (Guilherme et al. 2015), (Rezende et al. 2011)
Yam peels	14.7	20.02	(Mithra and Padmaja 2017)

The lignin content of the five biomass samples analyzed in this study shows no significant deviation from reported values from previous works. Rice husks biomass has the highest lignin value of 18.6 wt % in this study and has the highest value in the reported values from previous works, while cassava peels biomass has the lowest lignin value in this study. Table 2. 4 shows the comparison of lignin content with values obtained from previous studies. The main function of lignin in the plant cell wall is for structural support, impenetrability, and resistance against microbial attack and oxidative stress. It is also an amorphous heteropolymer that is insoluble in water. These factors make the degradation of lignin a very arduous task (Hendriks and Zeeman 2009). Pretreatment of the biomass is therefore of utmost importance to break the lignin barrier in the plant cell wall and make the cellulose and hemicellulose available during fermentation.

Table 2. 4: Comparison of lignin content with reported outcomes from literature

Lignocellulosic biomass	Present study Lignin content result	Reported values	References
Corn cobs	8.36	15.0, 11.9, 16.03	[72], [73], [74]
Cassava peels	4.29	16.89, 7.5	[26], [75]
Rice husks	18.6	28.03, 24.4	[76], [72]
Sugar cane bagasse	13.5	22.62, 17.79, 22.2	[76], [77], [78]
Yam peels	8.64	6.72	[79]

The ash content, as well as the crude protein contents of the biomass samples, analyzed in this work, showed a similar pattern with those reported from previous work. Tables 2.5 and 2.6 show a comparison of ash content and protein content with those reported in previous works respectively. Ash is referred to as the total content of dust and inorganic substances in biomasses and it is estimated to be around 10 % in lignocellulose biomass (He et al. 2014). In biomass pretreatment before fermentation, high ash content is a drawback as the ash particles absorb more steam, water, dilute acid solution, or solvent than the relatively larger lignocellulosic fibers. This, therefore, reduces the efficiency of the pretreatment process. With exception of yam peels biomass, the result of the ash content of the biomasses presented in this study is lower than that of those presented in previous studies. This further goes to confirm that the biomasses presented in this study are good feedstock for bioethanol production. The protein content of the biomasses presented in this study conforms with those from other previous researchers.

Table 2. 5: Comparison of ash content with reported outcomes from literature

Lignocellulosic biomass	Present study Ash content result	Reported values	References
Corn cobs	0.58	0.9	[72]
Cassava peels	0.33	11.38, 7.00, 4.5, 3.23	[26], [22], [75], [80]

Rice husks	0.36	14.89, 17.9, 20.26	[76], [72], [11]
Sugar cane bagasse	1.35	4.17, 8.80, 20.9	[76], [77], [78]
Yam peels	4.26	3.29, 1.40	[79], [81]

Table 2. 6: Comparison of protein content with reported outcomes from literature

Lignocellulosic biomass	Present study Protein content result	Reported values	References
Corn cobs	4.26		
Cassava peels	1.86	3.70, 3.50, 8.72	[26], [22], [80]
Rice husks	1.96	2.01	[76]
Sugar cane bagasse	1.79	1.45	[76]
Yam peels	0.31	0.087	[81]

The XRD diffractograms of biomass samples analyzed, are depicted in Figures 2.3 to 2.8. Except for yam peels biomass, the XRD patterns of all other biomasses in this study show a wide diffraction peak centered at $2\theta = 23^{\circ}$ which suggests their amorphous structure. From Figure 4, the XRD of all biomass samples measured shows the presence of native cellulose in high proportion. The presence of carbon is also confirmed in all the samples measured, confirming the ability of the bioethanol produced from these biomass samples as being fit to be used as fuel because of the high heating value of carbon. The presence of carbon is highly desirable in the lignocellulosic compounds being investigated because carbon is the main constituent of hydrocarbons which is the major component of fossil fuels, of which an alternative is being sought.

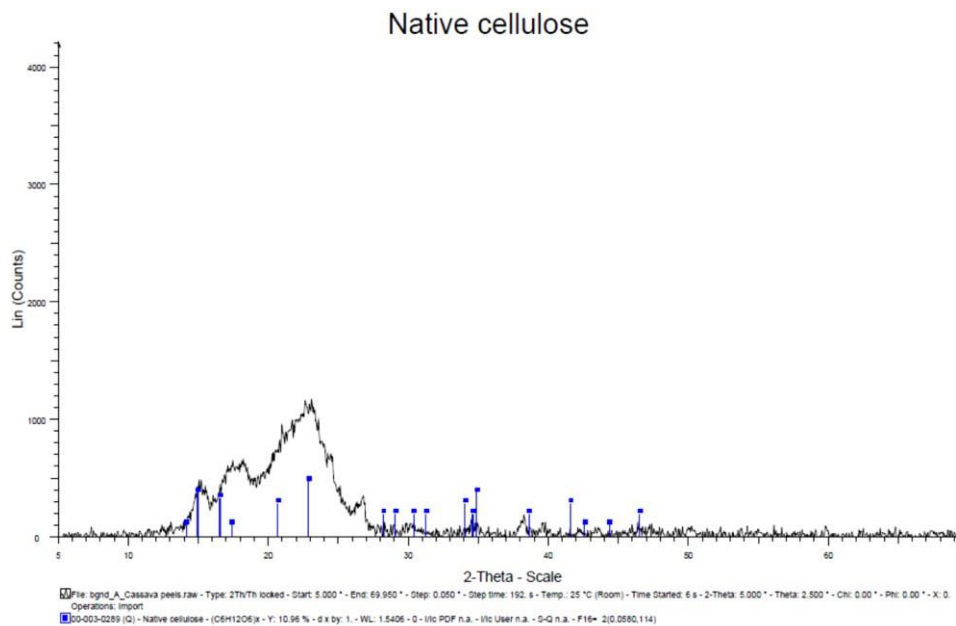


Figure 2. 3: XRD analysis of cassava peel biomass

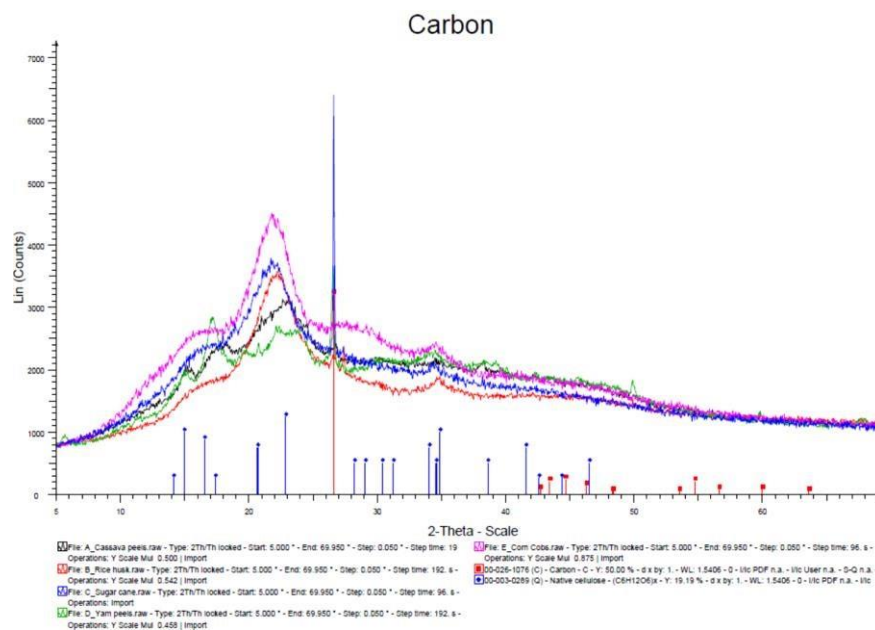


Figure 2. 4: XRD analysis of all biomasses studied as measured

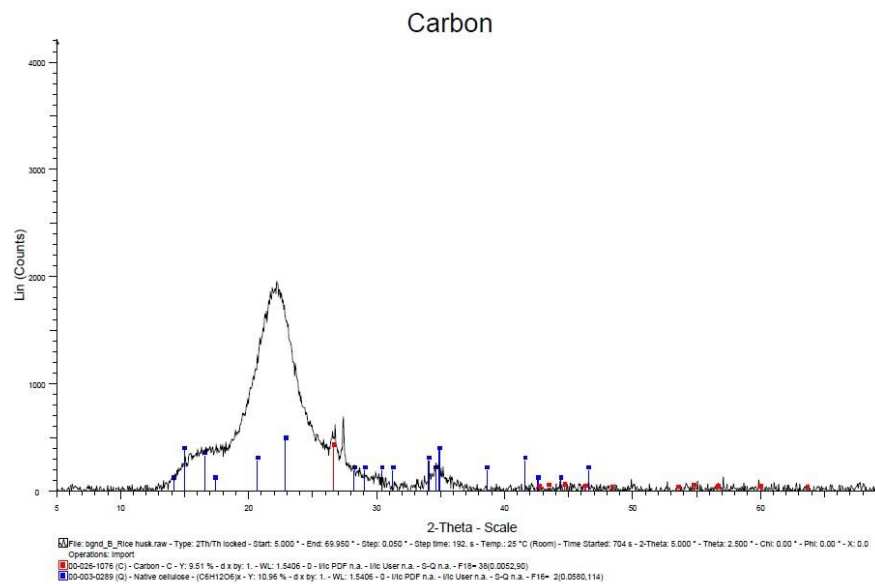


Figure 2. 5: XRD analysis of rice husk biomass

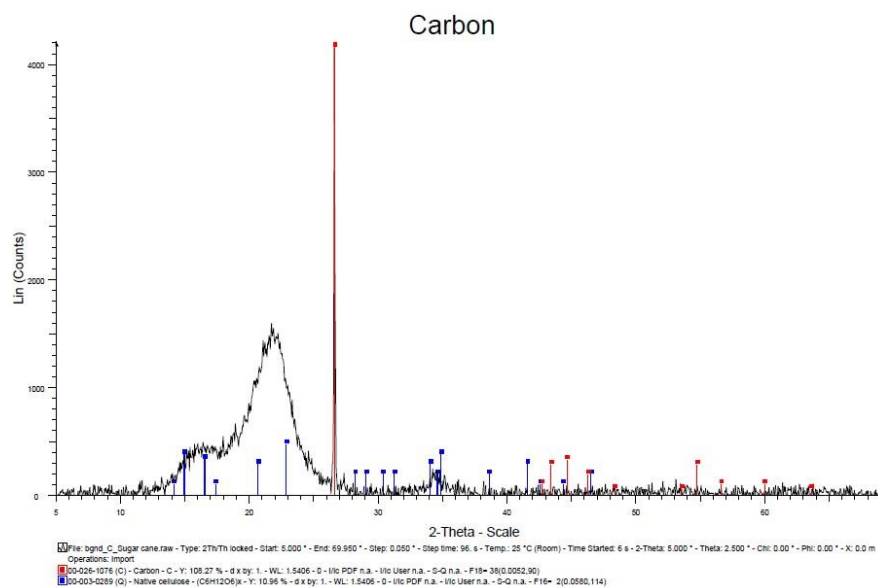


Figure 2. 6: XRD analysis of sugar cane biomass

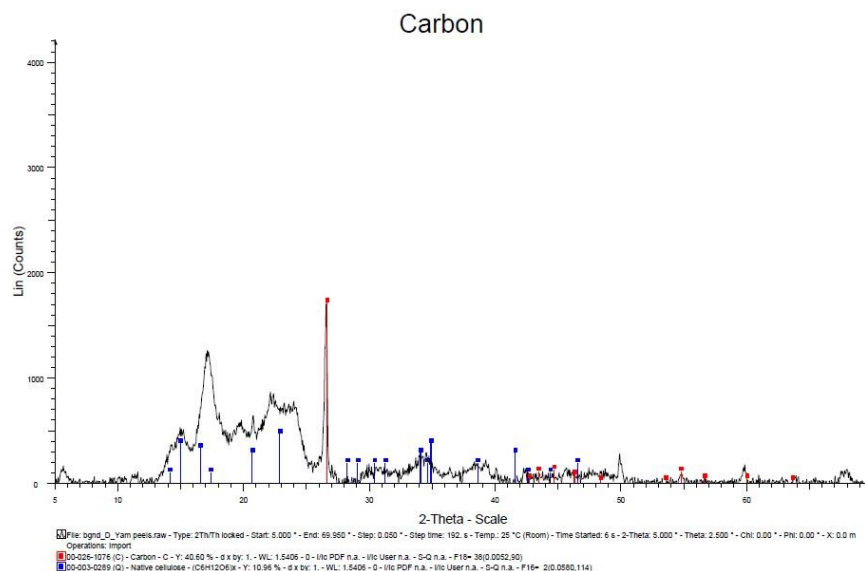


Figure 2. 7: XRD analysis of yam peel biomass

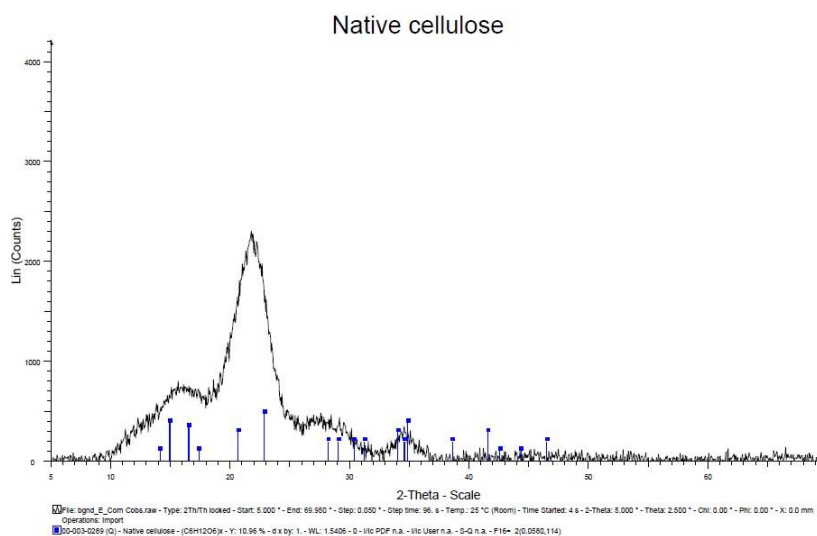


Figure 2. 8: XRD analysis of corn cobs biomass

3.2 Effect of composition of biomass on pretreatment and fermentation

As stated in the previous study (Awoyale and Lokhat 2019), lignocellulosic biomass is highly recalcitrant to being broken down to its constituent sugars as the lignin and hemicellulose component of the molecule hinder the activity of cellulose enzymes by joining themselves to the structure of the enzyme and thus behaving like an inhibitor. This inhibiting property of the lignin and hemicellulose is usually achieved by

a reduction in pore size of the molecule because cellulose, hemicellulose, and lignin are usually associated closely in a molecule. The results obtained in this study show that the lignin content is considerably lower than the cellulose and hemicellulose contents for all the biomasses analyzed.

The pretreatment methods currently being used include mechanical, thermal, chemical, and biological pretreatment. Mechanical pretreatment is usually the first step and can be combined with any one of the other three pretreatment methods. Mechanical pretreatment which usually involves milling, helps in crystallinity and particle size reduction, leading to an increase of available surface area and a reduction in the degree of polymerization of the biomasses (Hendriks and Zeeman 2009). Thermal pretreatment is a preferable combination with mechanical pretreatment. Steam explosion pretreatment is a good example of the thermal pretreatment method. It is a desirable method in terms of cost and effectiveness. Steam pretreatment involves putting the biomass in a large vessel and steam with temperatures up to 240 °C and medium pressure applied for a few minutes. The steam is released after a set time and the biomass is quickly cooled down. The main objective of a steam pretreatment is to solubilize the hemicellulose to make the cellulose better accessible for enzymatic hydrolysis thereby avoiding the formation of inhibitors. Since the cellulose content of all the biomass samples analyzed in this study is high, we can expect a good yield of bioethanol from the enzymatic hydrolysis (fermentation) of the biomasses after effective pretreatment. A combination of feedstocks (biomasses) can make the yield of bioethanol to be significantly improved.

4.0. Conclusion

The selected biomasses including sugar cane bagasse, corn cobs, rice husks, cassava peels, and yam peels were all obtained from different parts of Nigeria and were characterized for moisture content, cellulose content, hemicellulose content, lignin content, dry matter, crude protein, ash content and crude fat content using proximate analysis, TGA and XRD. The results obtained from all the analysis shows uniformity in the composition of the biomasses, with the TGA result also showing that cellulose, which is the most important part of the biomasses, has the highest weight percent in all the biomasses analyzed in this study. The cellulose content of the biomass samples analyzed fell within the range of 36.8 to 38.60 wt % with exception of cassava peels that have a lower cellulose content of 25.8 wt % which might be due to the species of cassava used in this study. From the XRD analysis also, the presence of carbon was confirmed in high proportion in the biomasses confirming that these biomass wastes can serve as effective precursors in the production of biofuels, and hence as replacement feedstock to fossil fuels. These results, therefore, buttress the fact that the biomasses presented in this study are good feedstock for bioethanol production.

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Chapter FOUR

Paper 3, EDEPSNLBBP

Experimental determination of the effects of pretreatment on selected Nigerian lignocellulosic biomass in Bioethanol production

Awoyale, Adeolu A. and, Lokhat, D.

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PAPER 3

Experimental determination of the effects of pretreatment on selected Nigerian lignocellulosic biomass in Bioethanol production

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ABSTRACT

In the present study, five lignocellulosic biomass namely, corn cobs (*Zea mays*), rice husks (*Oryza sativa*), cassava peels (*Manihot esculenta*), sugar cane bagasse (*Saccharum officinarum*), and white yam peels (*Dioscorea rotundata*) of two mesh sizes of 300 and 425 microns and a combination of some and all of the biomass were pretreated using combined hydrothermal and acid-based, combined hydrothermal and alkali-based and hydrothermal only processes. The raw and pretreated biomass were also characterized by Fourier transform infrared spectroscopy (FT-IR), Brunauer–Emmett–Teller (BET), X-Ray diffraction (XRD), and Scanning electron microscopy (SEM) to determine the effects of the various pretreatments on the biomass being studied. The cellulose values of the raw biomass range from 25.8 wt% for cassava peels biomass to 40.0 wt% for sugar cane bagasse. The values of the cellulose content increased slightly with the pretreatment, ranging from 33.2 to 43.8 wt%. The results of the analysis indicate that the hydrothermal and alkaline-based pretreatment shows more severity on the different biomass being studied as seen from the pore characteristics results of corn cobs + rice husks biomass, which also shows that the combination of feedstocks can effectively improve the properties of the biomass in the bioethanol production process. The FTIR analysis also showed that the crystalline cellulose present in all the biomass was converted to the amorphous form after the pretreatment processes. The pore characteristics for mixed corn cobs and rice husks biomass have the highest specific surface area and pore volume of 1837 m²/g and 0.5570 cc/g respectively.

Keywords: Pretreatments, lignocellulosic biomass, Bioethanol, Hydrothermal, Characterization.

1.0 INTRODUCTION

The use of non-environmentally friendly fossil fuel as a source of energy has continued to pose serious challenges to the environment coupled with their non-renewability. Researchers worldwide have over has been seeking alternative sources of fuels for energy generation [1], [2]. In the recent past, fuel bioethanol used to be produced from carbohydrate-rich foodstuffs like cassava, yam, wheat, sugar cane molasses, rice, barley, guinea corn, etc. However, the production of bioethanol from food sources impacts negatively on the food chain for humans and animals. The processing of crops leaves a huge mass of wastes that were hitherto burnt off or disposed of indiscriminately, thereby causing environmental pollution [3]. The waste material left behind from processing the carbohydrate-rich crops is known as biomass. The biomass is rich

in lignin, cellulose, and hemicellulose and is, therefore, more commonly referred to as lignocellulosic biomass [4]. Bioethanol produced from lignocellulosic biomass has been established to be a good alternative to fuels produced from fossil fuels. Examples of lignocellulosic biomass from which bioethanol can be produced include corn cobs, rice husks, cassava peels, yam peels, mango peels, sorghum straw, pineapple peels, potatoes peels, pawpaw peels, sugar cane bagasse among others [5]. Significant research is ongoing globally to develop industrial processes for bioethanol production from lignocellulosic biomass [6]. The major constituents in the make-up of lignocellulosic biomass are lignin, cellulose, and hemicellulose. As shown in previous study [3], the cellulose content of most of the lignocellulosic biomass found in Nigeria is very high, thereby making them potential raw materials for bioethanol production. Lignocellulosic biomass by nature is extremely resistant to being disintegrated to its component sugars owing to the lignin and hemicellulose parts of the biomass molecule hindering the access of the enzyme to the cellulose by joining themselves to the enzyme's structure, thereby acting as an inhibitor. Cellulose, hemicellulose, and lignin are usually connected tightly in a macromolecular structure, thereby making the pore size of the molecules to be reduced, this reduction in the pore size enhances the inhibiting characteristics of the lignin and hemicellulose. Pretreatment or 'prehydrolysis' is the process through which the cellulose constituent is subjected to and made susceptible to enzymatic hydrolysis. Lignocellulosic biomass requires suitable and adequate pretreatment for the resultant hydrolysis to take place since they are recalcitrant. The efficacy of the pretreatment process influences both the up-stream choice of feedstock, cellulose, hemicellulose, and lignin components recovery efficiently, the chemical and morphological features of the ensuing cellulosic component, which consequently controls downstream hydrolysis and ultimately fermentation to bioethanol [7] Lignocellulosic biomass feedstocks are diverse; therefore, it is not easy to specify a general pretreatment process for all of them. Several pretreatment technologies have been proposed in recent times. The pretreatment method selected for pretreating the biomass has a significant effect on bioethanol production cost and yield [8]. Pretreatment technologies can be categorized into physical, chemical, biological, and physio-chemical or a combination depending on the diverse forces or resources expended in the pretreatment process [9]. The liquid hot water (LHW) pretreatment is a form of hydrothermal pretreatment which requires no rapid decompression and no catalyst or chemical addition. Temperature and pressure range of between 170 to 230°C and >5 Mpa respectively are used in LHW pretreatment [10]. The LHW pretreatment eliminates hemicellulose from lignocellulosic materials and exposing the cellulose thereby making it available for fermentation. After the pretreatment, the resulting slurry can then be sieved to acquire a solid rich in cellulose and another liquid portion with hemicellulose sugars [11]. Acid pretreatment is a form of chemical pretreatment that involves chemical hydrolyses which solubilizes hemicellulose and lignin, thereby making the cellulose more open for enzyme action during the fermentation process. The acid pretreatment can be carried out with concentrated or dilute acid, but the use

of concentrated acid comes with a drawback of the formation of impeding compounds such as furfural and phenolic acids, besides, concentrated acids are noxious, corrosive, and usually hazardous. The pretreatment should, therefore, be carried out in corrosion-resistant equipment. Dilute acid pretreatment is the mainly suitable for large scale bioethanol production. Various reactors like the plug flow, shrinking bed, batch, flow-through, among others have been developed for this process [12].

An alkaline pretreatment is also a form of chemical pretreatment whereby a base like potassium, sodium, ammonium, and calcium hydroxides at standard pressure and temperature is used to treat the biomass. This pretreatment method has the advantage of being more efficient in removing lignin from the biomass. The method also eliminates acetyl and uronic acid groups existing on hemicellulose and therefore boosts the accessibility of the enzyme that breaks down hemicellulose [13]. Alkali pretreatment can also be applied at low temperatures, pressure, and time. Sodium hydroxide is more effective than others [14]. A few researchers have also attempted to combine two pretreatment processes for substantially increasing the yield of reducing sugars such as the combination of alkaline pretreatment (lime) with the oxidative delignification process [15]. To assess the efficacies of the various pretreatment techniques in the preparation of the lignocellulosic biomass for the transformation of the enzymes, the results of a standard cellulase treatment on the pretreated biomass can be compared [16]. The severity factor is frequently employed to explain lignin reduction and xylan solubility [17] [18]. Pereira et al, 2016 [19] worked on the ‘physical-chemical – morphological characterization of the whole sugarcane lignocellulosic biomass used for second-generation (2G) ethanol production by spectroscopy and microscopy techniques. Their work entailed detailed analysis of the bagasse, straw, and tops of sugarcane through NMR, FTIR, XRD, and SEM. The result from their work shows that skeletal aromatic and methoxyl groups attributable to lignin structure are present in sugarcane lignocellulosic biomass. In the work of Zhang et al, 2014 [20], ‘XRD was used to study the interactions of cellulose in lignocellulosic biomass with ionic liquids’. The result obtained from the study shows that with an increment in pretreatment temperature using an ionic liquid, there is a drop-in crystallinity index of the biomass, a phenomenon ascribed to the swelling of the crystalline cellulose.

Modern pre-treatment methods can take above 40% of the entire production costs and is the most energy-consuming part of converting lignocellulosic biomass to biofuels. Furthermore, the pre-treatment processes involve the use of substantial amounts of corrosive reagents as well as heating methods which can gravely impact negatively on the environment. It is therefore of utmost importance to reduce the pre-treatment costs and the negative impact they might have on the environment. A novel appraisal means currently being utilized for conversion studies is the life cycle assessment (LCA), which helps in the identification and evaluation of the environmental performance and sustainability of different pre-treatment techniques. The

LCA measures and decode information over a product and process life cycle, in terms of their production, use, and end-of-life. Numerous researches have been done in the past using the LCA procedure to analyze the environmental impact of bioethanol production from various lignocellulosic feedstocks [21].

The main highlight of this work is to bring home the pre-treatment technologies available in the research space to Nigeria which is just coming up in renewable energy development with slight modifications to the existing processes. To this end, multiple biomass readily sourced from Nigeria was used for this research and a combination of the biomass feedstock also gave valuable information that can be utilized in the lignocellulosic bioethanol development of the country. Moreover, there are not many reports about one of the biomass in this study, which is yam peels, in literature. 96 % of the world's yam production is from West Africa and two-thirds of these are from Nigeria. The data supplied by this manuscript on yam peels biomass as a feedstock for bioethanol production will go a long way in providing a reference for future research in this area. The same goes for the combinations of feedstocks which is more representative of a commercial application where a single processing plant would obtain a variety of different agricultural residues as reported in this study. There are no significant studies reported on these hybrid feedstocks and the characteristics of the pre-treated materials. This study also elaborately compares the effects of the three pre-treatment processes adopted on several biomass.

2. EXPERIMENTAL

Materials and Biomass preparation. The rice husks in this study were sourced from a rice mill in Ekperi Etsako Central Local government Area of Edo State Nigeria, the corn cobs were obtained from Ogume, Ndokwa West Local Government Area of Delta State Nigeria, the cassava peels, yam peels, and sugar cane bagasse were all sourced from Effurun, Uvwie Local Government Area of Delta State Nigeria. All the sourced biomass were then sundried for about seven days and then taken to the grinding mill for grinding after which they were sieved into two particle sizes of 300 and 425 microns respectively. Analytical grade chemical reagents such as sodium hydroxide pellets, hydrogen peroxide, and tetraoxosulphate (VI) acid were used. 1500 g each of the yam peels biomass, cassava peels biomass, rice husks biomass, corn cobs biomass, and 1000 g of sugarcane bagasse biomass were measured and kept inside different vessels. To study the effects of biomass combinations, 300 g each of the 300 microns for all the five biomass was measured and mixed in a vessel. Also, 750 g each of 300 microns particle size cassava and yam peels biomass and corn cobs and rice husks biomass was measured and transferred into different vessels.

2.1 Pretreatment Methods

In this study, three pretreatment methods were adopted to pretreat the biomass for comparison purposes. The pretreatment methods used include combined hydrothermal and acid-based pretreatment, combined hydrothermal and alkaline-based pretreatment, and hydrothermal only pretreatment.

2.1.1 Combined Hydrothermal and Acid Pretreatment

The method used by Utama et al [100] was adopted in this work with slight modification. H_2SO_4 (98 % analytical grade JHD) with 98.08 g/mol molecular weight was used. 80 ml of the sulphuric acid was measured and transferred into a 2000 ml volumetric flask and distilled water was used to make up the volume to 2000 ml. 0.75 M solution of the sulphuric acid was thus obtained. The prepared biomass (individual and combinations) were then soaked with the prepared acid solution in batches and for each case, about 4 to 6 liters of distilled water was used alongside the 2 liters of the 0.75 M solution of the H_2SO_4 based on the absorbing capacity of the biomass. The mixture was then thoroughly mixed in the vessel before being transferred to a pressure pot and allowed to boil for about an hour. It was allowed to cool to ambient temperature before filtering and then kept inside sampling plastics. The filtrate from each batch was also stored separately.

2.1.2 Combined Hydrothermal and Alkaline Pretreatment

160 g of NaOH pellets were dissolved in a beaker and then moved to a 2000 ml volumetric flask containing 60 ml of H_2O_2 and then thoroughly mixed in the volumetric flask before making it up to the 2000 ml mark with distilled water. The same procedure as that of acid pretreatment was then followed on all the biomass.

2.1.3 Hydrothermal Pretreatment

Hydrothermal only Pretreatment was also done on all the biomass as described in the combined hydrothermal and acid pretreatment as control.

2.2 Characterization of the raw and pretreated biomass

Characterization of biomass is essential to establish its capability for bioethanol production [23]. The raw and pretreatment biomass samples were then subjected to characterization to establish the impacts of the different pretreatment methods on the biomass meant for fermentation for bioethanol production. The following physicochemical analysis was carried out on the raw and pretreated biomass: proximate analysis, ultimate analysis, FT-IR, XRD, BET, and SEM. The characterization was done at the multiuser laboratory of Ahmadu Bello University, Zaria Nigeria, and the Chemical Engineering Department laboratory of Federal University of Technology Minna, Nigeria

2.2.1 Proximate Analysis

The gross composition of the biomass pre and post pretreatment was determined using the proximate analysis to ascertain their moisture content, ash content, lignin content, cellulose content, and hemicellulose content.

2.2.1.1 Determination of Lignin Content

The acid detergent fiber (ADF) residue earlier obtained was immersed in chill sulphuric acid. The mixture was blended to a smooth paste to breakdown all the arms. The residue in the crucible was dehydrated for 24 hours at 100 °C and then allowed to cool to around ambient temperature. It was then weighed and labeled (W1). The crucible plus oven-dried residue was moved to a muffle furnace fixed at 550 °C to ash for three hours till a white greyish residue was obtained, cooled in a desiccator, and then weighed and labeled (W2). The lignin content was then calculated using the equation;

$$\text{Lignin content} = \frac{W1-W2}{\text{weight of sample}} \times 100\% \quad (1)$$

2.2.1.2 Determination of Holocellulose

A solution of 80 ml acetic acid and 1 g of sodium chloride were put into 2.5 g of extractive free sample (after extracting the sample) in a water bath hourly for six (6) hours in a process known as chlorinating. Subsequently, after six (6) hours of chlorinating, the samples were then allowed to stay for a while in a water bath to lower the temperature, and then the holocellulose was filtered using a Buchner funnel. The initial and final weight of the holocellulose were taken and the holocellulose content was calculated using the following formula:

$$\text{Holocellulos content} = \frac{W1-W2}{\text{weight of sample}} \times 100\% \quad (2)$$

Where: W1 – the weight of the sample before the process

W2 – the weight of the Sample after the process

2.2.1.3 Determination of Hemicellulose

2 g of holocellulose which had earlier been dried in the oven was transferred into a 25 ml glass beaker, afterwards, 10 ml of 17.5 % sodium hydroxide (NaOH) solution was mixed with the holocellulose, it was then made to stay in a water bath, a flat end glass rod was employed in stirring it for it to be soaked with the NaOH, after adding of the initial portion of 17.5% (NaOH) solution every five (5) minutes, additional 5 ml of NaOH solution was then introduced and thoroughly agitated using a glass rod. Sodium hydroxide

was continuously added until all the NaOH solution was used up. Thereafter, the mixture was made to stay in a water bath for thirty (30) minutes.

2.2.1.4 Determination of cellulose

The cellulose content of the biomass was determined using the formula:

$$\text{Holocellulose} = \text{Cellulose} + \text{Hemicellulose}$$

$$\text{Cellulose} = \text{Holocellulose} - \text{Hemicellulose}$$

2.2.1.5 Determination of moisture content

The moisture content of the biomass was gotten by the method of oven drying. This was done at a temperature of 103 ± 2 °C following ASTM D1037 (1991). The moisture content was thereafter determined by using the equation:

$$M = \frac{W_f - W_i}{W_1} \times 100\% \quad (3)$$

Where W_i – the initial mass of the sample

W_f – the final mass of the sample

2.2.1.6 Determination of the ash matter

The Ash matter in the biomass samples was determined by the method described in ASTM D2017 (1998). 1 g each of the samples was put in a pre-weighed crucible and was then burnt in a muffle furnace at 760 °C until ashing was completed after which the container was then moved into a desiccator to lower the temperature. Three replicates were made. The samples were then weighed after cooling. The ash matter was determined by using the following equation:

$$\text{Ash content (\%)} = \frac{W_2 - W_0}{W_1 - W_0} \times 100\% \quad (4)$$

Where: W_0 = Weight of the container

W_1 = weight of the container + biomass sample before burning

W_2 = weight of the container + biomass sample after burning

2.2.2 FT-IR, SEM, BET, and XRD characterization of the raw and pretreated samples

The virgin and pretreated biomass were also characterized by Fourier transform infrared spectroscopy (FT-IR), Brunauer–Emmett–Teller (BET), X-Ray diffraction (XRD), and Scanning electron microscopy (SEM).

2.2.2.1 Fourier transform infrared (FT- IR) spectroscopy

The main function of FT-IR spectroscopy is for the detection of the different functional groups present in the virgin and pretreated biomass [24]. The main result of FTIR assays mainly deals with the lignin content of the biomass [25]. For this work, Agilent Cary 630 FTIR Spectrometer was used to characterize the virgin and the pretreated biomass. The FTIR was analyzed using Agilent MicroLab PC software equipped with the equipment.

2.2.2.2 Scanning electron microscopy (SEM)

This is an analytical procedure that scans a sample with an electron beam to produce a magnified image for assessment. SEM analysis makes the sample structures to be assessed and their elemental make-up determined [103]. The Phenom ProX desktop SEM with a magnification range of 20 – 100,000x and element detection range of C – Am and acceleration voltage of 10 kV was used in this study.

2.2.2.3 Brunauer–Emmett–Teller (BET) Analysis

The specific surface area of a biomass sample and the pore size distribution can be measured with this analysis, which can be used to forecast the dissolution rate as this rate is proportional to the specific surface area and the surface area, in turn, can be used to forecast bioavailability. The Nova 4200e BET analyzer was used in this study. The BET analyzer used nitrogen as an analysis gas. The outgas time was 3 hours at a temperature of 250 °C. The pressure tolerance for the analysis was 0.100/0.100 (ads/des). The equilibrium time was 60/60 seconds and the equilibrium time out was 240/240 seconds. The analysis time was 111.8 minutes.

2.2.2.4 X-ray Diffraction (XRD) analysis

This is a swift analytical procedure used to detect phases of lignocellulosic biomass and can also provide information on the cell composition of the biomass. The lignocellulosic biomass meant for analysis should be in fine particulate form and thoroughly mixed before the mean bulk components are determined. This test is centered on the applied intrusion of monochromatic X-rays and a lignocellulosic biomass sample. The X-ray is generated by the Cathode ray tube, which has a monochromatic radiation production. The X-rays are also collimated to meet and directed at the lignocellulosic biomass sample. The incident rays interact with the lignocellulosic biomass sample to enable the creation of positive interference and a diffracted ray after conditions meet Bragg's law ($n\lambda = 2d \sin \theta$). The diffracted X-rays are then detected,

sorted out and calculations carried out. The lignocellulosic biomass samples, with a range of 2θ angles for all likely diffraction paths of the lattice to be achieved, as the grounded lignocellulosic biomass is unsystematically orientated. As a result of every mineral having a set of exclusive d-spacings, diffraction peaks were converted to d-spacing to spot the minerals existing in the biomass. A comparison of the d-spacings with a standard reference pattern was then carried out.

2.3 Life Cycle Assessment (LCA)

The LCA is equipped with the data collection section, which is meant for the identification and accounting of all the input and output of a process. In building and analyzing LCA models, thorough, holistic, and generally admitted stock data is needed for the materials and processes used. SimaPro has been a leading LCA software package that is used to extract considerable amounts of related data from diverse production handling reports, which includes chemical and food production facilities and brings in the data from a varied collection of accessible databanks. Ecoinvent v3 LCI Databank was also employed in this study. This is the most frequently consulted and referenced [27].

2.4 Economic Consideration of the bioethanol production process

Bioethanol production process economic assessment usually includes the estimation of yields, financial considerations, and costs related to the investment and operating costs. To determine the efficiency of the pre-treatment process, the bioethanol yield from the hybrid biomass (cassava peels and yam peels biomass) for the acid-based and alkali-based pre-treatments was evaluated using the model used by Solarte-Toro et al [28]. The assessment also included obtaining the utility costs, which comprise the cooling water, steam, fuel, and electricity required for pre-treatment, fermentation, and distillation of the fermented hydrolysates, cost of raw materials and transportation, labor costs, operating charges, Fermentation, and distillation equipment Costs, administrative costs, cost of enzymes and depreciation. The data were evaluated using engineering economics and descriptive statistics. The bioethanol cost was then compared with the current market price of bioethanol as obtainable in Nigeria.

3. RESULTS AND DISCUSSION

3.1 Effects of different pretreatment rigorousness on the physicochemical properties of biomass

The results of the proximate analysis on the raw and pretreated biomass samples of different particle sizes are as shown in Tables 3.1 to 3.4. The cellulose values of the raw biomass range from 25.8 wt% for cassava peels biomass to 40.0 wt% for sugar cane bagasse. This result conforms with what is obtainable in literature as reported by Salihu et al [29]. The pretreated biomass shows a significant difference from the raw biomass with values ranging from 33.2 to 43.8 wt%. The values of the cellulose content increased slightly with the pretreatment and that of the lignin content decreasing significantly with pretreatment. High cellulose and

low lignin contents are a very desirable quality in lignocellulosic biomass for bioethanol production [30]. The hydrothermal and alkaline-based pretreatment shows more severity on the different biomass being studied with the lignin content reducing significantly with values ranging from 12.4 wt% for cassava peels to 22.8 wt% for a mixture of all the biomass. This also conforms to the findings of Sabiha-Hanim et al [30] and Chang et al [31] that both found out from their works that alkaline pretreatment is more efficient in lignin removal and considerably increases the degradability of cellulose even if only some part of the lignin is removed.

The ash content of the pretreated biomass except for rice husks biomass which remained the same were all reduced. It is worthy of note that elevated ash content is a problem as the ash particles in the biomass takes up more steam, H₂O, dilute acid solution, or diluent than the relatively larger lignocellulosic fibers [32]. The consequence of the ash content of the lignocellulosic biomass could also be seen in the moisture content results for the pretreated biomass. Since the ash particles in the biomass absorb the steam, water, dilute acid, and other solvents during pretreatment, the moisture content of the biomass increased slightly after pretreatment with the alkali pretreated biomass having the highest moisture content, again confirming the superior severity of the alkali pretreatment over the other pretreatment methods adopted in this study. The hemicellulose content also shows the same pattern by increasing slightly after the different pretreatments, with the values of the pretreated biomass ranging from 33.5 to 43.5 wt %. For the mixed biomass (cassava peels plus yam peels, corn cobs plus rice husks, and all five biomass combined), there is not much difference in the outcome of the different pretreatment methods. The values obtained for their cellulose and hemicellulose content for the three pretreatments fall within a very narrow range. Total delignification of the biomass is not easy due to the position of lignin in the macromolecular structure [33].

The proximate analysis parameters for the three pretreatment methods adopted in this study were analyzed statistically using analysis of variance (ANOVA) of Microsoft Excel and the results are as shown in Tables 5a -d. The p-values in the ANOVA results for the ash content, lignin content, hemicellulose content, and cellulose content were all greater than 0.05, showing that there is no significant difference in the effects of the different pretreatments on the biomass in this study. The statistical significance of the data obtained for the proximate analysis parameters was tested by F-test and shows that the effects of the three pretreatment methods were highly significant as suggested by the model F values on the tables.

Table 3. 1: Proximate analysis result for untreated (raw) biomass samples

Biomass	Moisture content (%)	Ash content (%)	Lignin content (%)	Hemi cellulose (%)	Cellulose (%)
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	300 μm	425 μm	300 μm	425 μm	300 μm	425 μm	300 μm	425 μm	300 μm	425 μm
Cassava Peels	18.3	15.0	8.0	2.0	20.2	21.6	36.0	35.3	25.8	33.2
Corn cobs	21.0	20.0	3.0	4.0	19.2	19.2	34.0	36.0	36.0	34.0
Rice husks	21.0	18.0	10.0	2.0	24.4	25.2	34.5	31.5	33.5	31.0
Sugar cane bagasse	22.0	17.0	6.0	6.0	9.2	12.4	45.0	45.0	39.8	40.0
Yam peels	26.0	27.0	6.0	10.0	21.6	20.0	39.0	28.0	36.8	38.1

Table 3. 2: Proximate analysis result for hydrothermal and acid-based pretreated samples

Biomass	Moisture Content (%)		Ash content (%)		Lignin content (%)		Hemi cellulose (%)		Cellulose (%)	
	300 μm	425 μm	300 μm	425 μm	300 μm	425 μm	300 μm	425 μm	300 μm	425 μm
Cassava Peels	19.0	18.0	4.0	10.0	20.8	24.4	35.0	34.5	33.0	36.5
Corn cobs	19.0	25.0	4.0	2.7	20.4	20.8	34.8	35.0	34.7	34.5
Rice husks	28.0	27.0	10.0	9.0	20.0	18.8	33.5	36.0	35.0	34.5
Sugar cane bagasse	19.0		10.0		17.6		43.5		42.0	
Yam peels	20.0		2.0		22.4		36.0		34.5	
Cassava peels + Yam peels	25.0		3.0		24.0		37.5		36.5	
Corn cobs + Rice husks	22.0		2.0		24.4		36.0		35.5	
The mixture of all the five biomass	22.0		10.0		24.0		37.9		36.1	

Table 3. 3: Proximate analysis result for hydrothermal and alkali-based pretreated samples

Biomass	Moisture content (%)	Ash content (%)	Lignin content (%)	Hemi cellulose (%)	Cellulose (%)
	300 μm	300 μm	300 μm	300 μm	300 μm
Cassava Peels	19.0	2.0	12.4	43.8	42.2
Corn cobs	27.0	6.0	20.8	34.0	35.0
Rice husks	27.0	10.0	19.6	35.3	33.2
Sugar cane bagasse	29.0	8.0	16.0	41.8	43.7
Cassava peels + Yam peels	25.0	8.0	22.0	35.0	36.0
Corn cobs + Rice husks	22.0	4.0	21.2	34.3	34.2
The mixture of all the five biomass	24.0	10.0	22.8	35.3	36.7

Table 3. 4: Proximate analysis result for hydrothermal only pretreated samples

Biomass	Moisture content (%)		Ash content (%)		Lignin content (%)		Hemicellulose (%)		Cellulose (%)	
	300 μm	425 μm	300 μm	425 μm	300 μm	425 μm	300 μm	425 μm	300 μm	425 μm
Cassava Peels	17.0	20.0	1.8	4.2	17.6	20.0	41.5	38.3	43.5	35.2
Corn cobs	26.0	20.0	10.0	10.0	17.6	15.6	43.5	43.2	41.5	43.8
Rice husks	21.0	20.0	10.0	7.0	21.6	21.6	33.5	35.0	34.5	33.5
Sugar cane bagasse	25.0	-	10.0	-	17.6	-	43.5	-	41.4	-
Yam peels	17.0	-	10.0	-	24.0	-	35.9	-	35.6	-
Cassava peels + Yam peels	23.0	-	3.0	-	24.4	-	35.5	-	36.5	-
Corn cobs + Rice husks	22.0	-	7.0	-	22.8	-	35.8	-	35.7	-
The mixture of all the five biomass	23.0	-	3.0	-	22.0	-	35.0	-	35.5	-

Table 3. 5: Statistical analysis of the pretreatment parameters

a. Ash Content

Groups	Count	Sum	Average	Variance
Acid Pretreatment	8	45	5.625	13.69643
Alkaline pretreatment	7	48	6.857143	9.142857
Hot water	8	54.8	6.85	13.55143

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	7.872205	2	3.936102	0.32054	0.729417	3.492828
Within Groups	245.59214	20	12.27961			
Total	253.46435	22				

b. Lignin Content

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Acid Pretreatment	8	173.6	21.7	5.794286		
Alkaline pretreatment	7	134.8	19.25714	13.99619		
Hot water	8	167.6	20.95	8.545714		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	23.07242	2	11.53621	1.251507	0.307534	3.492828
Within Groups	184.3571	20	9.217857			
Total	207.4296	22				

c. Hemicellulose Content

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Acid Pretreatment	8	294.2	36.775	9.427857		
Alkaline pretreatment	7	259.5	37.07143	15.88571		
Hot water	8	304.2	38.025	16.785		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	6.775714	2	3.387857	0.243028	0.786532	3.492828
Within Groups	278.8043	20	13.94021			
Total	285.58	22				

d. Cellulose Content

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Acid Pretreatment	8	287.3	35.912	7.198		
Alkaline pretreatment	7	261	37.285	16.454		
Hot water	8	304.2	38.025	12.265		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	18.339	2	9.169	0.780	0.471	3.492
Within Groups	234.972	20	11.748			
Total	253.312	22				

3.2 Effect of pretreatment on the composition of biomass

The major purpose of pretreatment is the breaking down of the lignin make-up and the disruption of the crystalline make-up of cellulose for improving enzyme ease of access to the cellulose in the hydrolysis step [34]. Tables 3.6 to 3.13 show the pore characteristics of the different biomass being analyzed in this study. The BET adsorption isotherms are also shown in appendix B1 to B8. The results show that generally for all the biomass under consideration, there is a significant reduction in specific surface area and pore volume with the different pretreatment methods carried out on them, with hydrothermal only pretreatment showing the least values. The results also show that cassava peels biomass of 300 microns particle size has the highest specific surface area and pore volume of 819.6 m²/g and 0.2031 cc/g respectively for individual pretreated biomass, thereby upholding the assertion by many researchers that cassava peel biomass is a very good feedstock for bioethanol production [35][36]. For the combined biomass, the combined corn cobs and rice husks biomass with specific surface area and pore volume of 1837 m²/g and 0.5570 cc/g respectively show promising potential for improved yield of bioethanol after the fermentation process.

Table 3. 6: Pore characteristics of cassava peels biomass (Raw and pretreated)

Sample	Specific surface area (m ² /g)			Pore volume (cc/g)		Pore diameter (nm)	V _{micro} /V _{Total} (%)
	S _{BET}	S _{micro}	S _{Ext}	V _{Total}	V _{micro}		
Raw (300 µm)	541.8	819.6	574.5	0.2552	0.2031	2.647	0.7958
Raw (425 µm)	293.0	525.7	530.4	0.1364	0.0743	2.647	0.5447
Acid pretreated (300 µm)	252.4	470.5	475.1	0.1201	0.0655	2.647	0.5453
Acid pretreated (425 µm)	301.1	502.1	399.3	0.1429	0.0890	2.647	0.6228
Alkali Pretreated (300 µm)	278.8	448.9	360.5	0.1319	0.0815	2.647	0.6178
Hot water pretreated (300 µm)	276.1	440.2	355.4	0.1316	0.0812	2.647	0.6170
Hot water pretreated (425 µm)	242.9	415.6	349.4	0.1150	0.0676	2.647	0.5878

Table 3. 7: Pore characteristics of corn cobs biomass (Raw and pretreated)

Sample	Specific surface area (m ² /g)			Pore volume (cc/g)		Pore diameter (nm)	V _{micro} /V _{Total} (%)
	S _{BET}	S _{micro}	S _{Ext}	V _{Total}	V _{micro}		
Raw (300 µm)	336.2	571.0	471.3	0.1599	0.0934	2.647	0.5841
Raw (425 µm)	304.3	498.8	434.9	0.1437	0.0817	2.647	0.5685
Acid pretreated (300 µm)	234.2	402.3	331.9	0.1104	0.0658	2.647	0.5960
Acid pretreated (425 µm)	239.1	405.5	360.7	0.1123	0.0646	2.647	0.5752
Alkali Pretreated (300 µm)	310.3	500.7	393.6	0.1473	0.0927	2.647	0.6293
Hot water pretreated (300 µm)	264.5	436.6	345.2	0.1255	0.0778	2.647	0.6199
Hot water pretreated (425 µm)	237.9	410.3	379.5	0.1112	0.0629	2.647	0.5656

Table 3. 8: Pore characteristics of Rice husks biomass (Raw and pretreated)

Sample	Specific surface area (m ² /g)			Pore volume (cc/g)		Pore diameter (nm)	V _{micro} /V _{Total} (%)
	S _{BET}	S _{micro}	S _{Ext}	V _{Total}	V _{micro}		
Raw (300 µm)	288.0	503.7	464.9	0.1343	0.0757	2.647	0.5636
Raw (425 µm)	227.2	871.4	418.3	0.1056	0.0575	2.647	0.5445
Acid pretreated (300 µm)	275.7	463.0	376.5	0.1303	0.0788	2.647	0.6047
Acid pretreated (425 µm)	279.1	453.3	363.2	0.1326	0.0822	2.647	0.6199
Alkali Pretreated (300 µm)	253.0	446.9	416.0	0.1198	0.0664	2.647	0.5542
Hot water pretreated (300 µm)	246.5	432.3	413.5	0.1151	0.0639	2.647	0.5551
Hot water pretreated (425 µm)	262.8	433.4	371.9	0.1234	0.0725	2.647	0.5875

Table 3. 9: Pore characteristics of sugar cane bagasse biomass (Raw and pretreated)

Sample	Specific surface area (m ² /g)			Pore volume (cc/g)		Pore diameter (nm)	V _{micro} /V _{Total} (%)
	S _{BET}	S _{micro}	S _{Ext}	V _{Total}	V _{micro}		
Raw (300 µm)	278.6	491.3	456.1	0.1322	0.0728	2.647	0.5506
Raw (425 µm)	241.9	422.8	392.5	0.1143	0.0629	2.647	0.5503

Acid pretreated (300 μm)	310.0	531.9	486.4	0.1469	0.0813	2.647	0.5534
Alkali Pretreated (300 μm)	252.5	407.1	341.0	0.1184	0.0699	2.647	0.5903
Hot water pretreated (300 μm)	229.6	411.4	385.3	0.1087	0.0600	2.647	0.5519

Table 3. 10: Pore characteristics of yam peels biomass (Raw and pretreated)

Sample	Specific surface area (m^2/g)			Pore volume (cc/g)		Pore diameter (nm)	$V_{\text{micro}}/V_{\text{Total}}$ (%)
	S_{BET}	S_{micro}	S_{Ext}	V_{Total}	V_{micro}		
Raw (300 μm)	269.1	488.1	475.1	0.1281	0.0701	2.647	0.5472
Raw (425 μm)	244.4	409.1	356.3	0.1147	0.0660	2.647	0.5754
Acid pretreated (300 μm)	239.1	406.2	348.6	0.1125	0.0650	2.647	0.5777
Alkali Pretreated (300 μm)	242.9	408.4	354.2	0.1156	0.0671	2.647	0.5804
Hot water pretreated (300 μm)	232.4	400.3	340.7	0.1098	0.064	2.647	0.5828

Table 3. 11: Pore characteristics of cassava plus yam peels biomass

Sample	Specific surface area (m^2/g)			Pore volume (cc/g)		Pore diameter (nm)	$V_{\text{micro}}/V_{\text{Total}}$ (%)
	S_{BET}	S_{micro}	S_{Ext}	V_{Total}	V_{micro}		
Acid pretreated (300 μm)	499.9	864.1	818.2	0.2337	0.1325	2.647	0.5669
Alkali Pretreated (300 μm)	738.3	1118.0	760.5	0.3478	0.3003	1.847	0.8634
Hot water pretreated (300 μm)	518.6	888.4	719.3	0.2455	0.1447	2.647	0.5894

Table 3. 12: Pore characteristics of corn cobs plus rice husks biomass

Sample	Specific surface area (m^2/g)			Pore volume (cc/g)		Pore diameter (nm)	$V_{\text{micro}}/V_{\text{Total}}$ (%)
	S_{BET}	S_{micro}	S_{Ext}	V_{Total}	V_{micro}		
Acid pretreated (300 μm)	1181.0	1837.0	1257.0	0.5570	0.4481	2.647	0.8044
Alkali Pretreated (300 μm)	440.9	811.3	833.2	0.2057	0.1124	2.647	0.5464

Hot water pretreated (300 μm)	402.2	748.4	800.5	0.2022	0.1004	2.647	0.4965
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Table 3. 13: Pore characteristics of the combination of all the biomass

Sample	Specific surface area (m^2/g)			Pore volume (cc/g)		Pore diameter (nm)	$V_{\text{micro}}/V_{\text{Total}}$ (%)
	S_{BET}	S_{micro}	S_{Ext}	V_{Total}	V_{micro}		
Acid pretreated (300 μm)	471.7	870.1	879.4	0.2223	0.1188	2.647	0.5344
Alkali Pretreated (300 μm)	489.4	893.3	889.9	0.2283	0.1252	2.647	0.5484
Hot water pretreated (300 μm)	516.8	895.0	791.5	0.2449	0.1411	2.647	0.5761

3.3 FTIR spectroscopy of raw and pretreated biomass

FTIR is mainly used to characterize the biomass based on the organic groups present [37]. The focal point of FTIR analysis is the lignin content, which is an aromatic biopolymer composed chiefly of phenylpropane substituted components attached to form a giant molecule of non-consistent crystallinity and optical activity [38]. The FTIR spectra of the untreated (raw), as well as the treated biomass, are shown in Figures 3.1 and Appendix C1 to C5. Table 3.14 displays the functional groups and vibration modes of the raw and pretreated biomass at standard temperature. The absorption band of the raw and pretreated biomass between 3200 and 3600 cm^{-1} is usually attributed to the O - H stretching vibrations of alcohols, carboxylic acids, and hydroperoxides [39] [40]. The FTIR spectrum shows a fingerprint region of 1420 – 670 for the source identification of the biomass. The results show that the O – H stretching of the hydroxy group of alcohol falls between 3693 to 3008 cm^{-1} for acid pretreated cassava peels and acid pretreated rice husks respectively. The methyl group of alkanes has a band of 2926 to 2855 cm^{-1} for water pretreated corn cobs and alkali pretreated corn cobs respectively. C = O stretching vibration can be attributed to ketones with a wavenumber of between 1636 and 1606 cm^{-1} . The band 1457 cm^{-1} shows the C - H bending or scissoring of alkanes found in acid pretreated corn cobs, cassava peels, and a combination of yam and cassava peels. Other organic compounds detected include ether, ether, and B-glucosidic bonds (864 cm^{-1}) between sugars [41]. For all the biomass, a very sharp peak of between 1006 to 1028 cm^{-1} of C – O – C is noted. The strong and sharp peak is attributed to the ether group. The hemicellulose content of the biomass can be ascribed to the wavebands at 1710 and 1028 cm^{-1} . There are also very sharp peaks noticed on the pretreated combined corn cobs and rice husks biomass, this is an indication of the fortification of the properties of the biomass by their combination.

Table 3. 14: Organic functional groups detected in the raw and pretreated biomass

Wavenumber (cm ⁻¹)	Organic functional groups	Description of vibration
3693 – 3008	Hydroxyl	This is due to the O–H stretching of the alcohol group present
2926 – 2855	Alkanes	This is as a result of the stretching of methyl (C–H) group
2855	Methoxy	The assigned peak is due to OCH ₃
1710 – 1718	Carboxylic acids	This band is due to the stretching vibration of C=O
1606 – 1636	Ketones	C = O stretching vibration attributed to ketones
1560 – 1610	Furan	C = C ring stretching vibrations
1420 – 670	Fingerprint region	
1457	Alkanes	C - H bending or scissoring of alkanes
1364 -1367	Phenolic	O-H phenolic group assigned peak
1159	Esters	O – C = O stretching of the esters group
1006 – 1028	Ethers	C – O – C The strong and sharp peak is credited to the ethers group
864	B-glucosidic bonds between sugars	

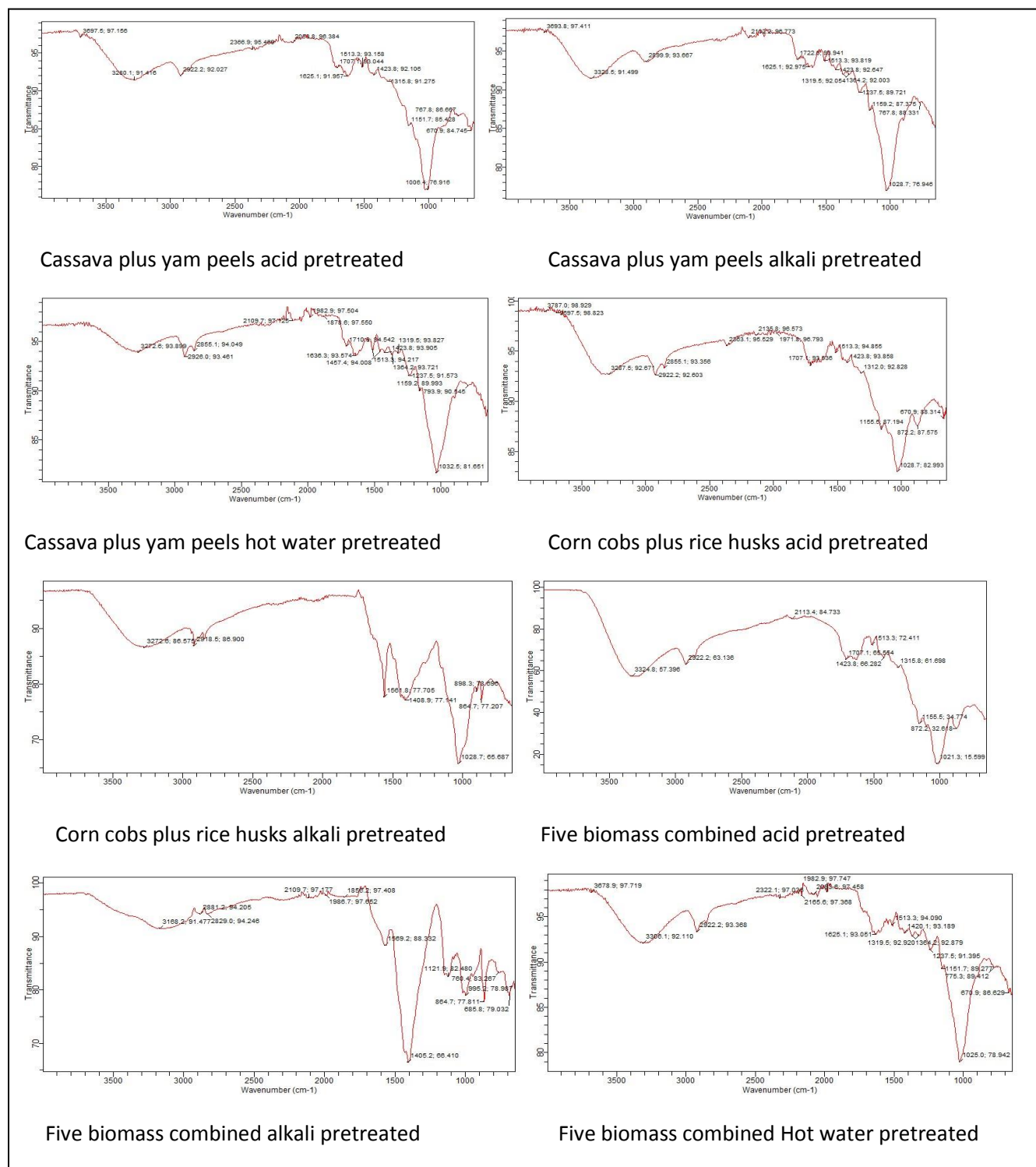


Figure 3. 1: FTIR of combined biomass (300 microns)

3.4 Inhibitor analysis of the different pretreatment process

The results of the proximate analysis as shown in Table 3.2 indicate that the combined hydrothermal and acid pretreated biomass has a very high recovery of hemicellulosic sugars with increased enzymatic convertibility. However, the process brings about the formation of inhibitory derivatives such as aliphatic carboxylic acids, furans, among others. The combined hydrothermal and alkali pretreatment was able to remove lignin and a small fraction of hemicelluloses, however with the formation of some side products such as acetic acid, hydroxy acids among others. Table 3.15 shows a summary of the inhibitory side products formed during the different pretreatment methods in this study.

In the work by Olsson et al [42], to reduce the effects of the inhibitors on the hydrolyzates for fermentation, two methods were proposed namely; detoxification and adaptation of the microorganism to the lignocellulosic hydrolysate. The latter is more cost-saving than the former. Other methods include overliming [43], charcoal adsorption, and ion exchange [44]

Table 3. 15: Summary of inhibitory side products formed during the different pretreatment methods

Pretreatment method	Main effects	Inhibitory products detected (From FTIR analysis)
Hydrothermal and acid-based pretreatment	Hydrolysis of hemicelluloses to monosaccharides	Carboxylic acids, Phenolic compounds
Hydrothermal and Alkali based Pretreatment	Removal of lignin and a minor part of hemicelluloses	Acetic acid, hydroxy acids, phenolic compounds
Hydrothermal only based pretreatment	Solubilization of hemicelluloses	Acetic acid, furan, aldehydes

3.5 Structural changes in the raw and pretreated biomass

The scanning electron micrograph (SEM) was employed to compare, study and analyze the untreated samples as well as morphological changes that had occurred on the pretreated samples as a result of the different pretreatments carried out on them. Figures 3.2 and appendix D1 to D7 show the images of the morphological analysis of the raw and pretreated biomass by SEM. The images show that all the raw biomass had a smooth intact structure with a rigid and fibrillary morphology, which had not been damaged by the crushing and grinding of the biomass. The images also revealed interesting transformations after the pretreatment processes were carried out on them. The morphologies of the pretreated biomass show that they were broken down and fragmented with the hitherto compacted and finely divided surfaces unsettled. The SEM images of the pretreated biomass also showed a cluster of globe-like micro grains deposited over

copious particles, this is an indication that cellulose and hemicellulose were disintegrated to a reasonable extent during the pretreatment processes [45]. However, it can be observed that the images of the alkali pretreated biomass show more of the globe-like clusters, indicating the severity of alkali pretreatment over the other pretreatment processes carried out in this study.

This result conforms with the pattern of results obtained by Chowdhury Z. Z [46] in their work on lignocellulosic biomass. Lignin, which is a non-saccharide fraction of biomass is more chemically rigid than the saccharide fraction, composed of cellulose and hemicellulose. The lignin was moderately disintegrated, and the initial properties of the grains were conserved. The images also show that pore volume with the surface area was considerably enlarged after the pretreatment process with the alkali pretreatment showing the highest severity. This observation was also supported by the results obtained from the BET analysis.

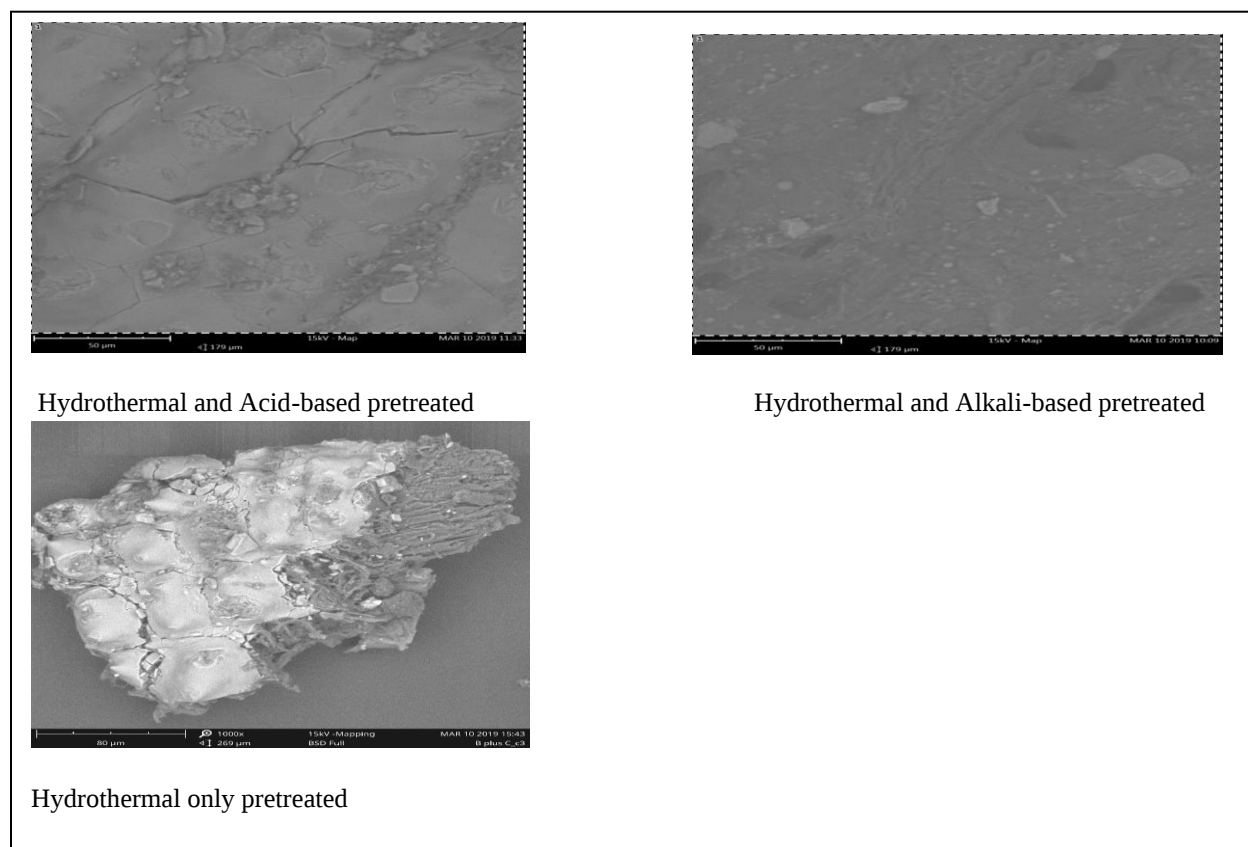


Figure 3. 2: SEM of pretreated corn cobs plus rice husks biomass (300 microns)

3.6 X-Ray diffraction (XRD)

The X-ray diffraction patterns of the raw and pretreated biomass are illustrated in Figure 3.3 and appendix E1 to E5. As can be seen, all the diffractograms showed the typical XRD peaks of cellulose. For the raw biomass, a sharp peak at 2θ values of between 18 and 22° was observed owing to the presence of crystalline cellulose in the biomass samples. This conforms to the results obtained from previous research [47]. The pretreated biomass samples showed diffractograms with peaks of somewhat reduced intensities, a demonstration of incomplete degradation of the cellulose with pretreatment [48]. The pretreated biomass samples had a broader peak at 2θ values of between 22 and 24° , which is an indication of atomic order in them. The non-existence of sharp peaks proves the amorphous texture of the pretreated biomass samples [49]. As opposed to amorphous cellulose, crystalline cellulose is more recalcitrant to enzymatic and microbial reactions [50] [51].

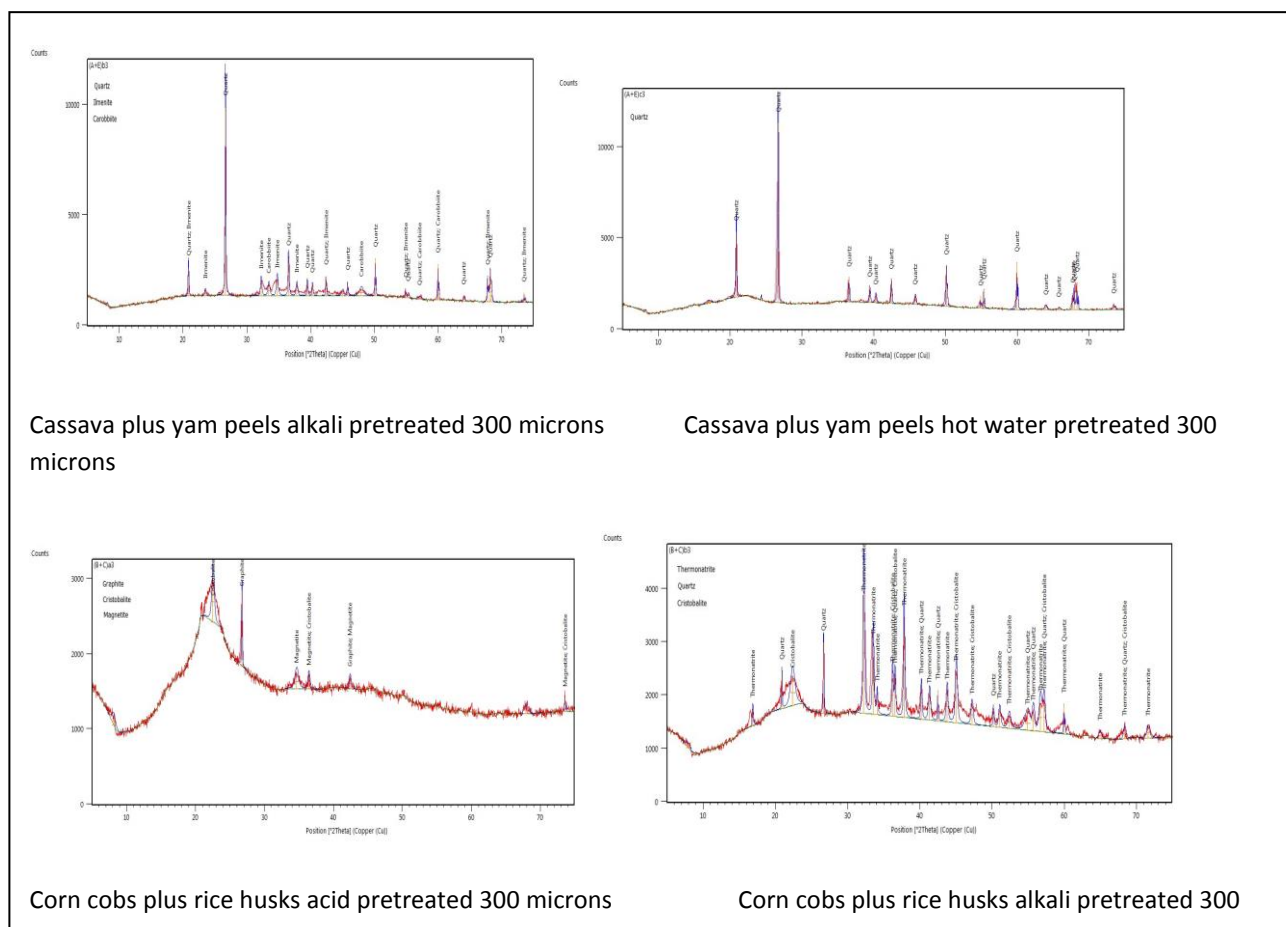


Figure 3. 3: XRD diffractograms of mixed biomass

3.7 Environmental Considerations

Many factors influence the determination of the life cycle effects from bioethanol production from different biomass; this includes cultivation of the feedstock, production of the enzymes and chemicals, pre-treatment, fermentation, delivery, and utilization of the bioethanol. Emissions to air, soil, and water during the pre-treatment process were assessed using SimaPro. The environmental impact categories include global warming potential (GWP)/climate change, eutrophication (EP), acidification (AP), photochemical oxidation demand (POD), and marine and human ecotoxicity. The emissions from the pre-treatment processes can be classified in terms of corresponding quantities using the CML (Centre of Environmental Science at Leiden University) method [52]. Potential for global warming is measured in Kg of CO₂ (eq), eutrophication is measured in Kg of PO₄⁻³ (eq), acidification is measured in Kg of SO₂ (eq), photochemical oxidation demand is measured in Kg of C₂H₄ (eq), while marine and human ecotoxicity are measured in Kg of dichlorobenzene (1,4 C₆H₄Cl₂) (eq). The GWP and the AP were considered on the mixed biomass of cassava peels and yam peels in this work.

3.7.1 Global warming potential (GWP)

The global warming potential (GWP) of a process or product and its consequent effect on the climate, have been the current subject of debate and regulation in the environmental performance assessment. GWP is an enumerated amount of the global mean comparative radiative driving effects of a certain greenhouse gas measured over a hundred-year timeline. The CML technique provides GHG emissions in units of corresponding liberated CO₂ via the global warming potential factors of 1, 25, and 298 for CO₂, CH₄, and NO₂, respectively. With an average value of 15.82 Kg CO₂ (eq), the alkaline pre-treatment using sodium hydroxide shows the highest discharge of GHG emissions, while acid pretreatment using dilute sulphuric acid generated an average of 8.68 Kg CO₂. Climate change is a very important consideration in lignocellulosic bioethanol production as one of the reasons for utilizing bioethanol for energy purposes is the reduction in GHG emissions.

3.7.2 Acidification Potential (AP)

Acidification stems from anthropogenic emissions involving sulfur (iv) oxide (SO₂), nitrogen oxides (NO_x), and ammonia (NH₃). The assessment of the acidifying potential of the sulphuric acid and sodium hydroxide used for pre-treatment of one of the biomass in this study (cassava peels biomass), show that sulphuric acid emitted 0.035 Kg SO₂ (eq), a value lower than that of sodium hydroxide emission, which was 0.087 Kg SO₂ (eq). This result is an indication that the acid pre-treatment is preferable in terms of environmental degradation owing to the effect of the biomass pre-treatment.

3.8 Economic consideration results and analysis

To be viable and economically acceptable, the expenditure for the processing of lignocellulosic biomass to fuel must not be up to the current gasoline cost. This is achievable owing to the efforts of researchers at improving the effectiveness of biomass processing technologies. The cost of feedstock, feedstock pre-treatment, and enzymes are important considerations for low-cost ethanol production. The use of hybrid (mixed) feedstocks and large-scale processing facilities coupled with low-cost feedstock as well as effective cellulases helps in making the process cost-effective. In converting biomass to bioethanol, some inputs result in environmental costs, the output from the process such as electricity generations and sales of the produced bioethanol would result in the recouping of the expenses of the production process. Table 3.16 shows the results of the techno-economic assessment of bioethanol produced from acid-based pre-treated cassava and yam peels biomass mixture. The bioethanol price of 0.41 USD/l is a good deal as it compares favorably well with the 0.45 USD/l price of ethanol in the Nigerian market. This value was obtained considering the total sugar present in the samples after the pretreatment. The produced bioethanol could also augment gasoline from crude oil, this would also reduce the drastic effects of the combustion of gasoline on the environment in terms of emissions and costs. The major advantage derivable from the use of biomass in bioethanol production is the limiting of greenhouse gases' environmental pollution [53]. The lignocellulosic bioethanol production, therefore, comes with more benefits in the long run in terms of economic and environmental considerations.

Table 3. 16: Techno-economic assessment results (Cassava and yam peels biomass mixture) *

Item	Acid-based pre-treated Cassava and yam peels biomass		References
	USD/year	(%)	
Raw materials ^a	1672	15.20	
Utilities ^b	1721	15.65	
Labour Cost	1707	15.52	
Maintenance	550	5.00	
Operating charges	522	4.75	
Plant Overhead ^c	578	5.25	
Administrative Costs	338	3.07	
Depreciation	1533	13.94	
Total production cost		100	
Total Project Capital Cost (USD) ^d	25923.86		
Bioethanol Cost (USD/l)	0.41		

Current Market Bioethanol Cost (USD/l)	0.45		[54]
Current Market Gasoline Cost (USD/l)	0.42 ^e		[55]

* The assessment is for a plant designed to process approximately 10 tons/year of cassava and yam peels biomass.

^a This includes costs of sourcing and transportation of the agricultural residues

^b This includes costs of cooling water, steam, fuel, and electricity required for pre-treatment, fermentation, and distillation of the fermented hydrolysates.

^c This includes costs of enzymes and reagents for pre-treatment

^d This includes costs of reactors, fermenters, distillation equipment, plant building, and furniture.

^e Subject to further increment as the government is reviewing the fuel subsidy policy.

4.0 CONCLUSION

Bioethanol production from lignocellulosic biomass such as corn cobs, rice husks, cassava peels, yam peels, sugar cane bagasse among others, is an emerging technology in the renewable energy field. However, this biomass must be pretreated before the fermentation process. The main goal aimed to be achieved by carrying out pretreatment is the breaking down of the lignin structure and the interruption of the crystalline make-up of cellulose for enhancing enzyme accessibility to the cellulose during the hydrolysis stage. Selected biomass was sourced locally, dried, and sieved into two mesh sizes and then pretreated using hydrothermal and acid-based based, hydrothermal, and alkali-based and hydrothermal-only processes. The raw, as well as the pretreated biomass samples, were then characterized by proximate analysis, SEM, FTIR, XRD, and BET. The cellulose values of the raw biomass range from 25.8 wt% for cassava peels biomass to 40.0 wt% for sugar cane bagasse. The pretreated biomass shows a significant difference from the raw biomass with values ranging from 33.2 to 43.8 wt%. The cassava peels biomass of 300 microns particle size has the highest specific surface area and pore volume of 819.6 m²/g and 0.2031 cc/g respectively for individual pretreated biomass, while for the combined biomass, the combined corn cobs and rice husks biomass with specific surface area and pore volume of 1837 m²/g and 0.5570 cc/g respectively. The combined corn cobs and rice husks biomass show a promising potential for improved bioethanol yield after fermentation. The organic compounds present in the biomass include hydroxyl, alkanes, methoxy, carboxylic acids, ketones among others. The use of a combined biomass feedstock would be preferred as

this will give greater flexibility in the bioethanol production from lignocellulosic biomass, and as was shown from this work, the combination of different biomass materials can result in favorable properties of the combined biomass feedstock after pretreatment.

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AUTHOR CONTRIBUTION

A.A.A and D.L conceptualized the research, A.A.A carried out the experimental work and developed the manuscript, D.L corrected and updated the manuscript.

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Chapter FIVE

Paper 4, IEPECADSNLB

Investigation of the effects of pretreatment on the elemental composition of ash derived from selected Nigerian lignocellulosic biomass

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PAPER 4

Investigation of the effects of pretreatment on the elemental composition of ash derived from selected Nigerian lignocellulosic biomass

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ABSTRACT

Lignocellulosic biomass is an important source of renewable energy and a potential replacement for fossil fuels. In this work, the X-ray fluorescence (XRF) method was used to analyze the elemental composition of raw and pretreated lignocellulosic biomass of cassava peels, corn cobs, rice husks, sugarcane bagasse, yam peels, and mixtures of cassava peels and yam peels, corn cobs and rice husks and all five biomass samples combined. The influence of particle size on elemental properties was investigated by screening the selected biomass into two size fractions, of an average of 300 and 435 μm , respectively. The total concentration of Mg, Al, Si, P, S, Cl, Ca, Ti, Cr, Mn, Fe, Co, Cu, Zn, Sn, Ni, Br, Mo, Ba, Hg, and Pb were determined for each of the biomass samples before and after the different pretreatments adopted in this study. From the results of the analysis, there was a significant reduction in the concentration of calcium in all the analyzed biomass after the alkaline pretreatment with rice husks biomass having the lowest concentration of 66 ppm after the alkaline pretreatment. The sulfur content of the acid pretreated biomass increased considerably which is likely due to the sulfuric acid used for the acid pretreatment. The fact that a mixture of biomass feedstock affects the properties of the biomass after pretreatment was validated in the mixed biomass of cassava peels and yam peels biomass as an example. The concentration of Mg in the mixed biomass was 1441 ppm but was 200 ppm and 353 ppm in individual cassava peels and yam peels respectively. The results of this study demonstrated that pretreated mixtures of biomass have varied elemental compositions, which could be an important factor affecting downstream processes, especially if a hybrid feedstock is used in a large-scale application.

Keywords: Biomass, Lignocellulosic biomass, biofuel, ash content, elemental composition

1. INTRODUCTION

The use of fossil fuels (petroleum, coal, and natural gas) from decomposing plants and animal remains for energy generation has posed several challenges to the environment due to their non-renewability nature and their adverse depletion of the ozone layer due to the release of greenhouse gases (GHG) [85]. The negative impacts of the use of fossil fuels on the environment have led to the introduction of biomass as a renewable and environmentally friendly fuel source. Biomass is a complex heterogeneous mixture of organic

materials, inorganic materials, containing various solid and fluid, intimately associated minerals of different sources [132]. The US patent on processing biomass defined biomass as any non-fossilized organic matter [133],[134]. They include cellulosic and lignocellulosic materials such as plant biomass, animal biomass, and municipal waste biomass [135]. They also include HAR-herbaceous and agricultural residues: shells, cobs, and husks of plants and others [136]. Sanderson, [137] defined lignocellulosic biomass as the inedible parts of plants that are feedstocks for the next generation of biofuels. Technically, lignocellulosic materials are a mixture of natural polymers (carbohydrates) such as lignin, cellulose, and hemicellulose, and tannins with more than two hydroxyl groups per molecule. Waste biomass is produced as a low value by-product of various industrial sectors such as agriculture. It includes corn stover, sugarcane bagasse, straws, sawmill, and paper mill discards.[138]

Typical lignocellulosic biomass contains 30-50% of cellulose, 15-35% of hemicellulose, and 10-30% of lignin [139]. With the complex nature of lignocellulosic materials, it poses resistance to chemical and biological degradation during enzymatic hydrolysis and subsequent fermentation. This highly recalcitrant nature of lignocellulosic materials in the production of biofuels, mainly ethanol makes the process economically unfeasible and thus, pretreatment is needed before saccharification and fermentation [140, 141]. The constituents of lignocellulosic biomass also include inorganic matters present in a trace concentration and are essential for plant growth. The inorganic constituents of biomass consist of macronutrients and micronutrients. The macronutrients are nutrients needed by plants in a very large concentration. They include nitrogen (N), potassium (K), magnesium (Mg), phosphorus (P), calcium (Ca), and sulfur (S). Micronutrients on the other hand are needed by plants in relatively small concentration. Examples of such micronutrients are copper (Cu), manganese (Mn), zinc (Zn), iron (Fe), boron (B), molybdenum (Mo), and chlorine (Cl). Other minerals that are of great benefit to plants but are not essential are sodium (Na), vanadium (V), nickel (Ni), cobalt (Co), aluminum (Al), silicon (Si), and selenium (Se). The elements function differently in plants, from the production of amino acids, protein synthesis, enzyme activation, nucleic acids, energy household, ATP, cell wall structure to photosynthesis reaction, among others.

In time past, the utilization of lignocellulosic biomass was restricted to combustion for domestic and industrial heating with attendant adverse effects on the environment. Problems such as land degradation and desertification have been associated with the use of lignocellulosic biomass. In recent times, researchers have come up with a better and more economically viable means of lignocellulosic biomass utilization with a minimal negative impact on the environment. Lignocellulosic biomass conversion routes such as thermochemical or biochemical processing methods afford their conversion to energy or energy carriers. The thermochemical processing route employs heat and chemical means such as combustion, pyrolysis,

gasification, and liquefaction in the production of energy products from lignocellulosic biomass whereas the biochemical processing route adopts the use of microorganisms or enzymes and bacteria in the decomposition of the biomass to obtain biofuels [142]. The thermochemical processes normally require a large amount of energy, as well as the inclusion of a solvent or catalyst. The biochemical approach has a longer cycle time and is less effective at breaking down resistant biomass components. By integrating the advantages of both ways in biofuel manufacturing, combining the two routes can be promising. When hydrothermal routes are utilized in the pretreatment stage to prepare the suitable biomass feedstock for the subsequent biological routes, the total process efficiency and final product yields are improved, and vice versa [143].

Depending on the various forces or resources utilized in the pretreatment process, pretreatment technologies can be classified as physical, chemical, biological, or physio-chemical, or a mix of these.

Liquid hot water (LHW) pretreatment is a type of hydrothermal pretreatment that does not require fast decompression or the addition of a catalyst or chemical. LHW pretreatment uses a temperature and pressure range of 170 to 230 °C and >5 Mpa, respectively. The LHW pretreatment removes hemicellulose from lignocellulosic materials, exposing the cellulose and allowing fermentation to take place [144].

Acid pretreatment is a type of chemical pretreatment involving chemical hydrolyses that solubilize hemicellulose and lignin, allowing enzymes to work on the cellulose during the fermentation process. Acid pretreatment can be done with concentrated or dilute acid, However, the use of concentrated acid has the drawback of the formation of inhibiting compounds such as furfural and phenolic acids; also, concentrated acids are unpleasant, caustic, and generally dangerous. As a result, corrosion-resistant equipment should be used for this pretreatment process. For large-scale bioethanol production, dilute acid pretreatment is the best option.

An alkaline pretreatment is a type of chemical pretreatment in which the biomass is treated with a base such as potassium, or calcium hydroxides at standard pressure and temperature. This pretreatment process offers the advantage of eliminating lignin from the biomass more effectively. The technique also removes acetyl and uronic acid groups from hemicellulose, increasing the enzyme's accessibility to break down hemicellulose Pretreatment with alkali can also be done at low temperatures, pressures, and times [145].

Shen et al., [146] worked on the prediction of the elemental composition of biomass based on proximate analysis. New correlations for determining elemental composition based on the proximate study of biomass was presented in the research work. The established correlations can be utilized for the accurate

computation of elemental composition of different biomass particularly for biomass with high ash content, after the proximate analysis.

Yusuf et al., [147] worked on the characterization of Ugandan biomass wastes as the potential candidates for bioenergy production. In the work, various analyses were performed on Mbwarzirume peel (MP) and Nakyinyika peel (NP) biomass, including proximal and ultimate such as TGA, FT-IR, AAS, and SEM-EDS. The result of analysis shows that the components identified in both ash deposits were sorted as follows during the EDS analysis: For MP, $O > K > C > Cl > Mg > P$, while for NP, $K > Cl > Mg > P > Al$.

Osman et al., [148] investigated the physiochemical characterization of miscanthus and its application in heavy metals removal from wastewaters. A novel alternative use was studied in this study, namely the direct use of dried miscanthus (DM) plant as an adsorbent for heavy metals removal (HMR) from wastewaters. XRD, S_{BET} , TGA, DSC, SEM-EDX, elemental analysis, halogen, and ICP techniques were used to investigate the physical, chemical, and leaching properties of DM. The results show that the DM sample had 42.85% carbon, 5.83 percent hydrogen, 1.21 percent nitrogen, 0.1 percent sulfur, and 50.01 percent oxygen.

The aim of this study is therefore to determine the effects of three pretreatment methods on the elemental composition of ash materials in selected Nigerian lignocellulosic biomass individually and as hybridized (mixed) feedstocks in bioethanol production. Knowledge of the ash content helps to estimate the possibility of slagging and scale formation in the process of combusting or gasification of biomass while the estimation of the elemental composition helps determine the conversion efficiency of the biomass in bioethanol production. Also, a hybridized (mixed) biomass feedstocks help to guarantee enough feedstock for large-scale bioethanol production plants, hence information on the mixed feedstocks would aid in equipment design and selection of process conditions/methods.

2.0 EXPERIMENTAL PROCEDURES/METHODS

2.1 Biomass preparation

The corncobs were obtained from Ogume, a village in Ndokwa West Local government area of Delta State, Nigeria. The rice husk used for this work was obtained from a rice mill in Ekperi in Etsako Central Local Government area of Edo State, Nigeria. The other biomass, yam peels, cassava peels, and sugarcane bagasse were locally sourced within Effurun in the Uvwie Local Government Area of Delta State, Nigeria. All biomass were sundried for about 7 days and then taken to the mill where they were ground into a powder. After grinding, the ground biomass were sieved into particle sizes of 300 μm and 425 μm . Pretreatment of biomass was carried out using analytical grade chemical reagents such as sodium hydroxide pellets, hydrogen peroxide, and tetraoxosulphate (VI) acid.

1500 g of each biomass was collected into different containers except for sugarcane bagasse for which 1000 g was collected. To verify the effect of biomass combination on the elemental composition, 300 g each of 300 µm sized particles for all five biomass samples were collected into another container and 750 g of 300 µm sized particles of cassava peels and yam peels combined and corn cobs and rice husks combined were measured into two containers respectively. Thorough mixing of the combined mixture of two or more biomass was ensured for uniformity in composition. Details of the pretreatment process can be obtained from previous work by the authors [145].

2.2 ASH CONTENT

1 g of sample was placed in a pre-weighed crucible and incinerated in a muffle furnace at 760 °C until complete ashing was achieved. The crucible was then transferred into a desiccator for cooling. Three replicates were made. The cooled samples were then weighed following ASTM D 2017 (1998). The ash content was calculated using the equation below.

$$\text{Ash Content (\%)} = \frac{\text{weight of ash}}{\text{original weight of sample}} \times 100 \quad (1)$$

$$= \frac{(W_2 - W_1)}{(W_1 - W_0)} \times 100 \quad (2)$$

Where W_0 = Weight of Crucible

$$\begin{aligned} W_1 &= \text{Weight of Crucible} + \text{Sample before incineration} \\ W_2 &= \text{Weight of the Crucible} + \text{Sample after incineration} \end{aligned}$$

2.3 XRF Analysis

X-ray fluorescence (XRF) spectroscopy is a rapid method used to determine the biomass ash composition. The XRF provides simple analytical solutions to a wide range of quality and process control requirements when compared to other analytical techniques. It can provide detailed analysis on non-destructive analysis, minimal preparation samples, simultaneous multi-element quantitative and qualitative analysis and these results are displayed in seconds. Analysis can be done on different types of samples ranging from solids, liquids, pastes, powders, films, slurries, filters, and oils, etc covering an elemental range of sodium to uranium in the periodic table. This accounts for the absence of the first 10 elements of the periodic table (mainly hydrogen, carbon, and oxygen). The chemical composition of different fuel ashes was measured by X-ray fluorescence (x-supreme 8000). To minimize the error, multiple experiments (triplicate) were carried out for each sample, and the average value was selected for the chemical composition of the samples. This was after the experiment to determine the quantity of ash contained in the different biomass had been done. The elemental composition in 1 kg of the different elements was given in mg (i.e. mg/kg) which is the same as ppm.

3.0 RESULTS

3.1 General observation

From the result of the experiment, it was seen that the chemical composition of the mineral matter of ashes depends largely on the biomass type, origin, and combustion conditions. This is following past researches on ash components and the varying composition of ash in comparison to the combustion temperature. The lignocellulosic biomass used in this project has varying ash chemical composition for cassava peel, corn cobs, rice husk, sugarcane bagasse, and yam peels as shown in Table 4.2. The main inorganic mineral constituents of the biomass are Ca, Fe, S, Si, Al, P, and Sn. The five major inorganic constituents of the biomass are shown in decreasing order of abundance as shown in Table 4.3.

Table 4. 1: Effect of acid, alkaline, and hydrothermal pretreatment on the raw biomass samples

Element	Mg	Al	Si	P	S	Cl	Ca	Ti	Cr	Mn	Fe	Co	Cu	Zn	Sn
CASSAVA PEELS (ppm)															
R	55	175	630	117	287	47	2014	284	9	19	1074	0	0	18	0
A	354	1111	4014	239	14044	18	1805	454	9	15	654	0	0	9	523
B	0	436	821	58	223	40	599	100	0	0	383	0	0	14	0
C	393	664	1104	219	333	57	1136	176	0	11	628	0	0	9	0
Mean	201	597	1642	158	3722	40	1389	253	4	11	684	0	0	12	131
CORN COBS (ppm)															
R	712	344	416	535	374	487	135	53	0	11	144	0	0	13	803
A	0	76	351	142	429	198	1242	146	0	10	317	0	0	8	0
B	41	137	194	43	81	85	84	41	0	0	124	0	0	14	1105
C	142	323	468	160	222	140	135	55	6	8	161	0	0	16	536
Mean	224	220	357	220	276	228	399	74	1	7	187	0	0	13	611
RICE HUSKS (ppm)															
R	511	324	1299	431	5083	33	68	29	0	18	36	0	0	0	0

A	774	490	4070	985	7517	14	65	58	0	28	72	0	0	9	0
B	53	28	376	356	54	30	66	34	0	45	81	0	0	18	430
C	1461	134	2303	1986	939	0	170	58	0	103	148	0	0	32	613
Mean	700	244	2012	940	3398	19	92	45	0	48	84	0	0	15	261
SUGARCANE BAGASSE (ppm)															
R	0	194	824	332	348	250	901	123	0	17	1368	0	10	14	612
A	0	796	1425	311	16197	15	382	128	0	98	955	0	34	15	0
B	0	1180	292	35	115	48	128	39	0	24	131	0	0	9	0
C	0	92	648	220	325	179	1009	121	0	20	1141	0	14	15	376
Mean	0	565	797	224	4246	123	605	103	0	40	944	0	15	13	247
YAM PEELS (ppm)															
R	0	331	631	334	263	74	272	589	0	61.5	1213	0	0	8	1516
A	287	1235	3574	353	7641	17	217	1085	0	33	1170	0	0	7	0
B	92	265	534	374	385	307	168	132	0	12	201	0	0	9	1364
C	1032	780	1356	1154	434	120	302	463	0	68	1411	11	0	15	0
Mean	353	653	1524	554	2181	130	240	567	0	44	999	3	0	10	720

Nomenclature:

R = Raw

A = Acid pretreated

B = Alkaline Pretreated

C = Hydrothermal Pretreated

Table 4. 2: Major inorganic constituents of the lignocellulosic biomass in this research.

Biomass (Raw Samples)	Elemental Composition (<i>decreasing order of abundance</i>)
Cassava peels	Ca > Fe > Si > S > Ti
Corn Cobs	Sn > Mg > P > Cl > Si

Rice husks	S > Si > Mg > P > Al
Sugarcane Bagasse	Fe > Ca > Si > Sn > S
Yam peels	Sn > Fe > Si > Ti > P

3.2. Effects of particle size

Particle size affected the elemental composition of the raw biomass being studied. This could be as a result of the presence of mineral matters of technogenic origin that are present in the biomass particles that were not broken to the particle size of $\leq 300 \mu\text{m}$ sizes or the presence of these minerals in $300 \mu\text{m}$ sized particles and absent in this same proportion in $425 \mu\text{m}$ sized particles. For example, from Table 4, Sn is present in the $425 \mu\text{m}$ micron-sized cassava particles but absent in the $300 \mu\text{m}$ sized cassava particles. Also, Cr, a trace element, was present in the $300 \mu\text{m}$ micron-sized cassava peel biomass particles but absent in the $425 \mu\text{m}$ micron-sized particles. Sn, a major constituent of unpretreated or raw $300 \mu\text{m}$ micron-sized corn cobs, was absent in the $425 \mu\text{m}$ size. This explains why varying particle sizes may also vary the inorganic mineral constituents or composition (ash content) of the biomass as corroborated by Lori et al [149] in their work on proximate and ultimate analyses of bagasse, sorghum and millet. Cassava peel biomass is typically rich in Ca with a 2014 ppm and 2098 ppm mass for $300 \mu\text{m}$ and $425 \mu\text{m}$ sized particles respectively. This kind of variation in the concentration of the elemental composition is observed all through the biomass considered in this research. The result also shows that magnesium has a higher concentration in the $425 \mu\text{m}$ microns sized biomass for all the biomass being studied except for corn cobs biomass where its concentration is higher in the $300 \mu\text{m}$ microns sized biomass. The concentration of zinc in all the biomass in this study is low and the effect of particle size on it is not too pronounced.

Table 4. 3: Effects of particle size

P.S*	Mg	Al	Si	P	S	Cl	Ca	Ti	Cr	Mn	Fe	Co	Cu	Zn	Sn
CASSAVA PEELS (ppm)															
300	55	175	630	117	287	47	2014	284	9	19	1074	0	0	18	0
425	753	851	521	244	431	42	2098	139	0	21	433	0	0	10	1028
CORN COBS (ppm)															
300	712	344	416	535	374	487	135	53	0	11	144	0	0	13	803
425	169	162	355	253	225	398	36	38	3	7	58	0	0	12	0
RICE HUSKS (ppm)															
300	511	324	1299	431	5083	33	68	29	0	18	36	0	0	0	0
425	5306	347	4605	3331	383	20	146	53	0	81	91	0	0	17	0
SUGARCANE BAGASSE (ppm)															
300	0	194	824	332	348	250	901	123	0	17	1368	0	10	14	612

425	204	311	644	341	353	207	690	66	0	10	548	0	0	9	397
YAM PEELS (ppm)															
300	0	331	631	334	263	74	272	589	0	61.5	1213	0	0	8	1516
425	1117	933	656	788	592	29	507	277	6	73	866	4	5	10	0

P.S* - Particle size

3.3 EFFECTS OF PRETREATMENT

The chemical pretreatment employed in this study was a determinant factor in the elemental composition of the ash content in the biomass being studied. The analysis of the effects of pretreatment on the elemental composition was focused on the 300 µm sized particles. The alkaline pretreatment was seen to reduce the Ca concentration in all the biomass tremendously. The acid pretreatment was seen to increase the sulfur content of the biomass. This is due to the presence of sulfur in the acid (H₂SO₄) used for the pretreatment. High concentrations of Si and Ca form low – melting–point eutectics, which can cause slagging. Salts of these elements do form surface deposits on heating equipment [150]. The five-biomass mixture has a relative concentration of chlorine with maximum concentration in corn cob and sugarcane bagasse with values of 487 ppm and 250 ppm respectively. Chlorine is a major parameter in ash deposits. Its presence reduces the melting point of ash and therefore allows for an easier deposition of ash. Al compounds also play a key role in reducing the melting point of ash. Sulfur oxides form sulphates and condense on the surface of heating equipment. They also form fly ash particles. Generally, fuels with high Ca content will have higher sulfur fixation in the ash [151], [20]. Ca and Mg in a biomass fuel increase the ash melting point temperature of the fuel, thus making it more suitable for power plant fuel as against the high concentration of potassium which will, in turn, lead to slagging and formation of hard deposit in the furnace and reboiler. The high phosphorus content of rice husks in hydrothermal pretreatment will influence the burning properties as well as cause the formation of low melting temperature ash [152].

Elements often associated with environmental toxicity are present in the biomass in very minute concentrations. Heavy elements such as Co and Cu were present only in sugarcane bagasse and yam peels and absent for all other samples. It was also noticed that Co and Cu which were present in raw sugarcane bagasse and yam peels were absent after both samples underwent alkaline pretreatment. The impact of alkaline pretreatment was evident in Mg and most of the biomass samples, reducing to 0.000 ppm or very low values. Alkaline pretreatment could be said to be relevant in removing some toxic minerals present in the biomass. Other toxic substances in the biomass include Al, Mn, Cr, and Zn but in trace concentration. It is important to also note that the presence of these substances in the given samples of sugarcane bagasse and yam peels does not validate their presence in all samples of yam peels and sugarcane bagasse as the elemental composition varies with growth processes, growing conditions (such as sunlight, geographical

location, climate, seasons), fertilizer and pesticides doses, plant distance from the source of pollution (plant environment), harvesting time, blending of different biomass types, and others [153] [132]. Sugarcane bagasse and rice husks which are characterized as herbaceous and agricultural residues (HAR) [132],[136] contained the highest concentration of silicon with values of 824 ppm and 1299 ppm respectively. Mg though present in all other samples was absent in sugarcane bagasse.

3.3.1 Acid Pretreatment

Table 4.5 and Figure 4. 1 show the elemental composition of the different inorganic matter present in the raw and pretreated samples of 300 µm sized particles of cassava peels, corn cobs, rice husks, sugarcane bagasse, and yam peels. From the table of results, it was seen clearly that acid pretreatment varied the elemental composition of the biomass by changing their amount and not necessarily the elemental constituents. Nevertheless, few samples showed the presence or absence of an element before or after pretreatment. Generally, Mg, Al, Si, P, S, Ti, and Mn showed an increase in amount across all biomass samples with exception of corn cobs for Mg, Al, Si, and P, and sugarcane bagasse for P alone. The raw sample of corn cobs contained 711.740 ppm of Mg whereas the acid pretreated sample contained 0.000 ppm of Mg. Also, the raw sample of yam peel biomass contained 0.000 ppm of Mg while the acid pretreated sample contained 286.914 ppm of Mg. This could be a result of experimental errors in introducing and removing different test samples from the XRF equipment. Further research can be carried out to verify this. The increase in the sulphur content of the acid pretreated sample against the raw sample was very high. This could be a result of the sulphur content in pretreating acid (H₂SO₄). There was a decrease in the amount of Cl and Ca for all samples after acid pretreatment. Acid pretreatment could be useful in reducing the number of certain minerals present in the biomass.

Table 4. 4: Effect of acid pretreatment on the raw biomass samples

	Mg	Al	Si	P	S	Cl	Ca	Ti	Cr	Mn	Fe	Co	Cu	Zn	Sn
CASSAVA PEELS (ppm)															
R	55	175	630	117	287	47	2014	284	9	19	1074	0	0	18	0
A	354	1111	4014	239	14044	18	1805	454	9	15	654	0	0	9	523
CORN COBS (ppm)															
R	712	344	416	535	374	487	135	53	0	11	144	0	0	13	803
A	0	76	351	142	429	198	1242	146	0	10	317	0	0	8	0
RICE HUSKS (ppm)															
R	511	324	1299	431	5083	33	68	29	0	18	36	0	0	0	0
A	774	490	4070	985	7517	14	65	58	0	28	72	0	0	9	0
SUGARCANE BAGASSE (ppm)															

R	0	194	824	332	348	250	901	123	0	17	1368	0	10	14	612
A	0	796	1425	311	16197	15	382	128	0	98	955	0	34	15	0

YAM PEELS (ppm)															
R	0	331	631	334	263	74	272	589	0	61.5	1213	0	0	8	1516
A	287	1235	3574	353	7641	17	217	1085	0	33	1170	0	0	7	0

R = Raw biomass

A = Acid pretreated biomass

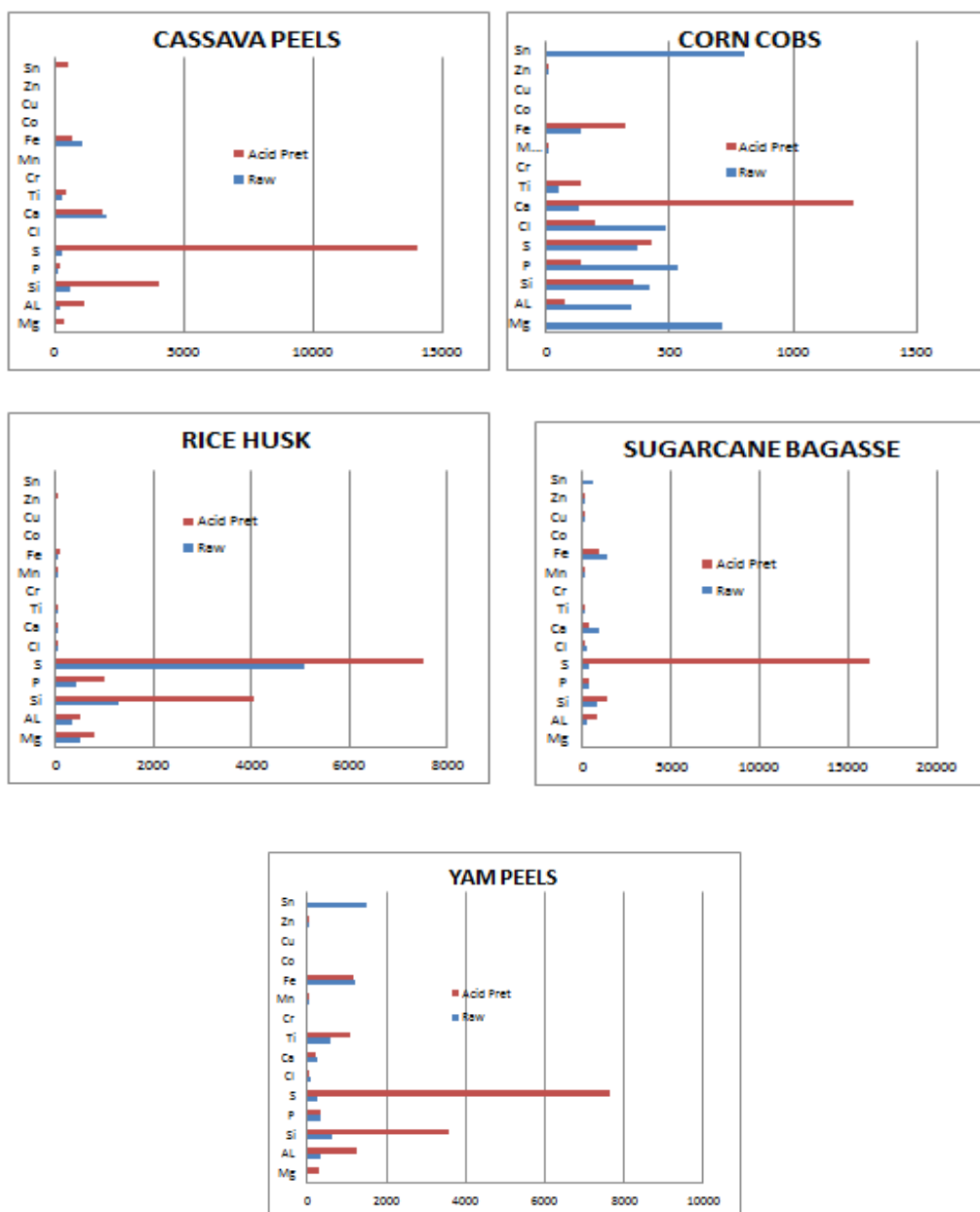


Figure 4. 1: Effect of acid pretreatment for all single samples

3.3.2 Alkaline Pretreatment

Table 4.6 and Fig. 4.2 show the results of alkaline pretreatment on the elemental composition of the different biomass. Alkaline pretreatment of the biomass samples brought about a different variation in the elemental composition of ashes from the different biomass samples. The concentration of Si and Cl in all five biomass samples decreased after alkaline pretreatment. Except for yam peels biomass, Mg, P, S, and Cl also show a decrease in concentration after pretreatment in an alkaline medium as well as Al, Cr, Mn, and Fe. Zn and Sn were seen to show a general increase in their concentration after alkaline pretreatment. The two elements were absent (showing 0.000 ppm for both) in the raw/unpretreated sample of rice husk but present after the samples were pretreated in an alkaline medium showing 18 ppm and 430 ppm respectively. The calcium concentration decreased drastically in all the biomass samples most probably because of the sodium hydroxide used for the alkaline pretreatment. Calcium is higher up the electrochemical series and for positive ions, the ones higher up the series displaces the ones lower in the series which could be the reason for the reduced calcium after the alkaline pretreatment. Another factor that could have led to the reduction of the calcium after the alkaline pretreatment could be leaching due to the high pH of the NaOH used for the pretreatment as corroborated by Osman et al. in their work [148] .

Table 4. 5: Effect of alkaline pretreatment on the raw biomass samples

	Mg	Al	Si	P	S	Cl	Ca	Ti	Cr	Mn	Fe	Co	Cu	Zn	Sn
CASSAVA PEELS (ppm)															
R	55	175	630	117	287	47	2014	284	9	19	1074	0	0	18	0
B	0	436	821	58	223	40	599	100	0	0	383	0	0	14	0
CORN COBS (ppm)															
R	712	344	416	535	374	487	135	53	0	11	144	0	0	13	803
B	41	137	194	43	81	85	84	41	0	0	124	0	0	14	1105
RICE HUSKS (ppm)															
R	511	324	1299	431	5083	33	68	29	0	18	36	0	0	0	0
B	53	28	376	356	54	30	66	34	0	45	81	0	0	18	430
SUGARCANE BAGASSE (ppm)															
R	0	194	824	332	348	250	901	123	0	17	1368	0	10	14	612
B	0	1180	292	35	115	48	128	39	0	24	131	0	0	9	0
YAM PEELS (ppm)															
R	0	331	631	334	263	74	272	589	0	61.5	1213	0	0	8	1516
B	92	265	534	374	385	307	168	132	0	12	201	0	0	9	1364

R – Raw biomass sample

B – Alkaline pretreated

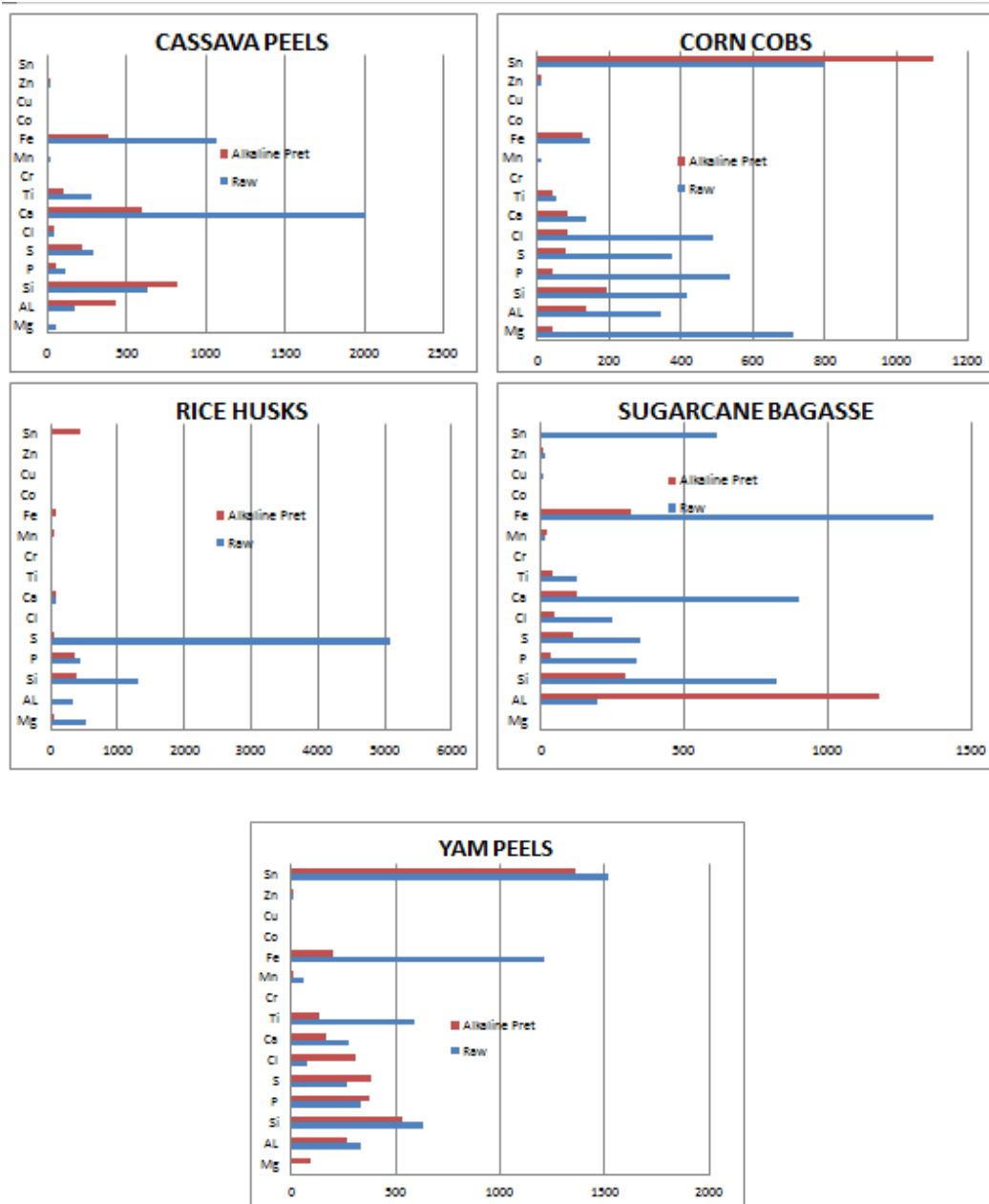


Figure 4. 2: Effect of alkaline pretreatment for all single samples

3.3.3 Hydrothermal pretreatment

Like alkaline pretreatment, hydrothermal pretreatment brought about a different variation in the elemental composition of the ash content of the five different biomass samples. Except for Co and Cu, all the other samples had no clear increase or decrease for all samples. Al, S, Cl, Ti, and Sn had a decrease in their amounts in the different samples but with exceptions in cassava peels, yam peels, for Al, S, and Cl; and corn cobs and rice husks for Ti and rice husks for Sn. Ca, Mg, Si, P, Mn, Fe, Co, Cu, and Zn all showed a general increase in their amount in the ash content of the biomass samples after pretreatment though with

exceptions. Co was 0.000 ppm in raw yam peels but 11.276 ppm in the hydrothermally pretreated sample of the same biomass. Table 4.7 and Figure 4.3 shows the effect of hydrothermal pretreatment.

Table 4. 6: Effect of hydrothermal pretreatment on the raw biomass samples

	Mg	Al	Si	P	S	Cl	Ca	Ti	Cr	Mn	Fe	Co	Cu	Zn	Sn
CASSAVA PEELS (ppm)															
R	55	175	630	117	287	47	2014	284	9	19	1074	0	0	18	0
C	393	664	1104	219	333	57	1136	176	0	11	628	0	0	9	0
CORN COBS (ppm)															
R	712	344	416	535	374	487	135	53	0	11	144	0	0	13	803
C	142	323	468	160	222	140	135	55	6	8	161	0	0	16	536
RICE HUSKS (ppm)															
R	511	324	1299	431	5083	33	68	29	0	18	36	0	0	0	0
C	1461	134	2303	1986	939	0	170	58	0	103	148	0	0	32	613
SUGARCANE BAGASSE (ppm)															
R	0	194	824	332	348	250	901	123	0	17	1368	0	10	14	612
C	0	92	648	220	325	179	1009	121	0	20	1141	0	14	15	376
YAM PEELS (ppm)															
R	0	331	631	334	263	74	272	589	0	61.5	1213	0	0	8	1516
C	1032	780	1356	1154	434	120	302	463	0	68	1411	11	0	15	0

R – Raw biomass sample

C – Hydrothermal pretreated

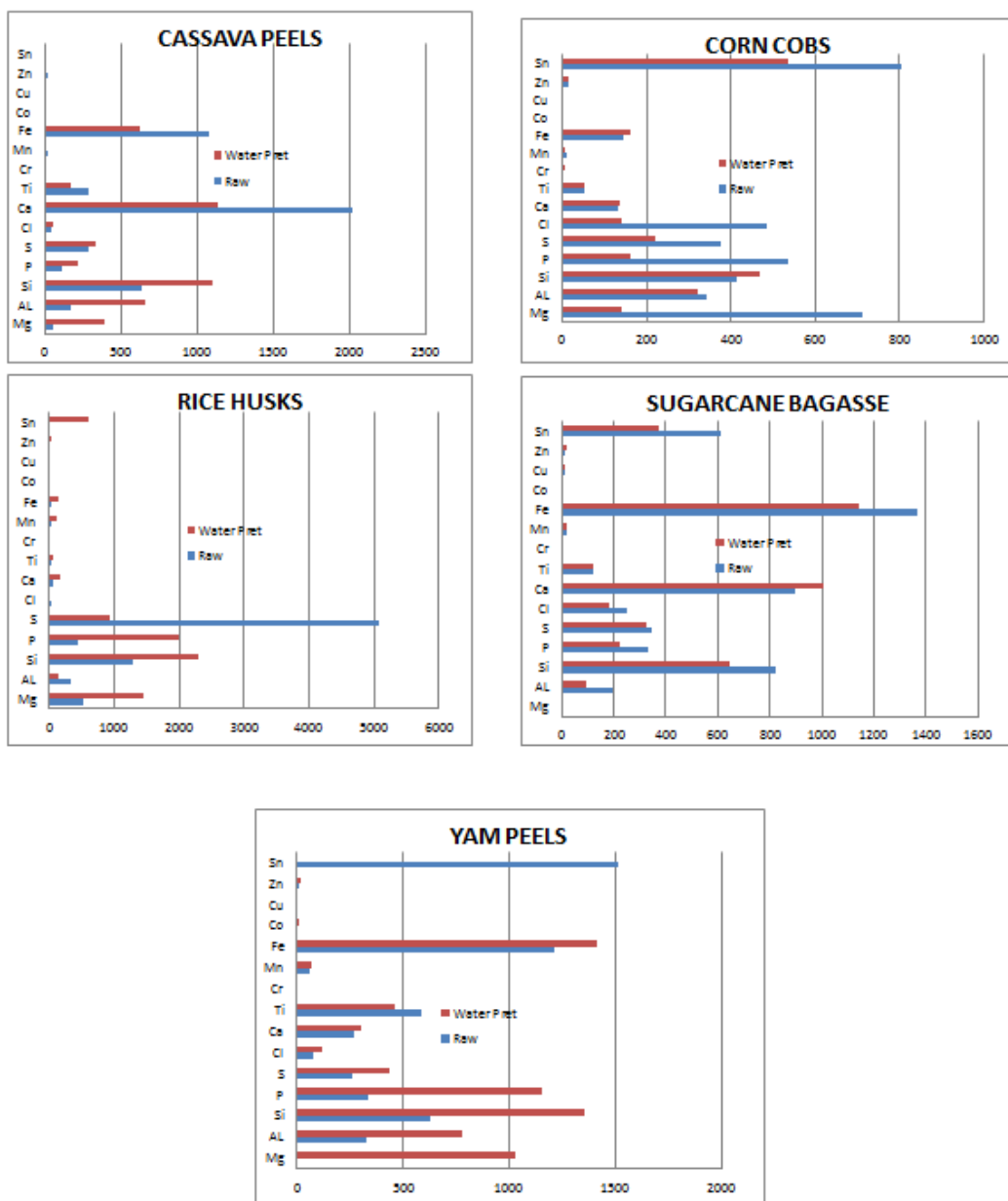


Figure 4. 3: The effect of Hydrothermal pretreatment for all single samples

3.4 EFFECTS OF MIXED BIOMASS

3.4.1 Cassava peels + Yam peels mixed biomass

Table 4. 8 and Figure 4.4 show the results of cassava + yam peels mixed biomass. The pretreated samples for cassava + yam peels mixed biomass have varying concentrations of the different elements. The average Mg, P, S, and Zn concentration in the cassava + yam peels mixed biomass was seen to have increased more than they were for the individual biomass. For example, the concentration of Mg in the mixed biomass was 1441 ppm but was 200 ppm and 353 ppm in cassava and yam respectively. A decrease in concentration was

however noticed in the cassava + yam peels mixed biomass for Al, Si, and Ti, when compared to the average composition of the elements in the individual cassava and yam, peels biomass. Ti in the cassava + yam peels mixed biomass was 181 ppm but was 253 ppm and 567 ppm in individual cassava and yam peels biomass respectively. The concentration of Cl, Ca, Cr, Mn, Fe, and Sn in the cassava + yam peels mixed biomass fell between the concentration of these elements in the individual cassava and yam peels biomass. As stated by Smith et al., [154] the composition of biomass changes when two or more biomasses are combined.

The choice of biomass for a given use depends on the requirements of the operation or use as each of these elements has its environmental and energy impact on the surroundings and the system. The results for magnesium, calcium and chlorine are in tandem with those obtained by Sadawi et al., [155] in their work on commodity fuels from biomass through pretreatment and torrefaction: effects of mineral content on torrefied fuel characteristics and quality.

Table 4. 7: Average composition of elements in cassava peels, yam peels, and cassava + yam mix

	Mg	Al	Si	P	S	Cl	Ca	Ti	Cr	Mn	Fe	Co	Cu	Zn	Sn
C (ppm)	200	597	1642	158	3722	40	1389	253	4	11	685	0	0	12	131
Y (ppm)	353	653	1524	554	2181	130	240	567	0	44	999	3	0	10	720
C + Y (ppm)	1441	523	1443	627	5421	60	492	181	3	38	685	0	3	13	648

C – Cassava peels biomass

Y – Yam peels biomass

C + Y – Cassava + yam peels biomass

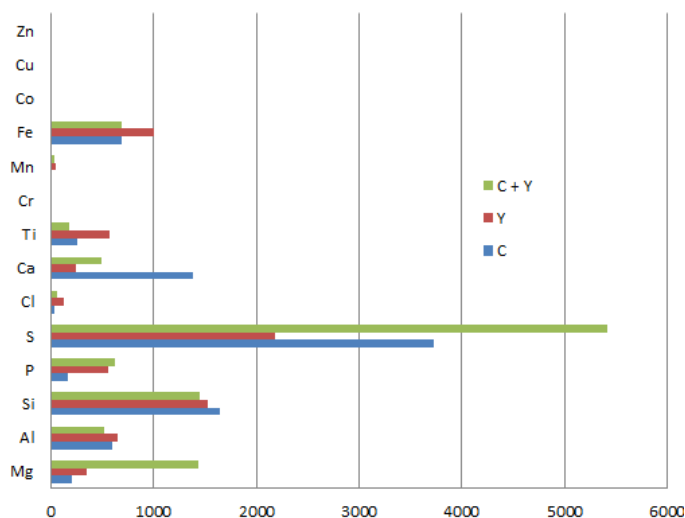


Figure 4. 4: The average elemental composition of elements in cassava peels, yam peels, and cassava + yam peels

3.4.2 Corn cobs + Rice Husks

The pretreated corn cobs + rice husks mixed biomass has a varying concentration of elemental composition as seen in Table 9 and Figure 5 respectively. The average Mg, S and Al concentration in the corn cobs + rice husks mixed biomass increased more than they are for the individual biomass. For example; the concentration of Mg in the mixed biomass was 800 ppm but 224 ppm and 670 ppm in individual corn cobs and rice husks respectively. Sulfur (S) in the mixed biomass was 9128 ppm but 276 ppm and 3398 ppm in individual biomass samples of corn cobs and rice husks respectively. A decrease in concentration was observed in the corn cobs + rice husks mixed biomass for Ca, and Sn when compared to the average composition of the elements in the individual biomass with 71 ppm for the mixed biomass and 399 ppm and 92 ppm for corn cobs and rice husks respectively. The other elements present in the biomass mixture took a position between the concentration of the individual biomass of corn cobs and rice husks with exception of Zn and Ti which had values equal to the least in the range. The result of the analysis shows there is a considerable change in the elemental composition of the mixture of the corn cobs and rice husks after pretreatment as compared to the singular biomass buttressing the fact that the combination of biomass feedstocks has a significant effect on their properties and subsequent output as corroborated in the previous work by the authors [144]. The high value of Phosphorus in the ash of individual and combined biomass of corn cobs and rice husks could be due to the use of fertilizers for crop cultivation [156].

Table 4. 8: Average composition of elements in corn cobs, rice husks, and corn cobs + rice husks mix

	Mg	Al	Si	P	S	Cl	Ca	Ti	Cr	Mn	Fe	Co	Cu	Zn	Sn
Cc	224	220	357	220	276	228	399	74	1	7	187	0	0	13	611
R	670	244	2012	940	3398	19	92	45	0	48	84	0	0	15	261
Cc + R	800	387	1370	867	9128	39	71	45	0	29	98	0	0	13	239

Cc – Corn cobs

R – Rice husks

Cc + R - corn cobs + rice husks mix

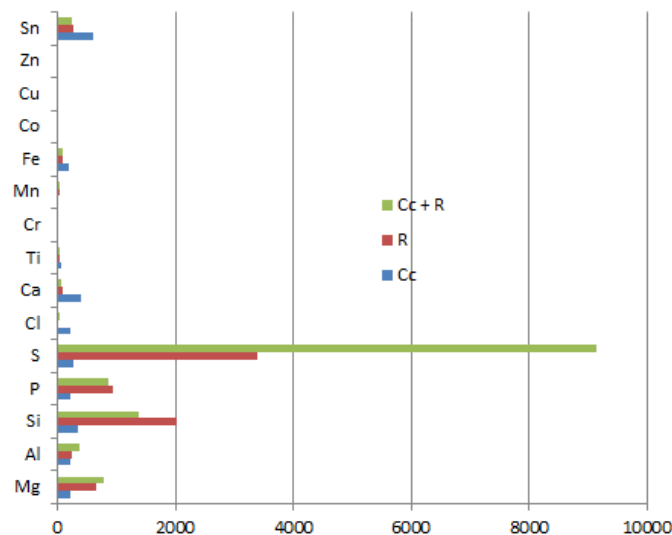


Figure 4. 5: The average elemental composition of elements in Cassava Peels, Yam peels, and Cassava+ Yam peels

3.4.3 Mixture of All 5 biomass

In analyzing the impact of the mixture of all 5 biomass samples on the overall elemental composition of the pretreated samples, the average values of each element in the combination of all the 5 biomass were included in Tables 4.10 and 4.11. This was also depicted in Figure 4. 6. From Table 4. 11, it was seen that the amount of Al, Si, and Ti in the different biomass considered was the peak in the overall mixture; with values of 909 ppm for the 5 biomass mixtures and 565 ppm, 523 ppm, and 387 ppm for S, C + Y, and Cc + R respectively for Al. It was also evident from Table 4.10 that Mg (with exception of sugarcane bagasse where it is 0 ppm), Mn, and Zn had the least values for the 5 biomass mixtures with 28 ppm in all mix and 40 ppm, 38 ppm, and 29 ppm for S, C + Y, and Cc + R respectively. Although the difference in the amount of Mn and Zn is insignificant, compared to the difference in the amount of Mg.

Table 4. 9: Composition of elements in cassava peels + yam peels mix, corn cobs + rice husks mix, and the mix of all 5 biomass samples after pretreatment.

	Mg	Al	Si	P	S	Cl	Ca	Ti	Cr	Mn	Fe	Co	Cu	Zn	Sn
CASSAVA PEELS + YAM PEELS (ppm)															
A	900	604	1021	591	1567	6	306	147	8	43	660	0	0	11	0
B	0	266	581	88	130	39	287	88	0	17	380	0	0	10	1045
C	1441	700	2726	1201	461	135	883	308	0	54	1015	0	10	18	899
Mean	780	523	1443	627	5421	60	492	181	3	38	685	0	3	13	648
CORN COBS + RICE HUSKS (ppm)															
A	1465	485	1856	1236	18135	9	87	55	0	41	138	0	0	15	0
B	136	288	884	497	120	69	56	34	0	17	59	0	0	12	479
Mean	800	387	1370	867	9128	39	71	45	0	29	98	0	0	13	239

ALL MIX (ppm)															
A	596	1644	3683	313	9867	38	479	782	0	23	794	0	0	7	494
B	445	931	2502	328	5864	143	223	335	0	9	207	0	0	7	664
C	632	151	1287	1139	308	119	156	60	0	53	170	0	0	18	587
Mean	558	909	2491	593	5346	100	286	392	0	28	390	0	0	11	582

A = Acid pretreated B = Alkaline Pretreated C = Hydrothermal pretreated

Table 4. 10: Average composition of elements in sugarcane bagasse, cassava peels + yam peels mix, corn cobs + rice husks mix, and the mixture of all 5 biomass samples.

	Mg	Al	Si	P	S	Cl	Ca	Ti	Cr	Mn	Fe	Co	Cu	Zn	Sn
S	0	565	797	224	4246	123	605	103	0	40	1368	0	10	14	612
C + Y	1441	523	1443	627	5421	60	492	181	3	38	685	0	3	13	648
Cc + R	800	387	1370	867	9128	39	71	45	0	29	98	0	0	13	239
All Mix	558	909	2491	593	5346	100	286	392	0	28	390	0	0	11	582

S = sugarcane bagasse C + Y = Cassava plus yam peels

Cc + R = Corn cobs plus rice husks

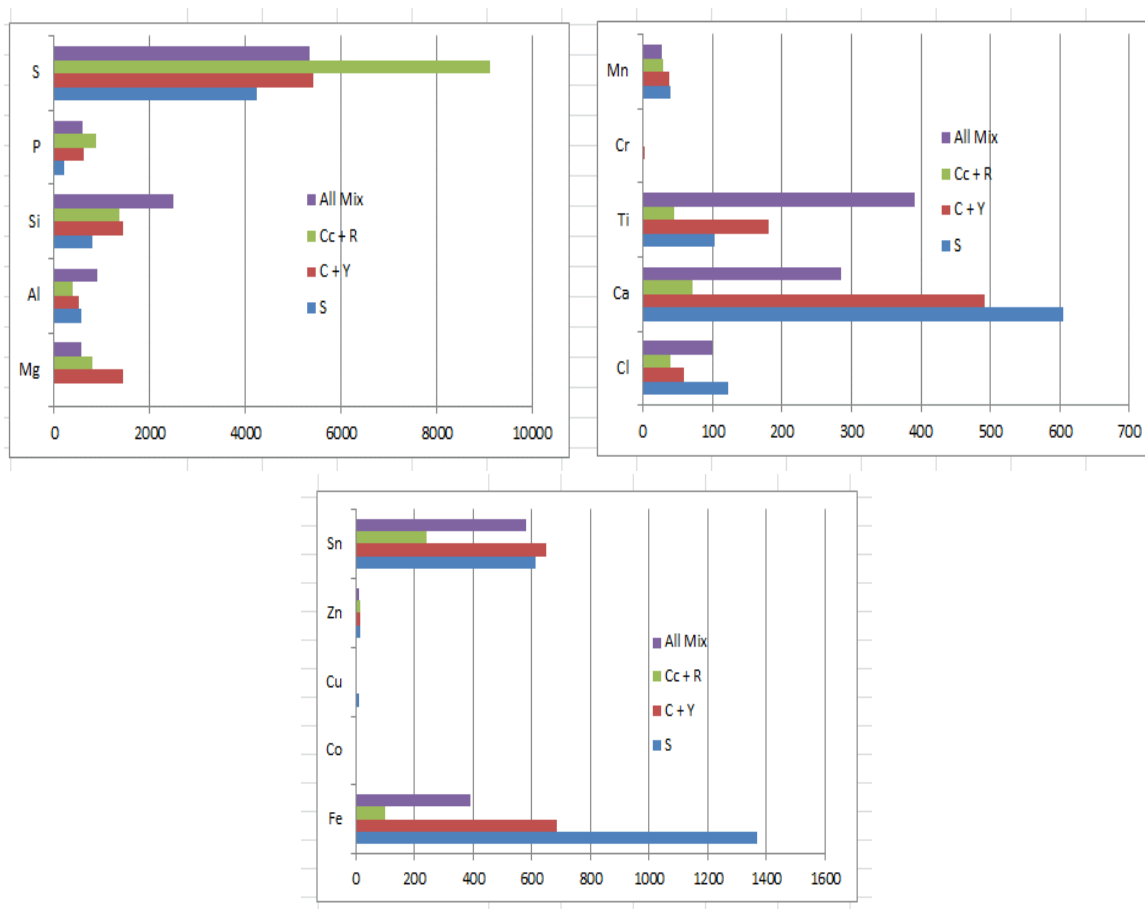


Figure 4. 6: The average elemental composition of elements in sugarcane bagasse, cassava peels + yam peels mix, corn cobs + rice husks mix, and the mix of all 5 biomass samples.

4.0 CONCLUSION

The results obtained from the XRF analysis of the five lignocellulosic biomass as well as their mixed forms show that pretreatment of samples plays a significant role in modifying the elemental composition of the biomass and this varies with pretreatment type as well. Thus, the effect of acid pretreatment on a biomass sample differs from that of alkaline pretreatment on that same sample. The alkaline pretreatment on the biomass samples show better results, especially on the hybridized (mixed) feedstocks with sulfur having lower concentration than that of acid pretreatment. In the hybridized biomass, the concentration of Al, Si, and Ti in the different biomass considered was the highest in the overall mixture of all the five biomass. For Aluminum, the reported values were 909 ppm for the five-biomass mixture and 565 ppm, 523 ppm, and 387 ppm for S, C + Y, and Cc + R respectively. Information on the elemental and ash composition of biomass is vital for a bioethanol processing plant as the elemental and ash components of the biomass should be given adequate consideration during process design and the selection of process parameters because certain elements may pose a harm to the ecosystem if their concentrations are too high.

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AUTHOR CONTRIBUTION

A.A.A and D.L conceptualized the research, A.A.A and P. O carried out the experimental work and developed the manuscript, D.L corrected and updated the manuscript.

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Chapter SIX

Paper 5, HSNLBFBP:MOPFPP

Hybridization of selected Nigerian lignocellulosic biomass feedstocks for bioethanol production: modeling and optimization of pretreatment and fermentation process parameters using response surface methodology

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PAPER 5

Hybridization of selected Nigerian lignocellulosic biomass feedstocks for bioethanol production: modeling and optimization of pretreatment and fermentation process parameters using response surface methodology

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ABSTRACT

In this study, hybridized feedstocks (mixtures of biomass) of cassava peels plus yam peels, as well as corn cobs plus rice husks biomass, were optimized using the response surface methodology centered on the statistical design of experiments (DOE) of the Box-Behnken design (BBD), to produce bioethanol. The feedstocks were locally sourced, hybridized (mixed), pretreated, and fermented before being distilled in a UOP3CC continuous distillation column. The BBD was applied using a 3-level, 3-factor process variables using pH, time, and particle size, and indicated as X_1 , X_2 , and X_3 , respectively. The bioethanol yield from the two hybridized biomass feedstocks was predicted by the developed quadratic polynomial models from BBD. For the hybridized biomass mixture of cassava peels plus yam peels, the optimal condition was statistically predicted as pH 5.00, fermentation time of 120.00 hours, and particle size of 362.5 microns, the predicted bioethanol yield under the optimal condition was 115.75 ml per 1500 g of hybridized biomass and the average volume of bioethanol obtained was 125.00 ml per 1500 g of biomass, which is within the projected range of the model equation, same applies to rice husks plus corn cobs hybridized biomass, but with a better prospect for bioethanol production.

Keywords: Bioethanol, Box–Behnken design, pretreatment, modeling, optimization

1. INTRODUCTION

Lignocellulosic biomass obtained from agricultural residues has been a good raw material in the production of fuel bioethanol as they are readily available, cheaply acquired (as they are waste materials that would have been discarded), and most importantly, they are very rich in polysaccharide (cellulose, hemicellulose,

and lignin) (1). Nigeria is blessed with a large expanse of arable land for crop cultivation from which lignocellulosic biomasses can be obtained as wastes after processing. Till now, the economy of Nigeria is worryingly dependent on crude oil with the country occupying 8th position among the largest global oil producers and accounts for close to 3% of the entire world's oil production (2). Crude oil being a fossil fuel is non-renewable and not environmentally friendly (3). The use of bioethanol produced from lignocellulosic biomasses such as cassava peels, yam peels, rice husks, corn cobs, among others as an alternative source of fuel for automobiles is a positive development and is currently being embraced by many countries of the world. Bioethanol is an evaporative, colorless liquid that is produced by the fermentation and subsequent distillation of starchy food crops such as yam, corn, cassava, potatoes, and so on. The production of bioethanol meant for augmentation/replacement of the conventional automobile fuel (gasoline) from lignocellulosic biomass is a process that is vigorously being pursued by many researchers world over because of its environmental friendliness and renewability (4,5). In comparison with gasoline fuel, bioethanol is more beneficial because of its very high-octane number, wider flammability limits, better heat of vaporization, and most importantly, the lesser release of acid gases such as carbon (iv) oxide and Sulphur (iv) oxide (6). Aside from its use as fuel, bioethanol obtained from lignocellulosic biomasses can be used for other important purposes in the food industry such as for food preservation (7).

At present globally, the use of combined lignocellulosic biomass feedstock for bioethanol production is not being adequately harnessed. There is a dearth of information globally on the performance of hybridized (mixed) feedstocks in bioethanol production, however, the little information that could be gathered from previous researches shows that the use of combined feedstock can influence massive savings in the production process when compared to the use of single feedstocks as it ensures availability of enough feedstocks for bioethanol production. Also, the information available shows that the use of mixed feedstock in bioethanol production gives a higher yield of bioethanol than the single feedstocks (8).

The first bioethanol production arrangement proposed involves cellulose hydrolysis followed by fermentation of the glucose generated during the enzymatic process. Separate hydrolysis and fermentation (SHF) is the name for this setup, which has a sequential nature. The fundamental advantage of SHF is that each phase can be carried out at its optimal operating temperature and pH (for example, cellulose hydrolysis at 50°C and pH of 4.5 and yeast fermentation at 32°C and pH of 4–5). SHF is the technology with the most potential for implementation in demonstration facilities, semi-industrial facilities, or on a commercial scale. In fact, this technology is used in most the existing demonstration plants for bioethanol production from lignocelluloses (9).

The modeling and optimization of the bioethanol production process help in the improvement of the process design and operation of process units for improved production of bioethanol from some of the available lignocellulosic biomass available in Nigeria. The importance of bioethanol in the emerging renewable energy development in Nigeria makes the optimization of its production process a worthy venture to provide information that serves as a reference for future researchers and industrialists in this field of study. Numerous designs of experiments (DOEs) notably Box-Behnken design (BBD), central composite design (CCD), and face-centered composite design (FCCD) can be used for research purposes (10).

Response surface methodology (RSM) is a compendium comprising of mathematical and statistical methods used for optimizing processes involving many process variables to give desirable responses and the major goal is to optimize the responses obtained. RSM expresses the impact of the identified process variables singly and/or their collaborative effects on the response of the process. From a few experiments, RSM develops a huge quantity of information that describes the characteristics of the system and develops a second-order polynomial projecting model that correlates the response of the process to the independent process variables (11,12).

Box -Behnken design (BBD) is a set of rotatable or almost rotatable second-order models centered on 3-level partial factorial models. The BBD is an effective design for response surface methodology as it evaluates factors of quadratic model, develops successive models, utilizes blocks, and identifies lack of fit of a model (13).

RSM comes with the advantages of requiring a much lower number of experimental runs required to assess numerous parameters (independent variables) and their relationships, also, the process can be easily modeled mathematically and is time and cost-saving. Other design methods such as artificial Neural network (ANN) has a major disadvantage of requiring numerous data points for the analysis (14)

Timung et al, (15) worked on the comparative study of the optimization of dilute acid and hot water pretreatment of different lignocellulosic biomass. The major focus of their work involved the comparison of the total reducing sugars (TRS) after the acid and hot water pretreatment on the different lignocellulosic biomass and the results obtained showed that the maximum production of TRS was noticed at run order 8 in both the acid and hot water pretreatment for sugar cane bagasse biomass.

Chen et al. (16), worked on understanding alkaline pretreatment parameters for corn stover enzymatic saccharification. The total number of experiments required to investigate the correlation between pretreatment parameters and compositional change of pretreated corn stover, as well as its glucan/xylan transformation was investigated using the central composite design to reduce the total number of

experiments. The result of their work showed that the sodium hydroxide loading is the most prevailing variable for enzymatic digestibility.

The objective of this work is to optimize the production of bioethanol from hybridized i.e a mixture of cassava peels plus yam peels and corn cobs plus rice husks biomass using the Box-Behnken design (BBD) of the response surface methodology (RSM). Hybridization of the biomass feedstock is expected to impart some unique behaviors to the process which ultimately improves the bioethanol yield and purity of the produced bioethanol. Furthermore, there is little information about the potentials of yam peels biomass for bioethanol production in the literature. The hybridized feedstocks are more representative of commercial practice, as it would be difficult to operate at scale with single biomass sources. This is the particularly novel component of this study. The BBD was used to generate the experimental runs to acquire data which were then used for the modeling and optimization of the pretreatment and fermentation parameters namely; time, pH, and particle size for response surface methodology. The success of this investigation will help to determine the best approach for bioethanol production from hybridized (mixed) biomass of different particle sizes.

2.0 EXPERIMENTAL SECTION

2.1. Materials and Biomass preparation and pretreatment.

Materials and Biomass provision. The rice husks were obtained from a local rice mill in Ekperi, Etsako Central Local government Area of Edo State Nigeria, the corn cobs were obtained from Ogume, Ndokwa West Local Government Area of Delta State Nigeria, the cassava peels, yam peels, and sugar cane bagasse were all sourced from Effurun, Uvwie Local Government Area of Delta State Nigeria. The biomass was afterward sundried for about seven days and then grounded in a mill after which they were sieved into two particle sizes of 300 and 425 microns respectively. Analytical grade chemical reagents such as sodium hydroxide pellets, hydrogen peroxide, and tetraoxosulphate (VI) acid were used. To study the effects of biomass combinations, 750 g each of 300 microns particle size cassava and yam peels biomass and corn cobs and rice husks biomass was measured and transferred into different vessels. The pretreatment and characterization of the biomass have been discussed in our previous work (17). Approximately 10 liters of each of the pretreated biomass were obtained and made up to 15 liters by the addition of water and transferred to the fermentation vessels.

2.2 Fermentation

Industrial grade *S. cerevisiae* Y-461159 yeast produced by Nike Chemical India was used for the fermentation of the pretreated hybridized biomass samples. The procedure used by Pratto et al [18] was followed in the activation of the *S. cerevisiae* yeast. The process was activated in an Erlenmeyer flask. 10 g/L yeast extract, 20 g/L peptones, and 40g/L glucose were thoroughly mixed in an incubator at a

temperature of 34°C for four hours before about 3 g/L dry cell weight each was introduced in the pretreated hybridized biomass after the pretreated samples were adjusted for pH in the range of 5.7 to 6.0. They were then left to ferment for 5 and 7 days at ambient temperature before being harvested for distillation.

2.3 Distillation of produced bioethanol

The fermented supernatants were harvested on the 5th and 7th day and made to undergo distillation in a UOP3CC continuous distillation column supplied by Armfield. The UOP3CC is equipped with a matching computer, fully installed with Armfield software, to supply output information received from its sensors. The distillate received from the UOP3CC continuous distillation column was measured with a measuring cylinder and recorded.

2.4 Optimization process using response surface methodology (RSM)

The use of the conventional approach to determine the optimum levels of all the process parameters comes with some disadvantages such as; waste of time, more experimental runs which may not be reliable and makes the total process cost unnecessarily over-bloated. All these shortcomings can be mitigated by the optimization of all the process conditions using RSM, which is an example of the statistical experimental model. This approach is most notably an expense of time and resources as well as more reliability. The Design-Expert 8.0.3.1 software (Stat-Ease Inc., Minneapolis, MN, USA) was used for the design, modeling, and optimization studies. Three different parameters were chosen as independent variables: pH, time, and Particle Size, and assigned as X_1 , X_2 , and X_3 , respectively in Table 5.1. The low, middle and high levels of each variable were coded as -1, 0, and +1, respectively. Box-Behnken was employed for the design of the experiment for the optimization studies involving Cassava peels plus yam peels biomass. In this study, Box-Behnken design (BBD), a class of rotatable or nearly rotatable second-order design based on three-level incomplete factorial designs, was selected for the experimental design. Box-Behnken can be applied as an effective method to develop the second-order response models. The Box-Behnken consists of three sections including the full or fractional factorial design points (where the factor levels were coded to the upper level to +1 and the lower level to -1 values), axial points (sometimes called “star” points), and the center point. A three-level-three-factor design was applied, which generated 17 experimental runs for bioethanol production as shown in Table 5.2. This included 12 factorial points and 5 central points to supply data involving the core of the experimental region. Response surface methodology (RSM) was used to optimize the bioethanol production process from the hybridized feedstocks and regression equation analysis was used to assess the response surface model. To relate the response variable to the independent factors, numerous regressions were used to match the coefficient of the polynomial model of the response. The property of the fit of the model was assessed using the test of significance and analysis of variance

(ANOVA). The generalized response surface model for describing the variation in the response variable is given as:

$$Y = b_0 + \sum_{i=1}^k b_i X_i + \sum_{i=1}^k b_{ii} X_i^2 + \sum_{i < j}^k b_{ij} X_i X_j + e \quad (2)$$

Where Y is the response variable, b_0 is the intercept value, b_i ($i = 1, 2, \dots, k$) is the first-order model coefficient, b_{ij} is the interaction effect, and b_{ii} represents the quadratic coefficients of X_i . X_i and X_j are the input variables that influence the response variable and e represents the random error.

Table 5. 1: Coding of Experimental Factors and Levels for Bioethanol Production from hybridized biomass samples

Variable	Unit	Symbols	Coded factors		
			-1	0	+1
pH		X_1	5	7.5	10
Time	hr	X_2	120	144	168
Particle Size	Microns	X_3	300	362.5	425

Table 5. 2: Box Behnken for Three Independent Factors for Bioethanol Production showing Coded and Actual Value (in bracket)

Std Run	X_1	X_2 (hr)	X_3 (microns)
1	-1 (5)	-1 (120)	0 (362.5)
2	1 (5)	-1 (120)	0 (362.5)
3	-1 (5)	1 (168)	0 (362.5)
4	1 (10)	1 (168)	0 (362.5)
5	-1 (5)	0 (144)	-1 (300)
6	1 (10)	0 (144)	-1 (300)

7	-1 (5)	0 (144)	1 (425)
8	1 (10)	0 (144)	1 (425)
9	0 (7.5)	-1 (120)	-1 (300)
10	0 (7.5)	1 (168)	-1 (300)
11	0 (7.5)	-1 (120)	1 (425)
12	0 (7.5)	1 (168)	1 (425)
13	0 (7.5)	0 (144)	0 (362.5)
14	0 (7.5)	0 (144)	0 (362.5)
15	0 (7.5)	0 (144)	0 (362.5)
16	0 (7.5)	0 (144)	0 (362.5)
17	0 (7.5)	0 (144)	0 (362.5)

2.5 Analysis of the produced bioethanol

The produced bioethanol samples were then taken to Lighthouse Petroleum Engineering Company Limited, Effurun GRA, Delta State, Nigeria for characterization for physical and fuel properties using the test methods as shown in Table 5.3.

Table 5. 3. Test methods used for characterization of the produced bioethanol

Parameter	Test method
Boiling Point, °F	ASTM D2892
Density, lb/gal	ASTM D1298
RVP, psi	ASTM D 323-99a
Flashpoint, °C	ATM D93 -13
Heat of vaporization, Btu/lb	ASTM E2071
Autoignition point, °F	ASTM E659, ASTM D1929
Flammability Limit, %	ASTM E918
Air: Fuel Ratio, Weight %	
Flame Temperature, °F	
Heat of Combustion, Btu/gal	ASTM D240
Octane number	ASTM D 2700
Lower Heating Value, Btu/lb	ASTM E711, ASTM D5865

Higher Heating value, Btu/lb	ASTM E711, ASTM D5865
Melting point, °F	ASTM D 87
Specific Gravity	ASTM D 1298-99

3.0 RESULTS AND DISCUSSION

3.1 Regression model and statistical analysis for the produced bioethanol from the hybridized feedstocks

Tables 5.4 and 5.5 depict the data for actual, predicted and residual values gotten and the parity curve is shown in Figure 5.1. Tables 5.6 and 5.7 show the results of BBD analysis of variance (ANOVA) for the response surface quadratic model. The outcomes revealed that the p-values of X_1 , X_2 , and X_1^2 of the model terms were significant (i.e. $p < 0.05$) while others are non-significant for the two hybridized feedstocks. The high Fisher test F-value of 11.98 and 22.80 as shown in the analysis of Variance (ANOVA) for Regression Equation of Tables 5.6 and 5.7 for cassava plus yam peels and rice husks plus corn cobs respectively implies the model is significant which is also reflected in X_1 , X_2 , and X_1^2 . A similar result (F-value of 11.08) was obtained by Maurya et al (2013) in their work on bioethanol production sugarcane bagasse [19]. F-value is a statistically acceptable degree of how perfectly the factors depict the variation in the data about its mean [20]. The data obtained for both feedstocks fitted best to a linear and quadratic mathematical model and exhibited a low standard deviation. The values of the coefficient of determination (R^2) in both cases indicate uniformity between the experimental values and the predicted values. Guan and Yao (2008) [21], recommended that R^2 should be at least 0.80 for a reliable fit of a model. In this case, R^2 of the models obtained were 0.9390 and 0.9670 for the cassava peels plus yam peels biomass and rice husks plus corn cobs biomass respectively, which indicated that the sample variation of 93.90 % and 96.70 % for bioethanol volume was attributed to the independent variables. The result of R^2 obtained in this study conforms with that obtained by Lina et al (2020) in their work on corn and rice straw which was approximately 93 %. This observation implies that the models have proven fit for the suitable representation of the definite relationship among the selected factors as corroborated by Jeya et al 2009 [22]. Similarly, the adjusted determination of coefficient, R^2_{adj} of 0.8606 and 0.9246 proves that the model has a high significance (Table 5.8 and 5.9). A large difference between R^2 and R^2_{adj} indicates that non-significant terms are involved in the model [23]. Adequate precision evaluates the signal to noise ratio, a ratio greater than 4 is desired [24]. Hence, the adequate precision of 10.664 and 15.655 indicates an adequate signal, and these models can be used to navigate the design space. Therefore, the quadratic mathematical models obtained in this study could be used in the theoretical prediction of bioethanol production from a liquid extract containing a mixture of cassava plus yam peels biomass as well as rice husks plus corn cobs biomass. The

final equation in terms of coded factors for the Box-Behnken design response surface quadratic model for cassava peels plus yam peels biomass can be expressed as:

$$Y = 9.00 - 34.25X_1 - 4.00X_2 + 3.25X_3 + 17.25X_1X_2 - 4.75X_1X_3 - 9.75X_2X_3 + 37.38X_1^2 + 13.87X_2^2 + 3.3X_3^2 \quad (3)$$

While that of rice husks plus corn cobs biomass is expressed as:

$$Y = 26.00 - 13.50X_1 - 40.50X_2 + 4.50X_3 + 9.00X_1X_2 - 6.00X_1X_3 - 34.00X_2X_3 + 12.00X_1^2 + 49.00X_2^2 + 10.00X_3^2 \quad (4)$$

Where Y is the bioethanol volume yield, X_1 is pH, X_2 is time and X_3 is particle size. The low standard error observed in the intercept and all the model terms in Tables 5.10 and 5.11 prove that the regression model suits the data favorably and the prediction is okay. The Variance Inflation Factor (VIF) gotten in this work indicated that the center points are orthogonal to all other factors in the models.

Table 5. 4: Experimental Data for Observed Yield, Predicted Yield, and Residual Values for Bioethanol Production from cassava peels plus yam peels

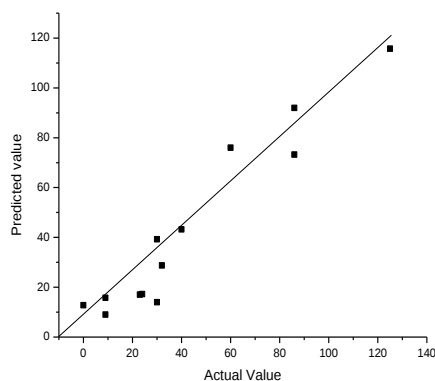
Std	Observed Volume (ml/1500 g biomass)	Predicted Volume (ml/1500 g biomass)	Residual Values
1	125.00	115.75	9.25
2	0.000	12.75	-12.75
3	86.00	73.25	12.75
4	30.00	39.25	-9.25
5	60.00	76.00	-16.00
6	23.00	17.00	6.00
7	86.00	92.00	-6.00
8	30.00	14.00	16.00

9	24.00	17.25	6.75
10	32.00	28.75	3.25
11	40.00	43.25	-3.25
12	9.00	15.75	-6.75
13	9.00	9.00	0.000
14	9.00	9.00	0.000
15	9.00	9.00	0.000
16	9.00	9.00	0.000
17	9.00	9.00	0.000

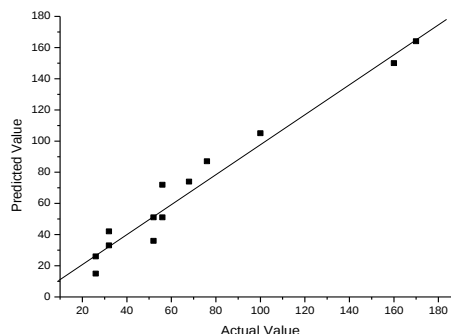
Table 5. 5: Experimental Data for Observed Yield, Predicted Yield, and Residual Values for Bioethanol Production from rice husks plus corn cobs biomass

Std	Observed volume (ml/1500 g biomass)	Predicted volume (ml/1500 g biomass)	Residual value
1	160.00	150.00	10.00
2	100.00	105.00	-5.00
3	56.00	51.00	5.00
4	32.00	42.00	-10.00
5	52.00	51.00	1.00
6	52.00	36.00	16.00
7	56.00	72.00	-16.00
8	32.00	33.00	-1.00
9	76.00	87.00	-11.00
10	68.00	74.00	-6.00
11	170.00	164.00	6.00
12	26.00	15.00	11.00
13	26.00	26.00	0.000
14	26.00	26.00	0.000
15	26.00	26.00	0.000
16	26.00	26.00	0.000

17	26.00	26.00	0.000
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A: Cassava peels plus yam peels biomass



B: Rice husks plus corn cobs biomass

Figure 5. 1: Parity plot showing the predicted values versus actual values.

Table 5. 6: Analysis of variance (ANOVA) for response surface quadratic model (Cassava peels plus yam peels).

Source	Sum of Squares	Df	Mean Square	F-value	p-value
X_1	9384.50	1	9384.50	55.09	0.0001
X_2	128.00	1	128.00	0.75	0.4148
X_3	84.50	1	84.50	0.50	0.5040
X_1X_2	1190.25	1	1190.25	6.99	0.0333
X_1X_3	90.25	1	90.25	0.53	0.4903
X_2X_3	380.25	1	380.25	2.33	0.1788
X_1^2	5881.64	1	5881.64	34.53	0.0006
X_2^2	810.59	1	810.59	4.76	0.0655
X_3^2	49.96	1	49.96	0.28	0.6121

Table 5. 7: Analysis of variance (ANOVA) for response surface quadratic model (Rice husks plus corn cobs).

Source	Sum of Squares	Df	Mean Square	F-value	p-value
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X ₁	1458.00	1	1458.00	9.47	0.0179
X ₂	13122.00	1	13122.00	85.21	< 0.0001
X ₃	162.00	1	162.00	1.05	0.3392
X ₁ X ₂	324.00	1	324.00	2.10	0.1902
X ₁ X ₃	144.00	1	144.00	0.94	0.3658
X ₂ X ₃	4624.00	1	4624.00	30.03	0.0009
X ₁ ²	606.32	1	606.32	3.94	0.0876
X ₂ ²	10109.47	1	10109.47	65.65	< 0.0001
X ₃ ²	421.05	1	421.05	2.73	0.1422

Table 5. 8: Analysis of Variance (ANOVA) for Regression Equation (Cassava peels plus yam peels biomass)

Source	Sum of Squares	Df	Mean Square	F-value	p-value
Model	18363.03	9	2040.34	11.98	0.0018
Pure Error	0.0000	4	0.0000		
Cor Total	19555.53	16			

$R^2 = 0.9390$, $\text{Adj.}R^2 = 0.8606$, Adequate Precision = 10.664

Table 5. 9: Analysis of Variance (ANOVA) for Regression Equation (Rice husks plus corn cobs)

Source	Sum of Squares	Df	Mean Square	F-value	P-value Prob > F
Model	31600.12	9	3511.12	22.80	0.0002
Pure Error	0.000	4	0.000		
Cor Total	32678.12	16			

R-Squared = 0.9670, Adj R² = 0.9246, Adequate Precision = 15.655

3.2 Effects of process parameters on bioethanol production

The three-dimensional response surface plots were obtained by plotting the response (bioethanol yield) on the z-axis against two variables on the x- and y-axis.

3.2.1 Interactive effect of Time and pH on hybridized Biomass

The response surface plot of the interaction of time and pH on bioethanol production is presented in Figure 5.2. For the acid pretreated biomass mixtures, the maximum bioethanol of 125 ml/1500g of hybridized cassava plus yam peels biomass was obtained at a time of 120 hours and a pH of 5.0 while that of rice husks plus corn cobs biomass was 160 ml/1500 g at a time of 120 hours and a pH of 5.0. The plots show that increasing the time of fermentation with a corresponding increase in the pH above 6.0 in an acidic pretreatment condition is unfavorable to bioethanol production from the hybridized mixture of yam peels plus cassava peels as well as rice husks plus corn cobs biomass. Besides, in a basic pretreatment condition, for hybridized cassava peels plus yam peel biomass, maximum bioethanol yield is 30 ml/1500 g biomass at a pH of 10.0 and at a time of 168 hours while that of rice husks plus corn cobs biomass was 52 ml/1500 g biomass at a pH of 10.0 and a time of 144 hours. However, in a hot water hydrolysis condition near-neutral pH, maximum bioethanol yield is 40 ml/1500 g biomass at a pH of 7.5 and a time of 120 hours for cassava peels plus yam peel biomass, while that of rice husks plus corn cobs biomass was 170 ml/1500 g biomass mixture at a pH of 7.5 and time of 120 hours. This indicates that maximum bioethanol production for cassava peels plus yam peels biomass is dependent on the time and pH, which should be between 120 hours and 136 hours and 5.0 and 6.0 for time and pH respectively, while for rice husks plus corn cobs biomass, there is greater flexibility in the optimum pH for bioethanol production from the hybridized biomass, ranging between 5.0 and 7.5.

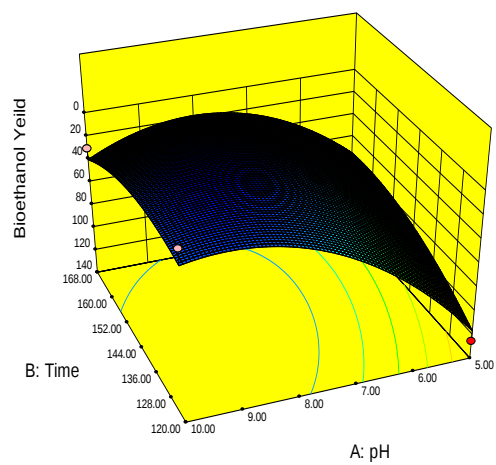
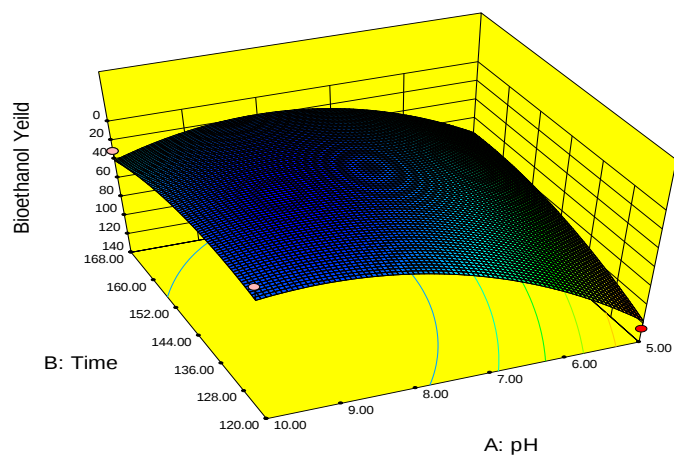
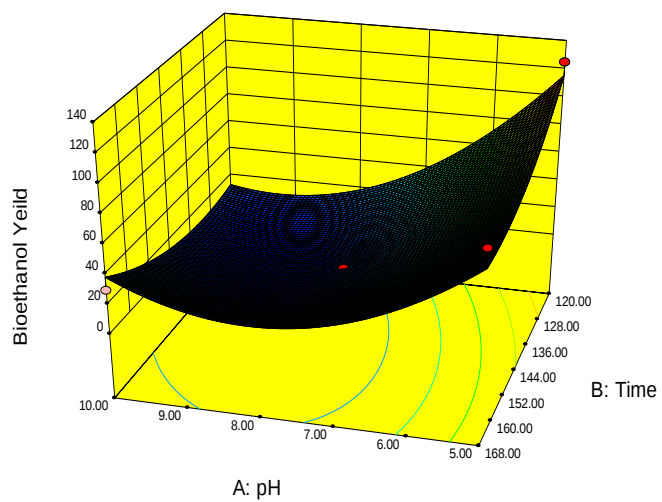
Table 5. 10: Regression Coefficients and Significance of Response surface Quadratic Model for Cassava peels plus yam peels

Factor	Coefficient	Df	Standard Error	95% CI		VIF
	Estimate			low	High	
Intercept	9.00	1	5.84	-4.80	22.80	-
X ₁	-34.25	1	4.61	-45.16	-23.34	1.00

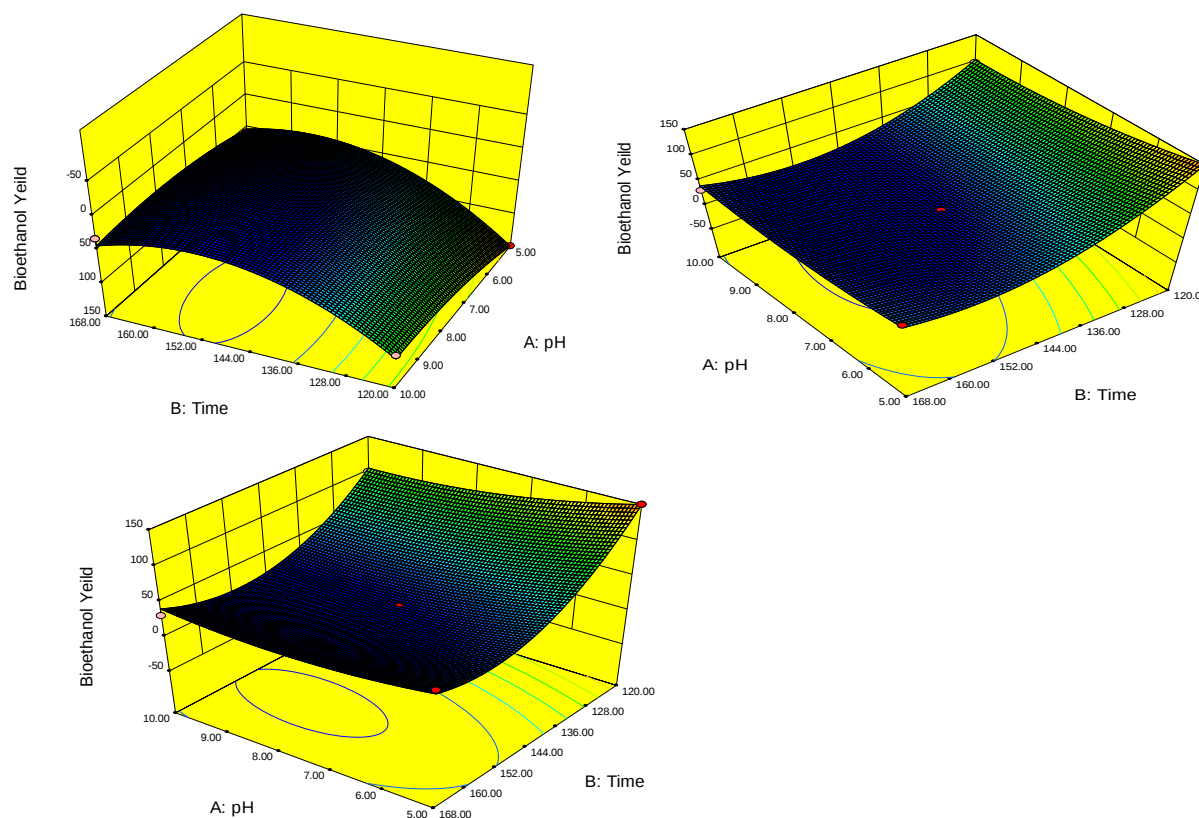
X_2	-4.00	1	4.61	-14.91	6.91	1.00
X_3	3.25	1	4.61	-7.66	14.16	1.00
X_1X_2	17.25	1	6.53	1.82	32.68	1.00
X_1X_3	-4.75	1	6.53	-20.18	10.68	1.00
X_2X_3	-9.75	1	6.53	-25.18	5.68	1.00
X_1^2	37.38	1	6.36	22.33	52.42	1.01
X_2^2	13.87	1	6.36	-1.17	28.92	1.01
X_3^2	3.37	1	6.36	-11.67	18.42	1.01

Table 5. 11: Regression Coefficients and Significant of Response surface Quadratic Model for Rice husks plus corn cobs

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	26.00	1	5.55	12.88	39.12	
X_1	-13.50	1	4.39	-23.87	-3.13	1.00
X_2	-40.50	1	4.39	-50.87	-30.13	1.00
X_3	4.50	1	4.39	-5.87	14.87	1.00
X_1X_2	9.00	1	6.20	-5.67	23.67	1.00
X_1X_3	-6.00	1	6.20	-20.67	8.67	1.00
X_2X_3	-34.00	1	6.20	-48.67	-19.33	1.00
X_1^2	12.00	1	6.05	-2.30	26.30	1.01
X_2^2	49.00	1	6.05	34.70	63.30	1.01
X_3^2	10.00	1	6.05	-4.30	24.30	1.01



A: Cassava peels plus yam peels



B: Rice husks plus corn cobs

Figure 5. 2: Response surface plots for the interactive effect of Time and pH on Bioethanol production.

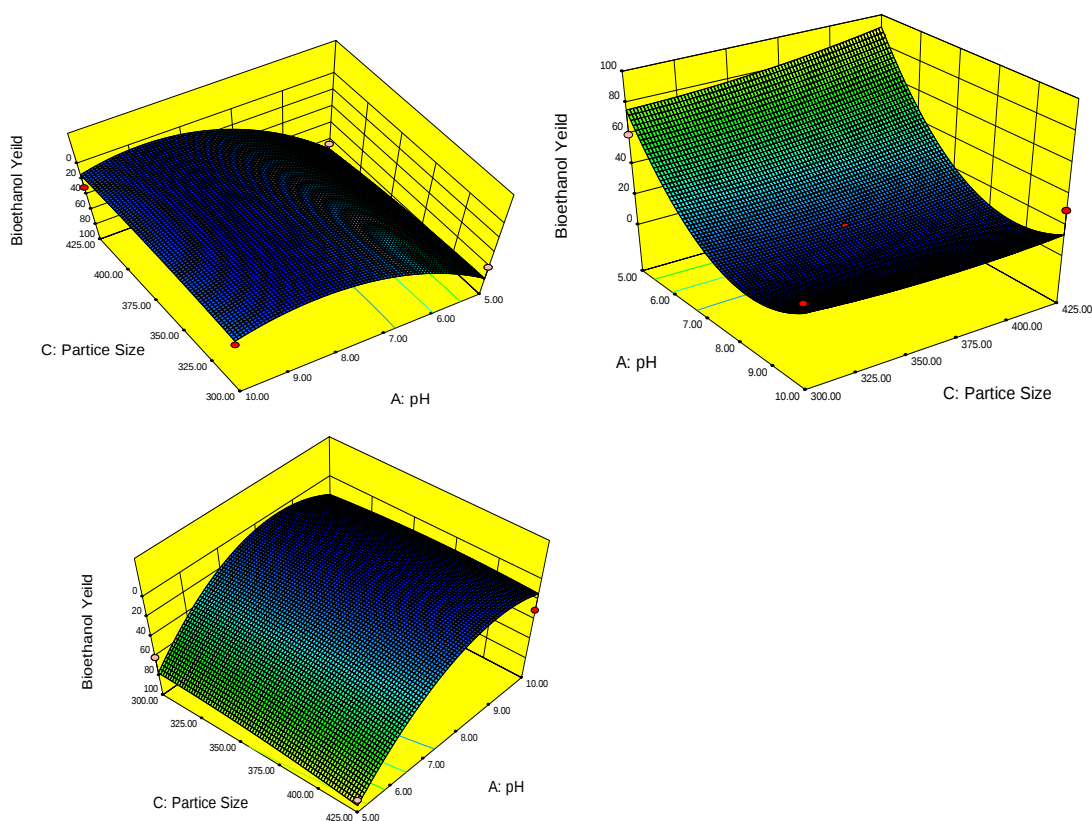
3.2.1 Interactive effect of Particle Size and pH on bioethanol production from hybridized biomass

The response surface plot of the interaction of particle size and pH on bioethanol production is presented in Figure 5. 3. The maximum bioethanol yield of 125 ml/1500 g from hybridized cassava plus yam peels biomass was obtained at a particle size of 362.50 microns and a pH of 5.0, while that of rice husks plus corn cobs biomass was 160 ml/1500 g.

For both hybridized biomass samples, the plots show that increasing the particle size during pretreatment with a corresponding increase in the pH above 6.0 in an acidic pretreatment is unfavorable to optimum bioethanol yield. Besides, in a basic pretreatment condition maximum bioethanol yield is 30 ml/1500 g cassava plus yam peels biomass at a pH of 10.0 and a particle size of 362.50 microns whereas rice husks plus corn cobs hybridized biomass gave a bioethanol yield of 68 ml/1500 g biomass. However, in a hot water hydrolysis condition near neutral pH for cassava peels plus yam peel biomass, maximum bioethanol yield is 40 ml/1500 g biomass at a pH of 7.5 and a particle size of 425 microns whereas the rice husks plus corn cobs biomass gave a maximum yield of 170 ml/1500 g biomass. It can therefore be deduced that increasing the particle size with a decrease in the pH will result in optimum bioethanol yield for both

hybridized biomass. Singh et al 2014, [25] in their review on delignification of lignocellulosic biomass to enhance their capability for bioethanol production showed that a good yield of bioethanol (0.47 g/g glucose) was attained from cashew apple bagasse pretreated with dilute H_2SO_4 at a temperature of $121.1^{\circ}C$ for 15 minutes.

The result from this study indicates that maximum bioethanol formation is also a function of the particle size and pH, which should be between 362.50 micros and 425 micros and 5.0 and 7.5 for particle sizes and pH respectively. Therefore, maximum bioethanol yield is achieved in an acid pretreated condition than basic and hot water pretreatment conditions.



A: Cassava peels plus Yam peels

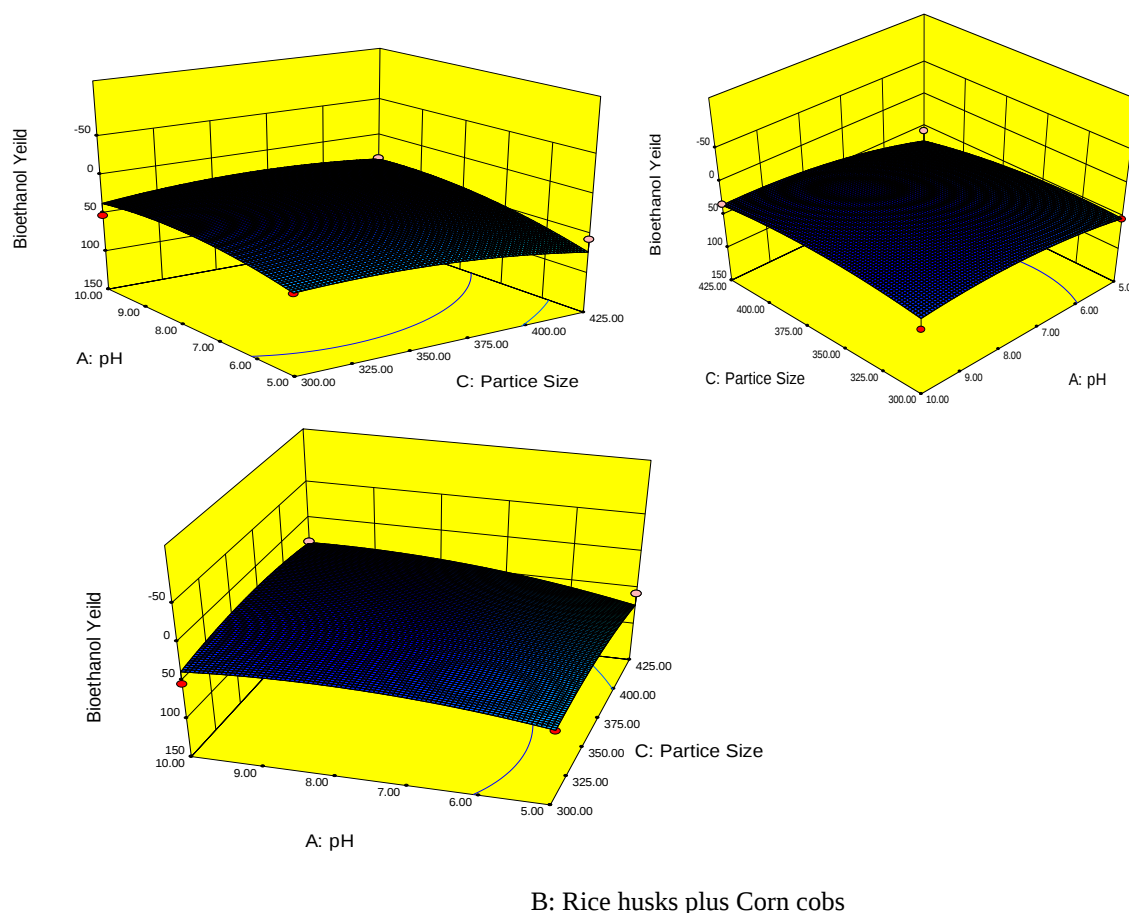
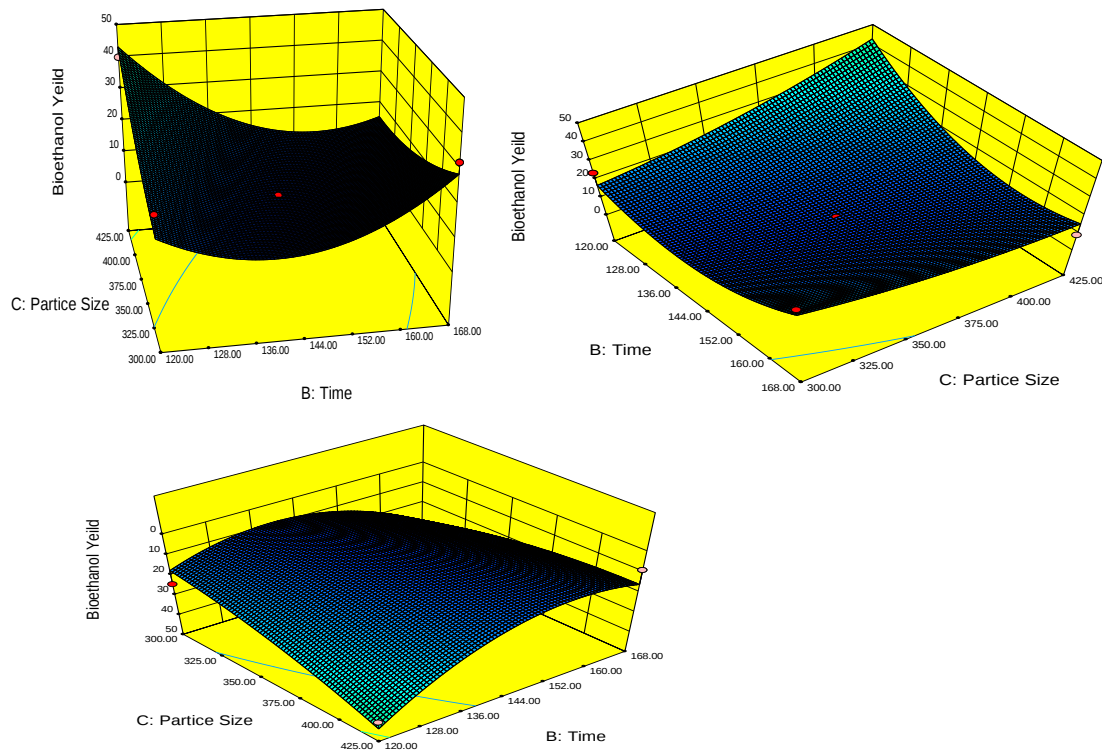


Figure 5. 3: Response surface plot for the interactive effect of Particle Size and pH on Bioethanol production

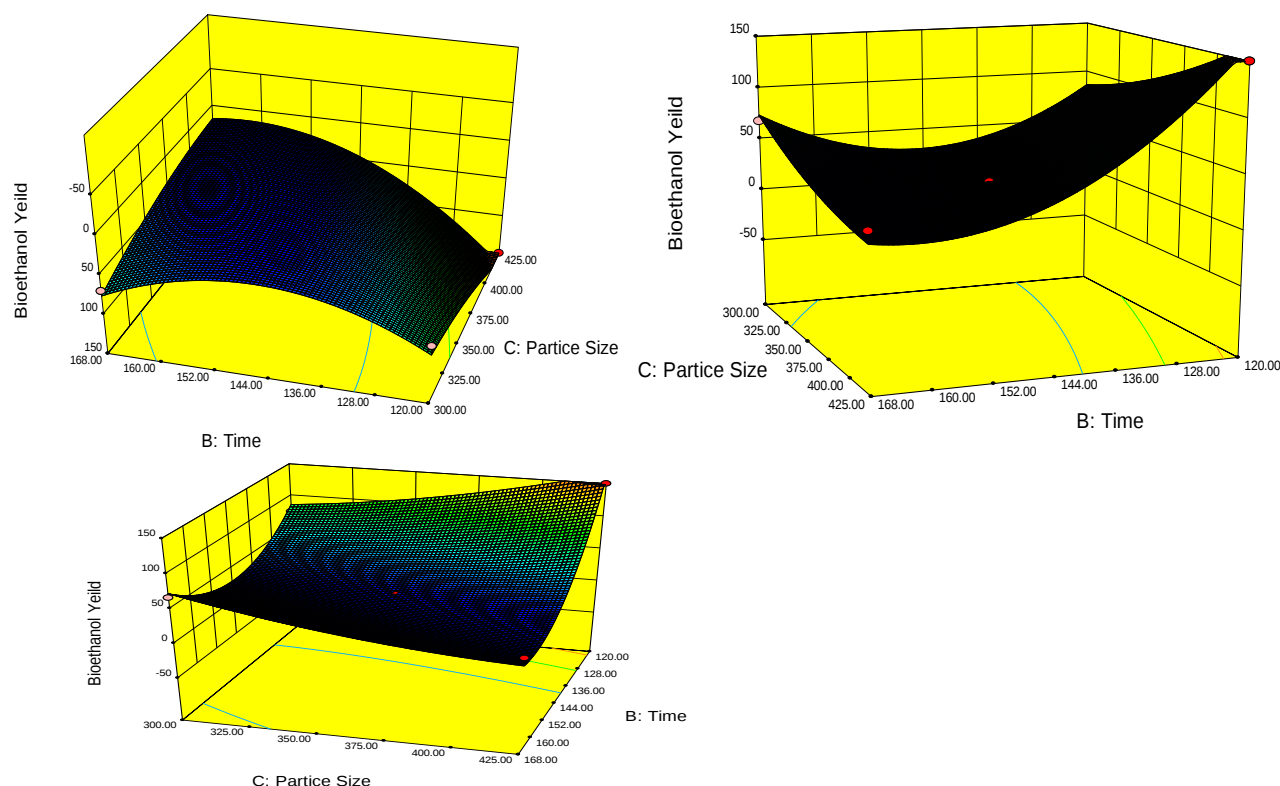
3.2.1 Interactive effect of Particle Size and Time on bioethanol yield from hybridized feedstocks

The response surface plot of the interaction of particle size and time on bioethanol production is presented in Figure 5.4. Bioethanol yield of 40 ml/1500 g biomass was obtained at the highest particle size of 425 microns and at a time of 120 hours for cassava plus yam peels biomass while for that of rice husks plus corn cobs was 170 ml/1500 g biomass. The plot shows that maintaining a range of particle sizes between 350 microns and 375 microns with a corresponding increase in fermentation time results in a decrease in bioethanol yield which is unfavorable. However, increasing the particle sizes beyond the stipulated range or a decrease beyond the stated range with a corresponding decrease in fermentation time results in an increase in bioethanol yield. This indicates that maximum bioethanol formation is dependent on the particle size and time especially for the rice husks and corn cobs hybridized biomass which has been corroborated by Ojewumi et al, 2018 [26] in their work on the bio-conversion of sweet potato peel waste to bioethanol with the aid of *saccharomyces Cerevisiae*. The result from the work shows that optimum bioethanol yield

is dependent on time. Longer fermentation time results in a decline in the yield of bioethanol as the yeast cells get used up as fermentation progresses.



A: Cassava peels plus Yam peels



B: Rice husks plus corn cobs

Figure 5. 4: Response surface plot for the interactive effect of Particle Size and Time on Bioethanol production

3.3 Characterization of the produced bioethanol

Table 5.12 shows the fuel properties of bioethanol produced via different pretreatment processes from the hybridized mixture of cassava peels plus yam peels biomass as well as corn cobs plus rice husks biomass. The values obtained for different parameters analyzed in this study compare favorably to the normal ethanol and gasoline values in the literature as shown in Table 5.13.

Table 5. 12. Fuel properties of the produced bioethanol

Parameters	Acid-based Pretreated		Alkaline-based Pretreated		Hot Water Pretreated	
	Cassava plus yam peels	Corn cobs plus rice husk	Cassava plus yam peels	Corn cobs plus rice husk	Cassava plus yam peels	Corn cobs plus rice husk
Boiling point, °F	172	173	173	172	171	172
Density, lb/gal	6.611	6.900	6.600	6.900	6.611	6.900
RVP, psi	4.37	4.86	4.34	4.90	4.35	4.86
Flash Point, °	48	43	48	56	48	50
Heat of vaporization, Btu/lb	N/D	386.17	N/D	N/D	N/D	N/D
Autoignition point, °F	285	310	288	300	295	304
Flammability Limit, %	3.10	3.36	3.15	3.35	3.10	3.35

Air: Fuel Ratio, Weight %	8.71	7.83	8.68	7.80	8.73	7.79
Flame Temperature, °F	N/D	2997	N/D	N/D	N/D	N/D
Heat of Combustion, Btu/gal	N/D	13156	N/D	N/D	N/D	N/D
Octane number	120	N/D	N/D	117	N/D	N/D
Lower Heating Value, Btu/lb	11215.46	11711.89	11622.87	11711.89	11534.33	11629.42
Higher Heating Value, Btu/lb	9866.57	9987.11	9713.24	10116.93	9713.24	10036.71
Melting Point, °F	144	148	140	150	142	149
Specific Gravity	0.7909	0.8268	0.7915	0.8268	0.7909	0.8268

Table 5. 13. Comparison of fuel properties of ethanol, and gasoline

Fuel Parameter	Ethanol	Gasoline	Reference
Boiling point, °F	172 - 173	80 - 437	[27]
Density, lb/gal	6.6	6.0 - 6.5	[27]
RVP, psi	2.3 – 2.5	8 - 15	[27]
Flash Point, °F	53.6 -68	-49	[28]
Heat of vaporization, Btu/lb	362 - 400	140 -170	[27]
Autoignition point, °F	689 - 797	442.4 - 878	[27], [28]
Flammability Limit, %	3.3 – 19.0	1.0 – 8.0	[27]
Air: Fuel Ratio, Weight %	8.97 – 9.0	14.5 – 14.7	[27]
Flame Temperature, °F	3506	3591	[27]
Heat of Combustion, Btu/gal	75,700 – 76,000	109,000 – 119,000	[27]
Octane number	96 - 113	85 - 96	[27]
Lower Heating Value, Btu/lb	10748.1 – 11564.92	17798.8 – 19045.57	[28]
Higher Heating Value, Btu/lb	12768.7	20335.34	[28]
Melting Point, °F	-173.2		[29]
Specific Gravity	0.794	0.70 – 0.78	[28]

4 CONCLUSIONS

The optimal values of the independent factors selected for the fermentation process of the hybridized biomass using RSM were obtained by analyzing the regression equations from the RSM technique. For the rice husks plus corn cobs biomass, the optimal condition was statistically predicted as pH 7.5,

fermentation time of 120 hours, and particle size of 425 microns. The predicted bioethanol yield under the optimal condition was 164 ml/1500g biomass. To verify the prediction of the model, the optimal condition values were applied to three independent replicates and the highest bioethanol yield obtained was 170 ml/1500 g biomass, which was well within the estimated value of the model equation.

For cassava peel plus yam peel hybridized biomass, the optimal condition was statistically predicted as pH 5.00, fermentation time of 120.00 hours, and particle size of 362.50 microns. The predicted bioethanol yield under the optimal condition was 115.75 ml/1500 g biomass. To verify the prediction of the model, the optimal condition values were applied to three independent replicates and the average bioethanol volume obtained was 125.00 ml/1500 g biomass, which was well within the estimated value of the model equation.

The results of this research showed that RSM with appropriate experimental design can be effectively applied to the optimization of the process variables in bioethanol production using biomass mixture of corn cob plus rice husk as well as cassava peels plus yam peels as feedstocks for the process. This may provide useful information regarding the development of an economic and efficient fermentation process as well as information on the behavior of hybridized feedstocks for industrial bioethanol production.

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▪ **AUTHOR CONTRIBUTION**

A.A.A and D.L conceptualized the research, A.A.A carried out the experimental work and developed the manuscript, D.L corrected and updated the manuscript.

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Chapter SEVEN

CONCLUSION AND RECOMMENDATIONS

CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

This study focused on the production of bioethanol from selected Nigerian lignocellulosic biomass as a way of contributing to the renewable energy development of the nation, which is currently at its infancy stage. Cassava peels, yam peels, corn cobs, rice husks, and sugarcane bagasse biomass, which are usually produced in large quantities as by-products of foodstuff processing, were adopted in this study to harness their potential for bioethanol production.

The major highlight of this study is the hybridization (combination) of the biomass to strengthen their properties for bioethanol production and guarantee enough feedstock for bioethanol production plants gradually emerging in Nigeria. The hybridized feedstocks were hypothesized to offer enhanced properties for the synthesis of bioethanol. To this end, the virgin biomass of two-particle sizes of 300 microns and 425 microns (individual and hybridized) were firstly characterized using proximate, ultimate, and analytical techniques such as XRD, SEM, FTIR, BET, and XRF to determine their composition and potential for bioethanol production. After which the individual and hybridized biomass were pretreated using three pretreatment methods for comparison purposes viz: Hydrothermal and acid pretreatment, hydrothermal and alkali pretreatment, and hydrothermal only pretreatment. The pretreated biomass was further characterized to determine the effects of the various pretreatment on them. Results of the analysis show the cellulose values of the raw biomass in a range between 25.8 wt% for cassava peels biomass to 40.0 wt% for sugarcane bagasse. However, pretreatment of the biomass enhanced their cellulose component with values ranging from 33.2 to 43.8 wt% for cassava peels and sugarcane bagasse respectively. The results of analysis of the pretreated hybridized biomass show that they have very good potential for better yield of bioethanol after fermentation compared to single feedstocks as there was a favorable change in their composition after pretreatment. Also, it was established that hydrothermal plus alkali pretreatment is more effective than hydrothermal plus acid pretreatment and hydrothermal only pretreatment for singular and hybridized biomass feedstocks.

The Box Behnken Design (BBD) and Response Surface Methodology (RSM) was also used to design an experiment to obtain the optimal conditions for bioethanol production from hybridized biomass of cassava peels + yam peels biomass and rice husks + corn cobs biomass. Three independent factors were considered in the design namely, pH, reaction time, and particle size. The optimal values of the independent factors chosen for the fermentation of the hybridized biomass utilizing the BBD were gotten by evaluating the regression equations from RSM. The rice husks plus corn cobs hybridized biomass showed better potential

for bioethanol production than the cassava plus yam peels biomass as the rice husks plus corn cobs biomass had the highest bioethanol yield obtained at 170 ml/1500 g biomass, while the cassava peels plus yam peels biomass had the highest bioethanol yield of 125.00 ml/1500 g biomass after the experiments. However, both biomass mixtures had bioethanol yields after the experiments falling well within the projected values of the model equation. It was also established from the yield that lignocellulosic biomass particle size plays a major role in their processing to obtain the optimum quantity and quality bioethanol. The outcomes of this study indicated that hybridization (mixture) of two or more lignocellulosic biomass enhances their properties and potentials of bioethanol production in terms of yield and quality.

The findings from this research work will undoubtedly supply valuable data for future researchers and industrialists delving into bioethanol production from lignocellulosic biomass.

7.2 Recommendations

Based on the research findings from this study, the following recommendations are made:

The use of other lignocellulosic biomass such as maize and guinea corn straw after harvesting, wood, among others, which are generated in Nigeria to produce bioethanol should be explored to guarantee adequate feedstock for bioethanol production plants springing up in the country.

Further research should be carried out on the microorganism used for the fermentation of lignocellulosic biomass to improve the yield and quality of bioethanol. The use of other microorganisms such as *Aspergillus niger* and *Candida utilis* can be explored for the fermentation of biomass to produce bioethanol.

A more detailed study on the biomass composition and effects of the different pretreatments carried out in this study on the glucose release after the saccharification can be carried out.

A quantitative study of the inhibitors formed during the pretreatment stage can be carried out

The optimized dosage of enzymes in the saccharification and fermentation stages can be investigated.

Further research should be carried out for information on the basic density of the different biomass in this study

Appendices

The content of these appendices can be summarized as follows:

Appendix A shows the certificate and congratulatory letter from Wiley publishers for the first paper for this thesis.

Appendix B (B1-B8) shows different figures of the BET absorption isotherms of raw and pretreated biomass which supports and serves as supplementary data for paper 3.

Appendix C (C1-C5) shows different figures of the FTIR of raw and pretreated biomass which supports and serves as supplementary data for paper 3.

Appendix D (D1-D8) shows different figures of the SEM images of raw and pretreated biomass which supports and serves as supplementary data for paper 3.

Appendix E (E1-E5) shows different figures of the XRD diffractograms of raw and pretreated biomass which supports and serves as supplementary data for paper 3.

Appendix F shows the UOPCC distillation column used in the study

Appendix G shows the acceptance letter for paper 5

Appendix A: Congratulatory/Recognition certificate and letter



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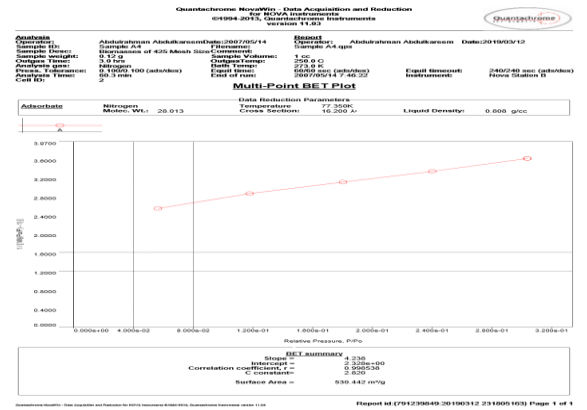
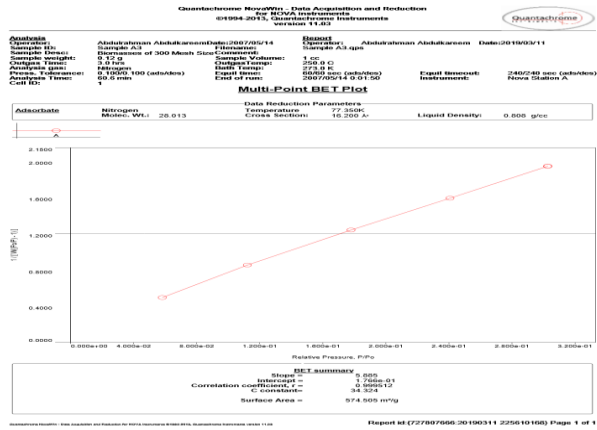
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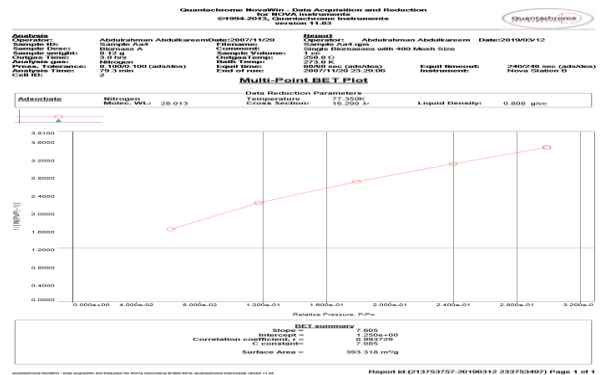
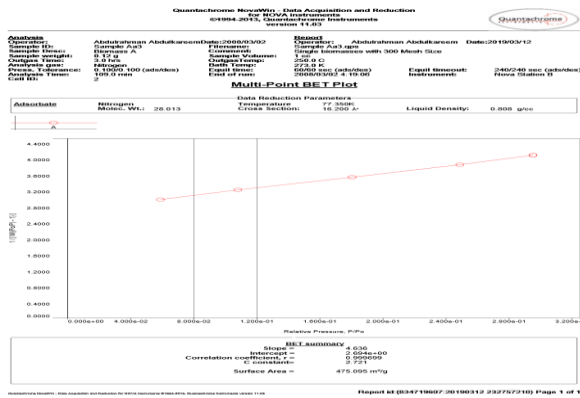
Best wishes,
Biofuels, Bioproducts, and Biorefining

Appendix B: BET absorption isotherms of raw and pretreated biomass



Raw Cassava peels 300 microns

Raw Cassava peels 425 microns



Acid pretreated Cassava peels 300 microns

Acid pretreated Cassava peels 425 microns

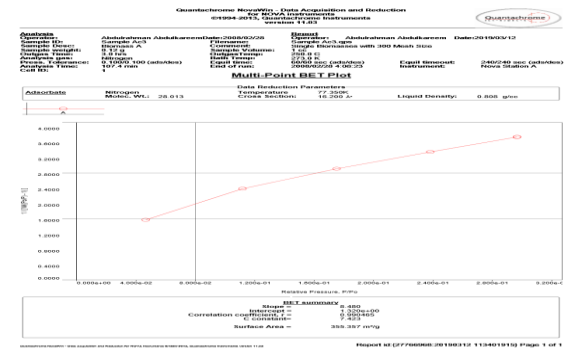
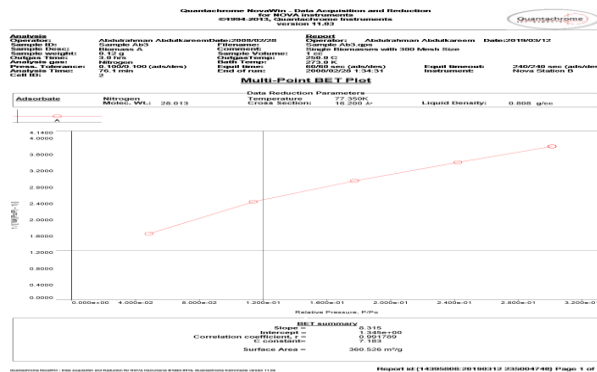
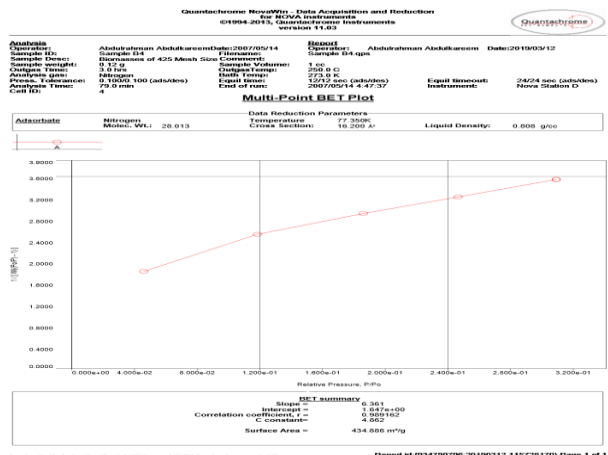
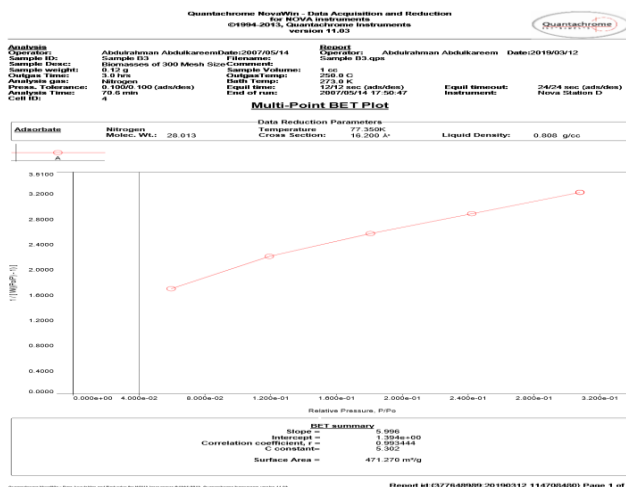
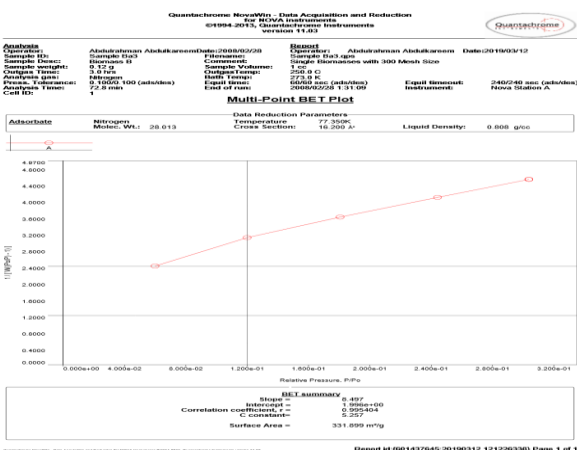


Figure B1: BET absorption isotherms of raw and pretreated cassava peels biomass

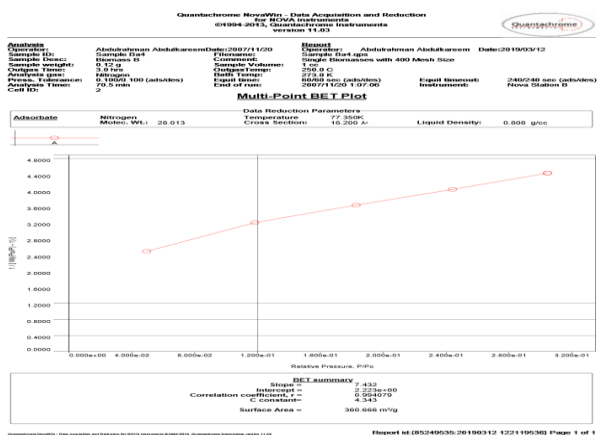


Raw corn cobs biomass 300 microns

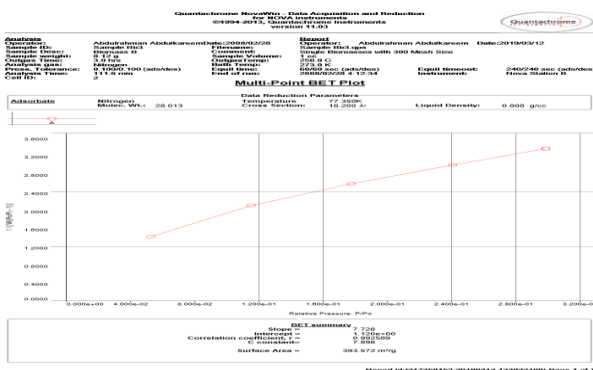
Raw corn cobs biomass 425 microns



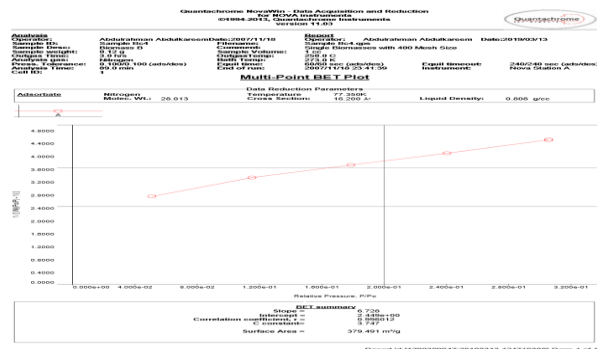
Acid pretreated corn cobs 300 microns



Acid pretreated corn cobs 425 microns

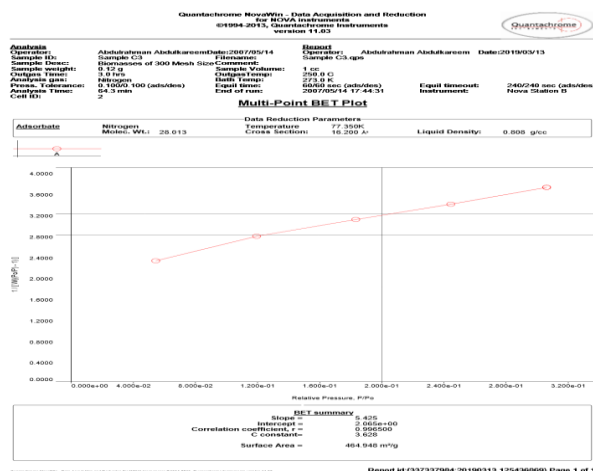


Alkali pretreated corn cobs 300 microns

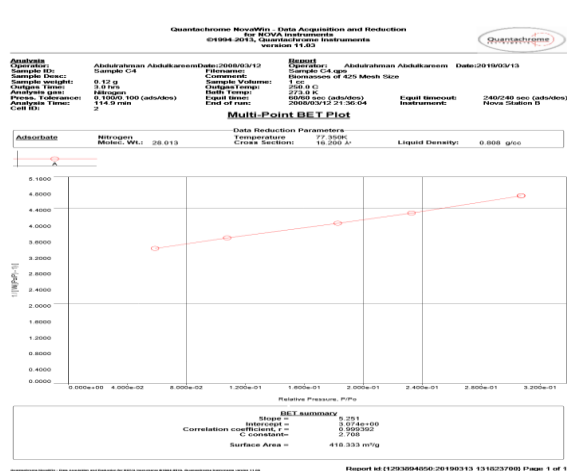


Hot water pretreated corn cobs 300 microns

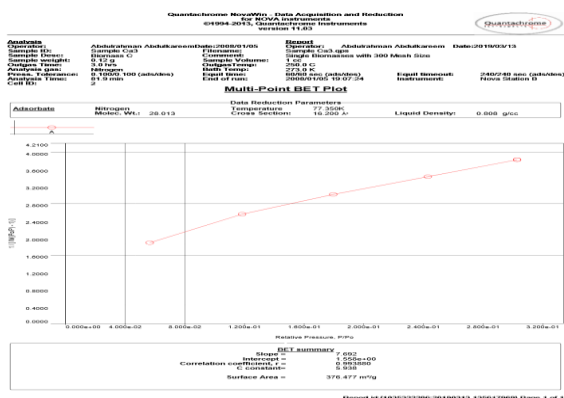
Figure B2: BET absorption isotherms of raw and pretreated corn cobs biomass



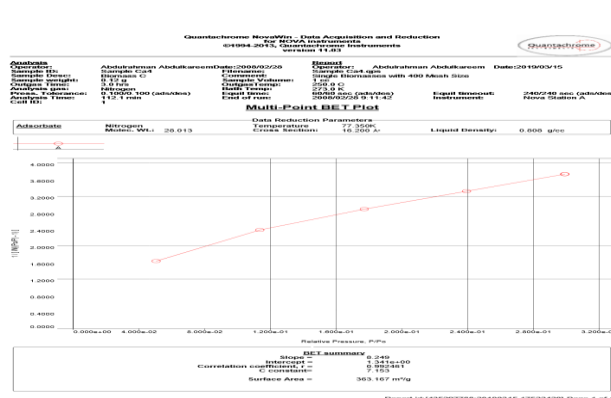
Raw rice husks 300 microns



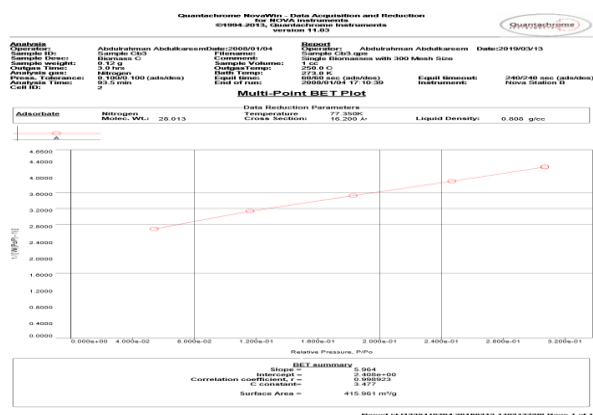
Raw rice husks 425 microns



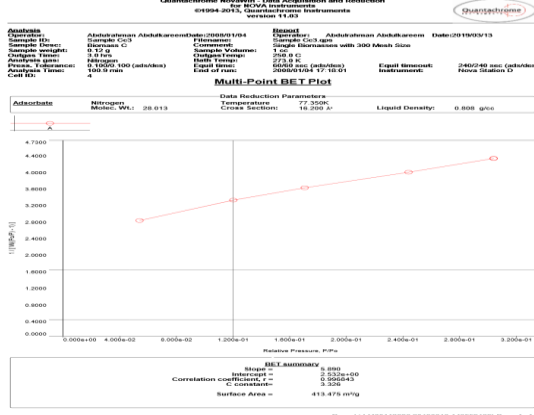
Acid pretreated rice husks biomass 300 microns



Acid pretreated rice husks biomass 425 microns

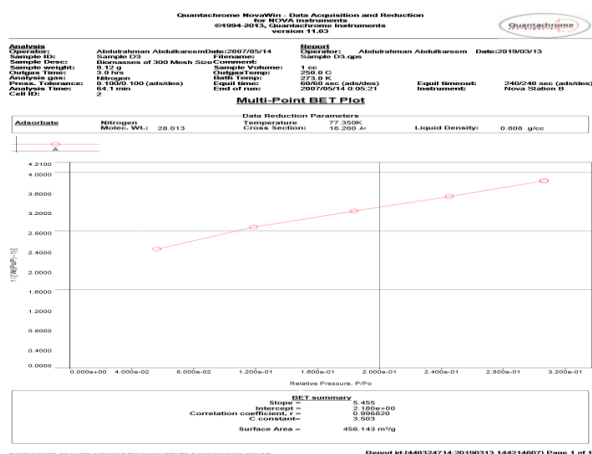


Alkali pretreated rice husks biomass 300 microns

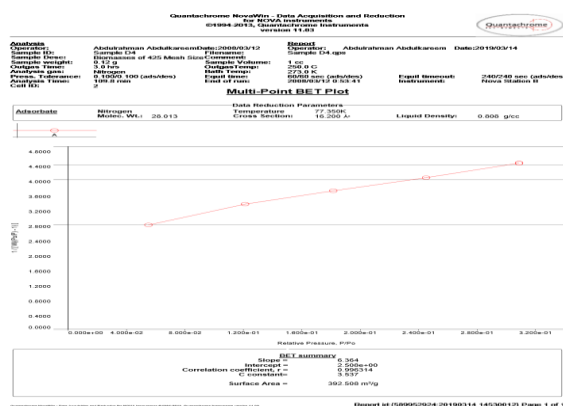


Hot water pretreated rice husks biomass 300 microns

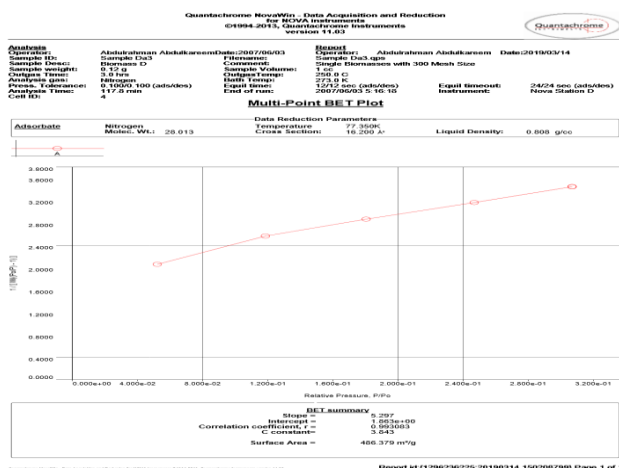
Figure B3: BET absorption isotherms of raw and pretreated rice husks biomass



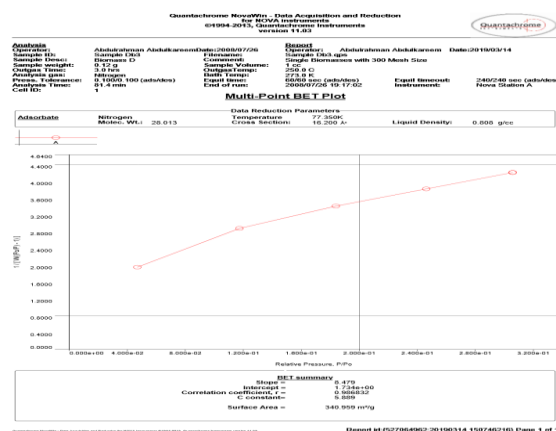
Raw sugar cane bagasse 300 micron



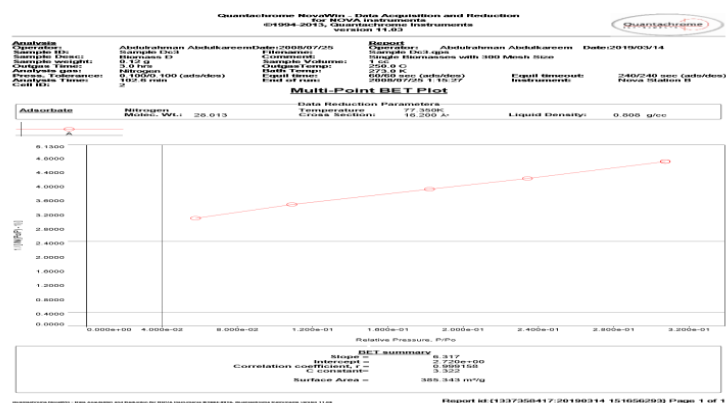
Raw sugar cane bagasse 425 micron



Acid pretreated sugar cane bagasse 300 microns

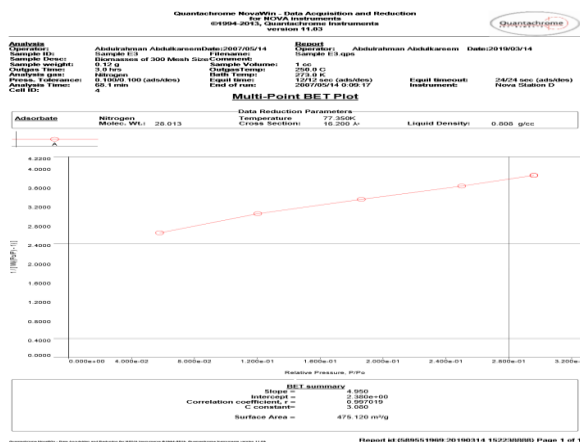


Alkali pretreated sugar cane bagasse 300 microns

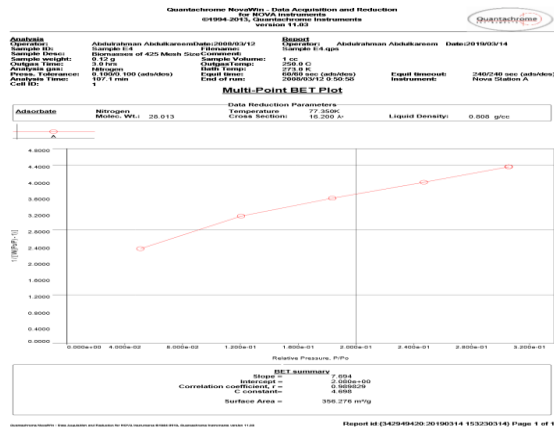


Hot water pretreated sugar cane bagasse 300 microns

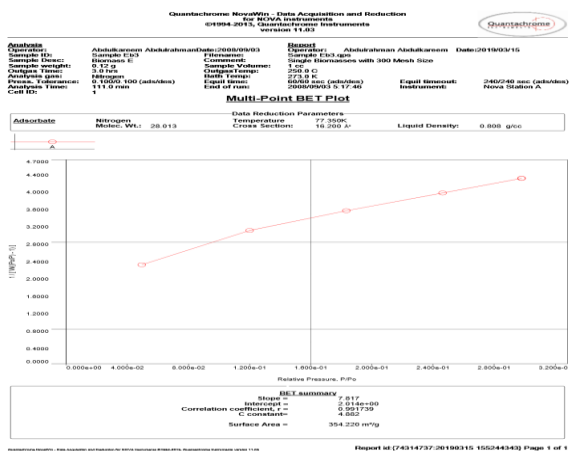
Figure B4: BET absorption isotherms of raw and pretreated sugar cane bagasse biomass



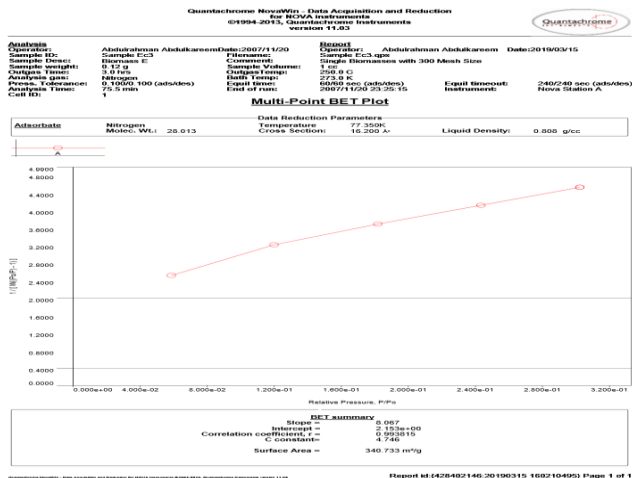
Raw yam peels biomass 300 microns



Raw yam peels biomass 425 microns

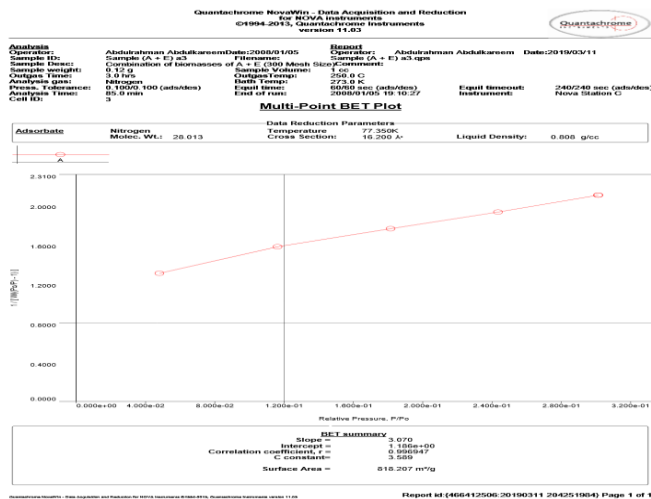


Alkali pretreated yam peels biomass 300 microns

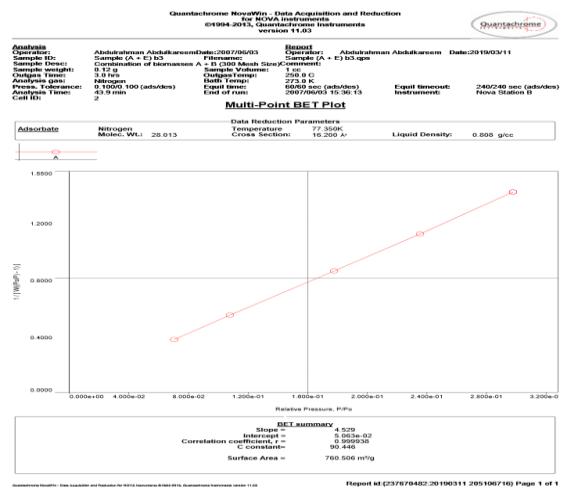


Hot water pretreated yam peels biomass 300 microns

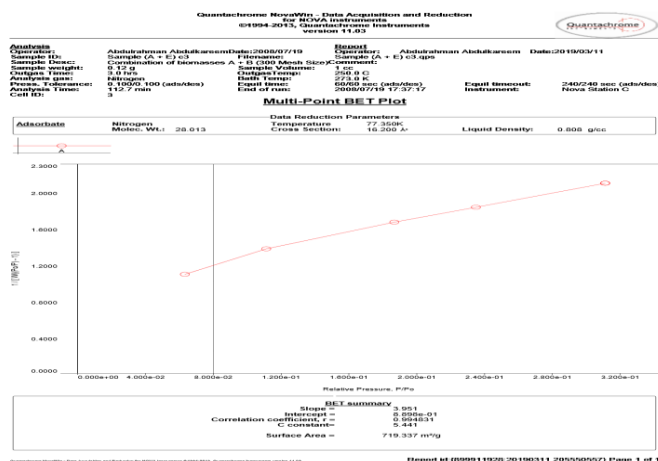
Figure B5: BET absorption isotherms of raw and pretreated yam peels biomass



Acid pretreated 300 microns

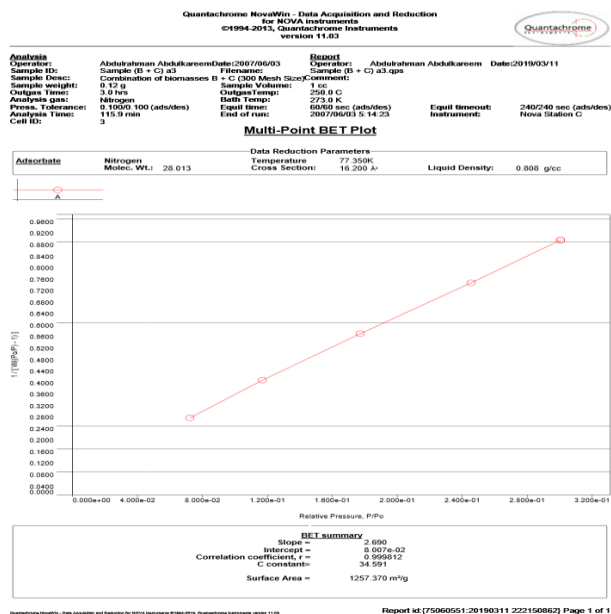


Alkali pretreated 300 microns

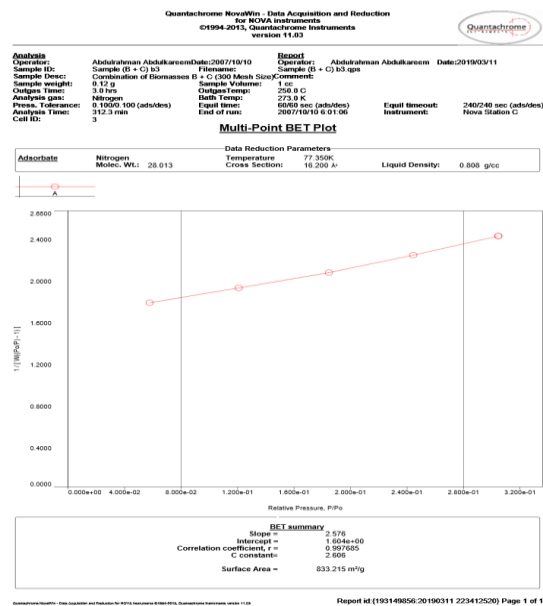


Hot water pretreated 300 microns

Figure B6: BET absorption isotherms of pretreated cassava plus yam peels biomass

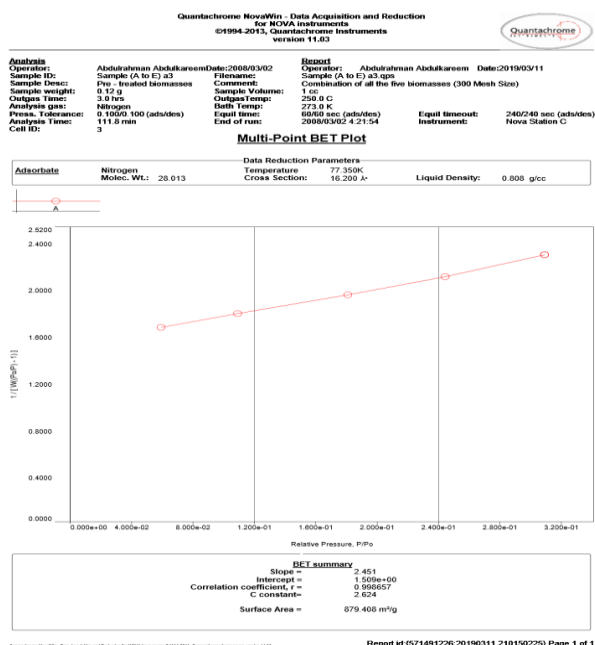


Acid pretreated

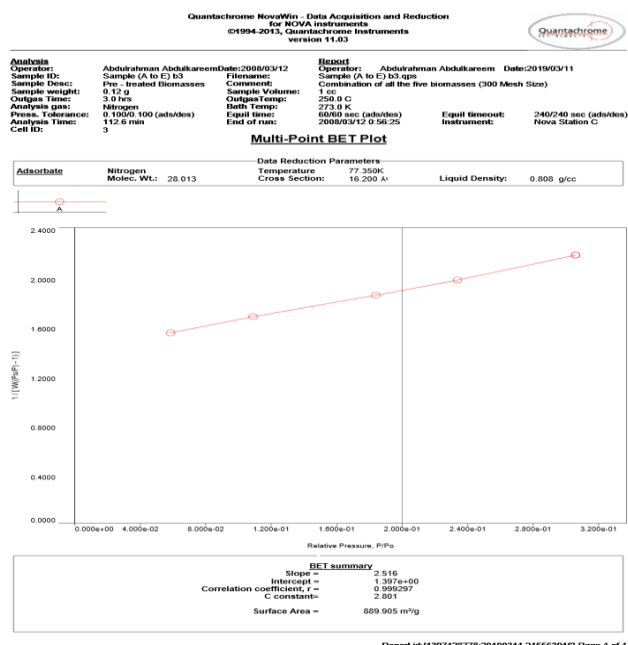


Alkaline pretreated

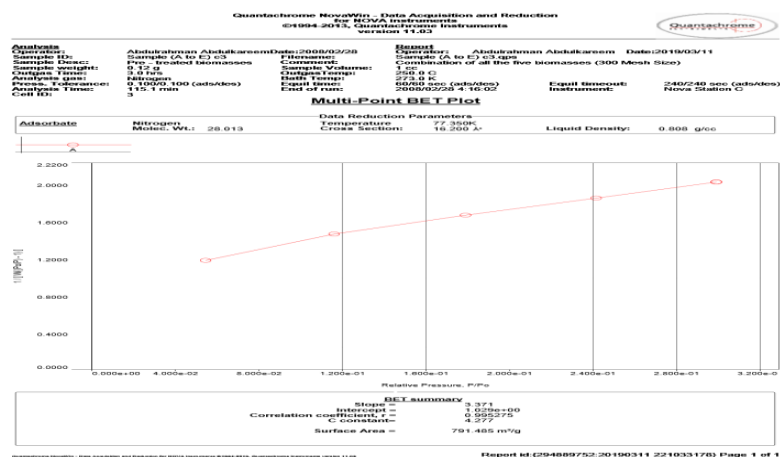
Figure B7: BET absorption isotherms of pretreated corn cobs plus rice husks biomass



Acid pretreated



Alkali pretreated



Hot water pretreated

Figure B8: BET absorption isotherms of the mixture of all the biomasses

Appendix C: FTIR spectra of raw and pretreated biomass

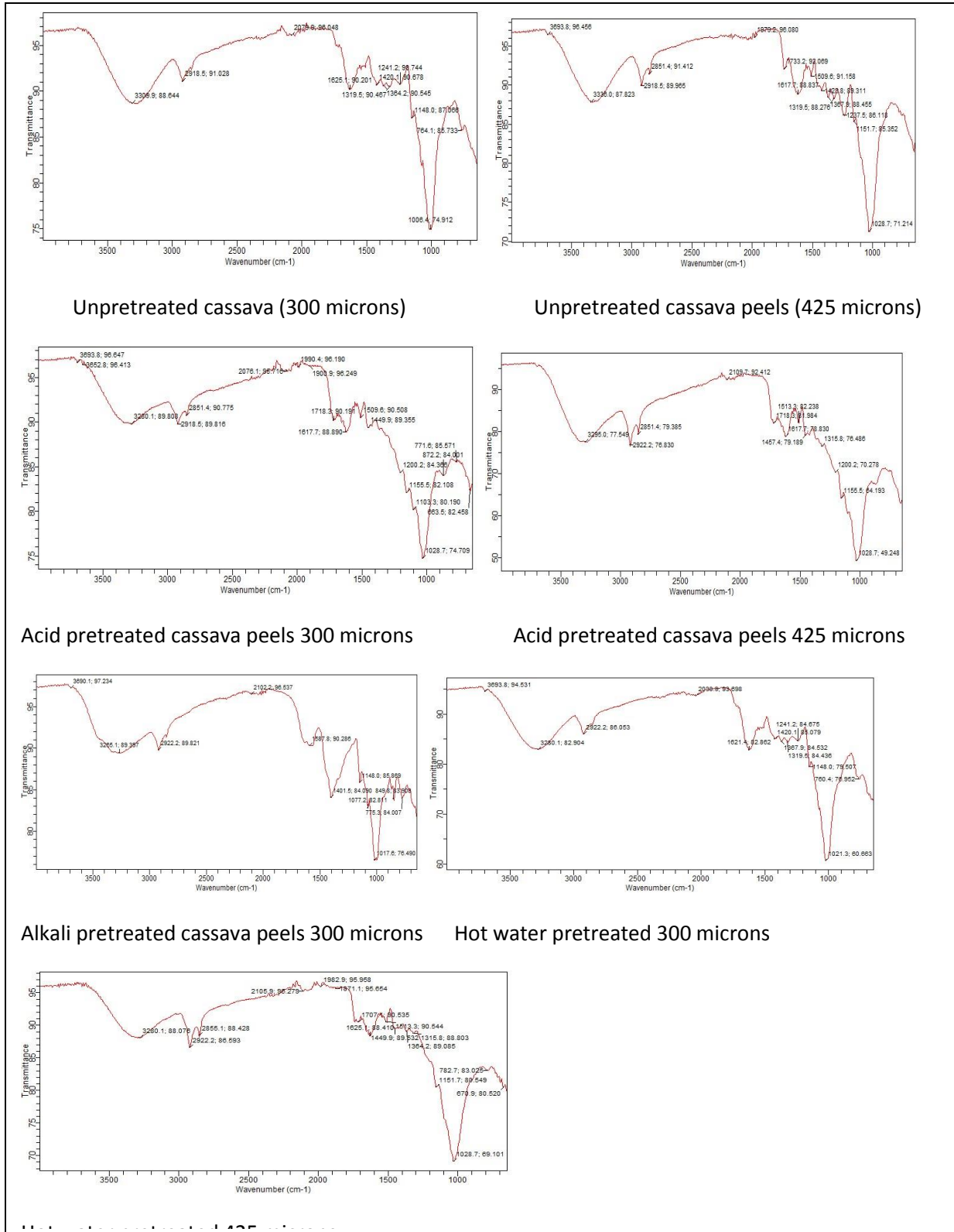
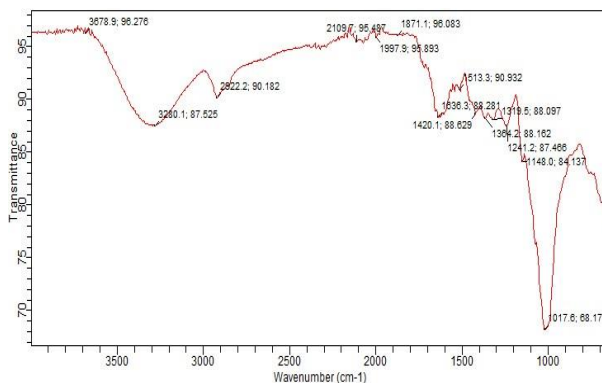
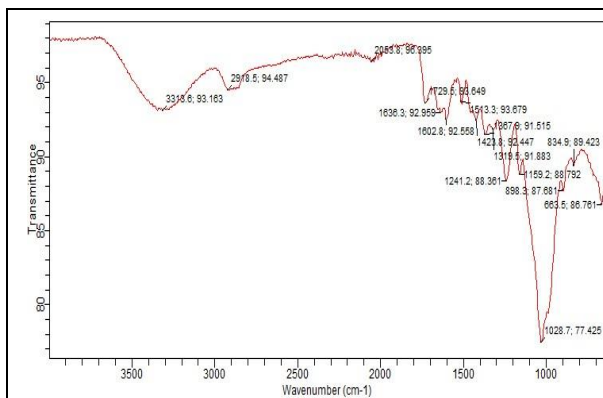
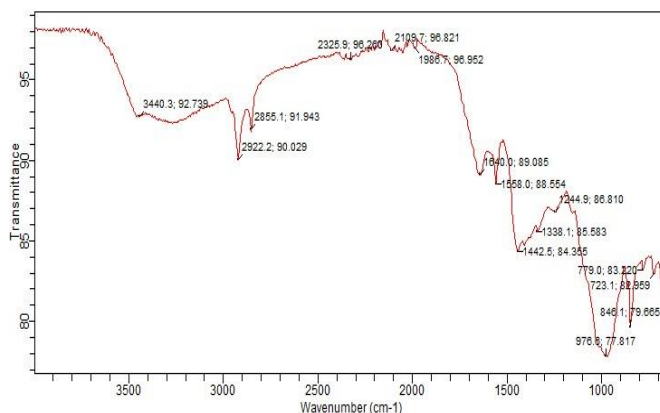
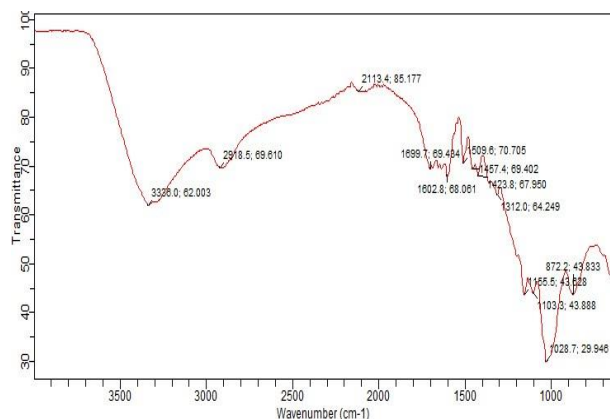


Figure C1: FTIR of cassava peels biomass (Raw and pretreated)



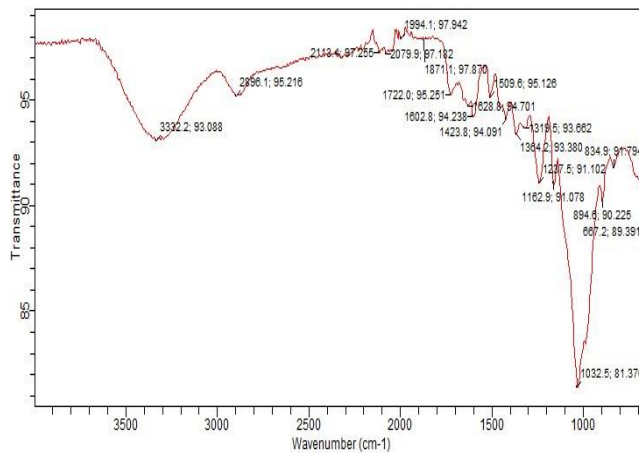
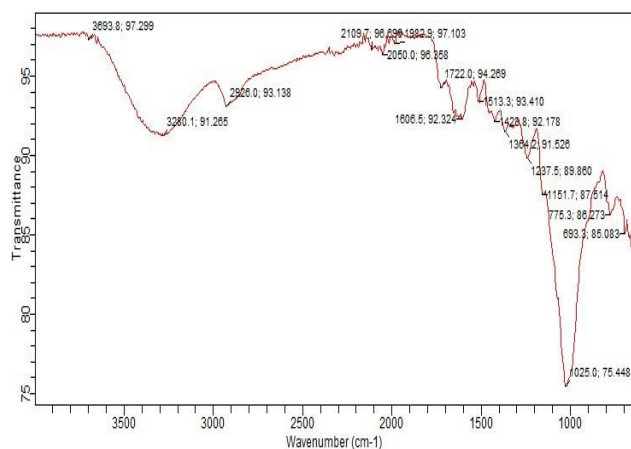
Corn cobs raw sample 425 microns

Acid pretreated 300 microns



Acid pretreated 425 microns

Alkali pretreated 300 microns



Hot water pretreated 300 microns

Hot water pretreated 425 microns

Figure C2: FTIR of corn cobs biomass (Raw and pretreated)

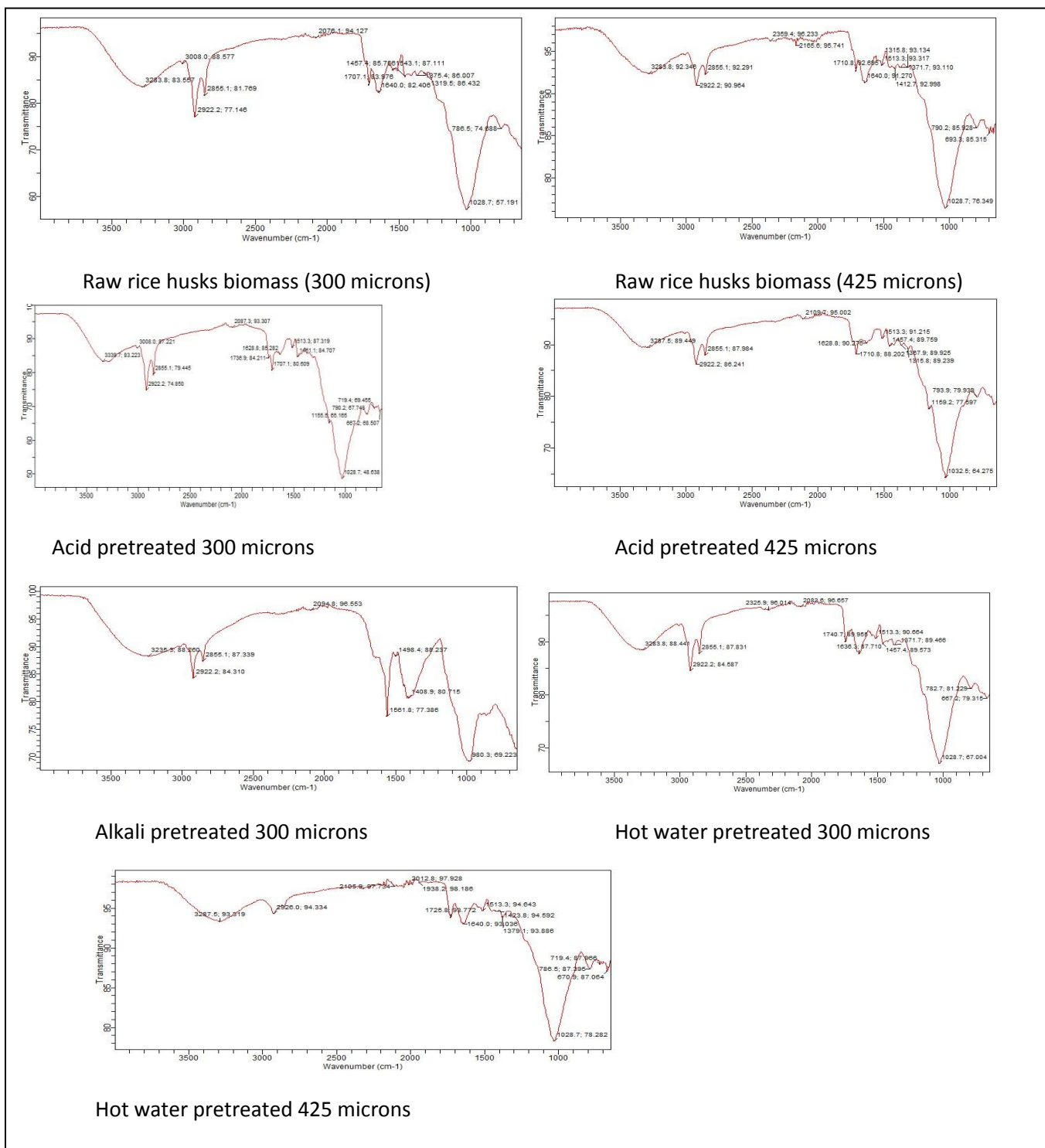


Figure C3: FTIR of rice husks biomass

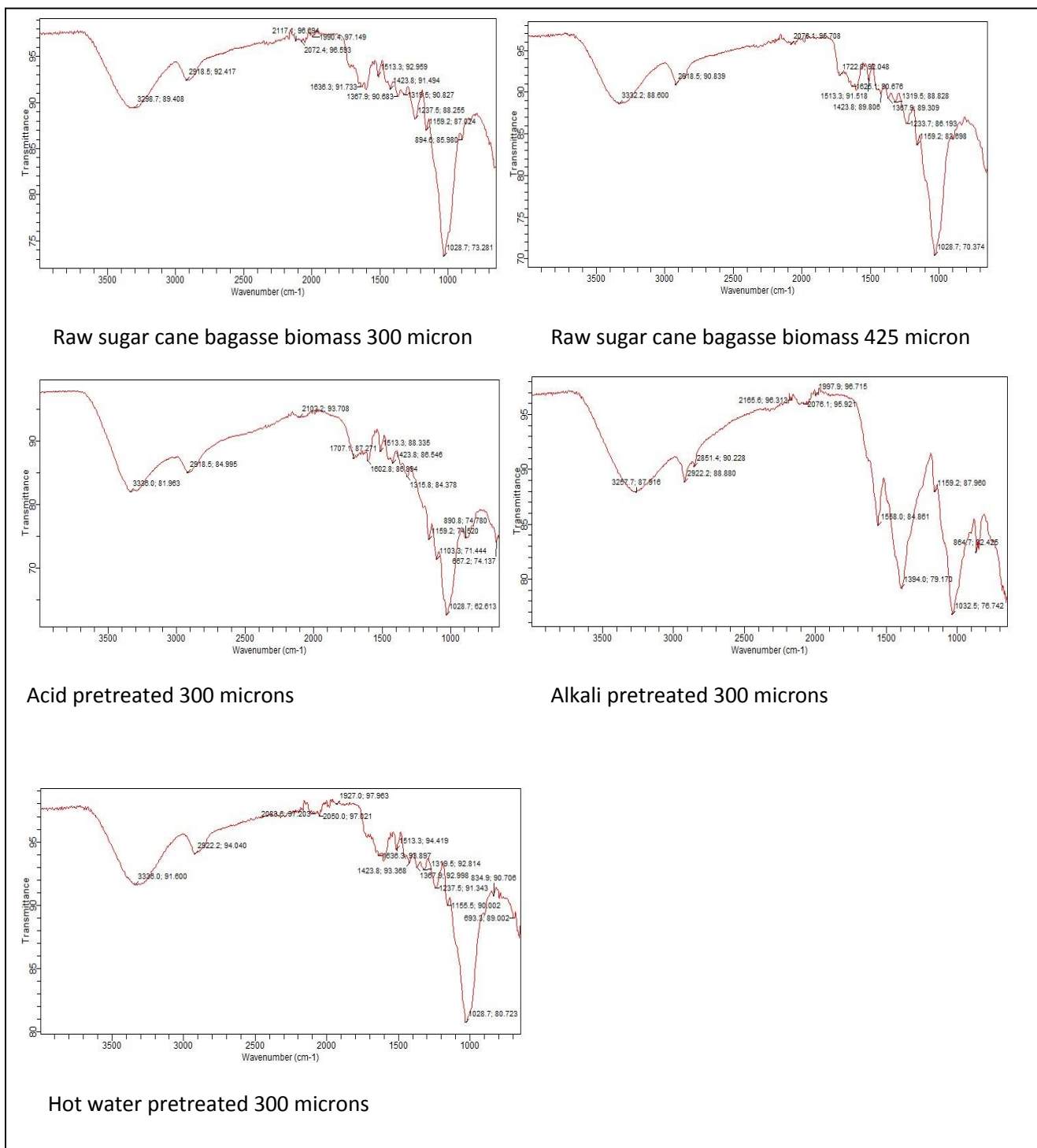
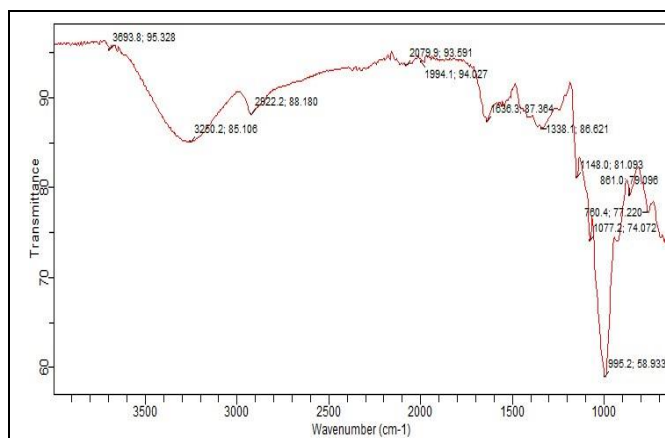
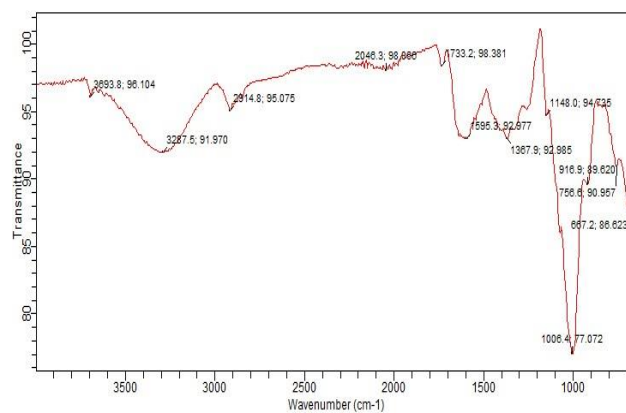


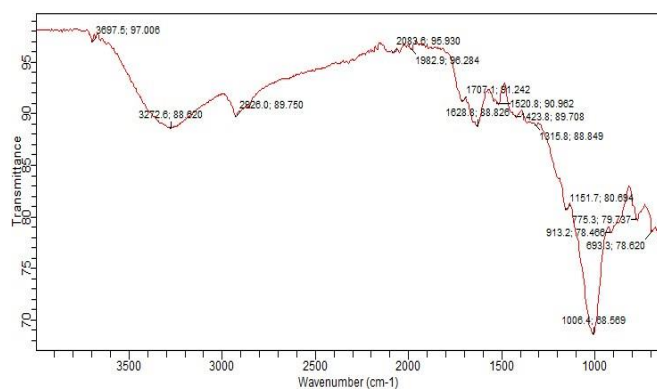
Figure C4: FTIR of sugar cane bagasse biomass (Raw and pretreated)



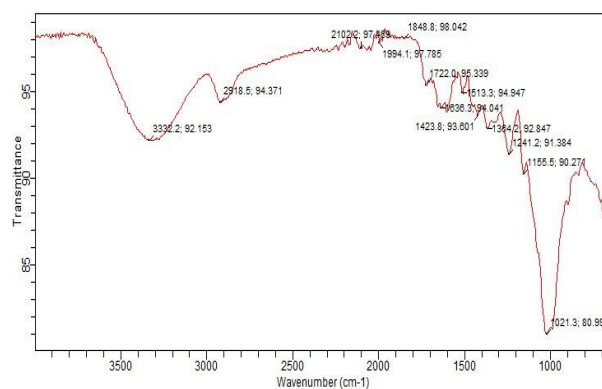
Raw yam peel biomass (300 micron)



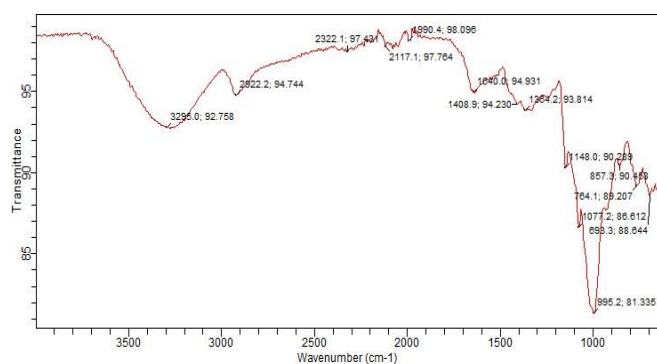
Raw yam peel biomass (425 micron)



Acid pretreated 300 microns



Alkali pretreated 300 microns



Hot water pretreated 300 microns

Figure C5: FTIR of yam peels biomass

Appendix D:

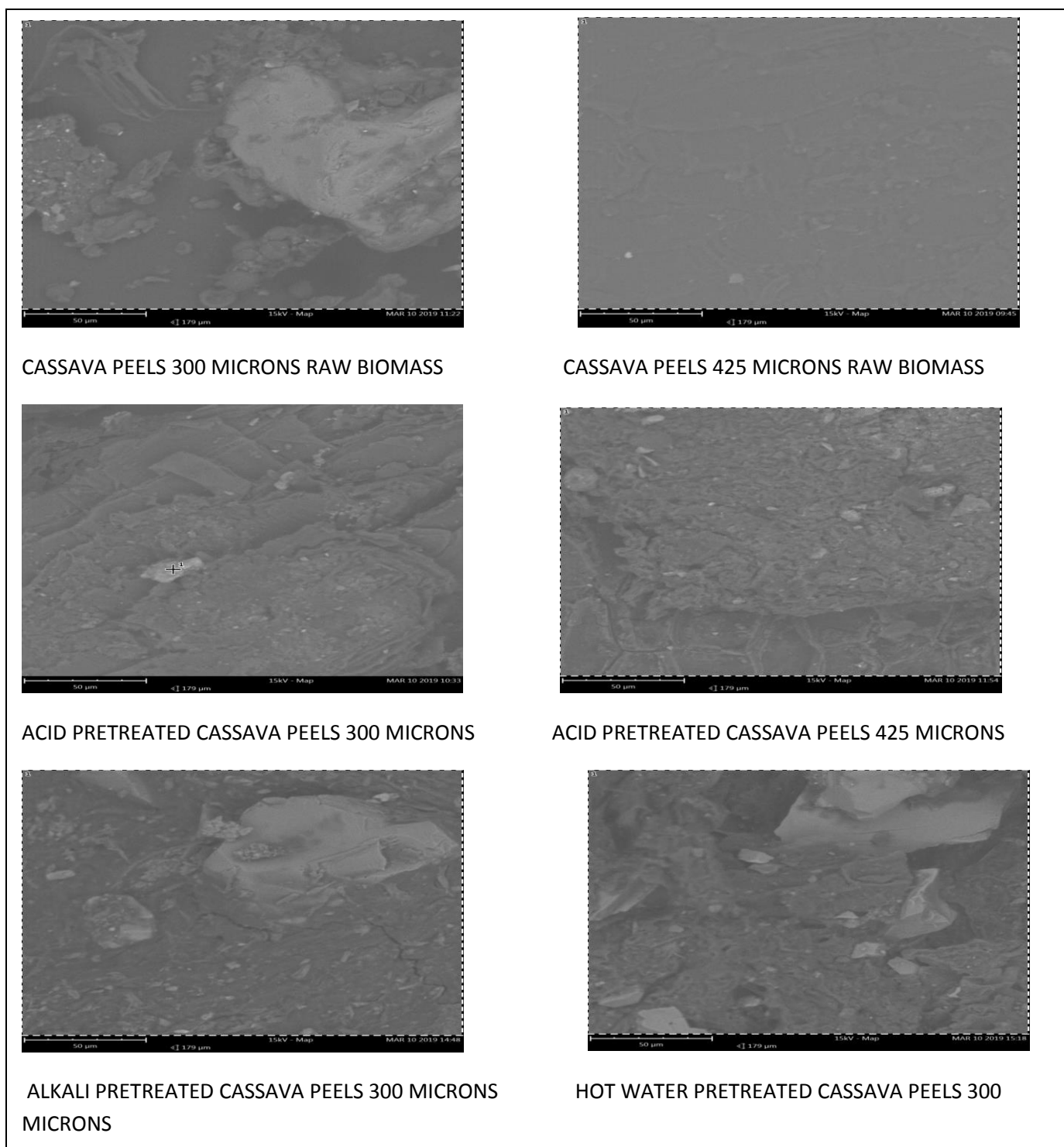
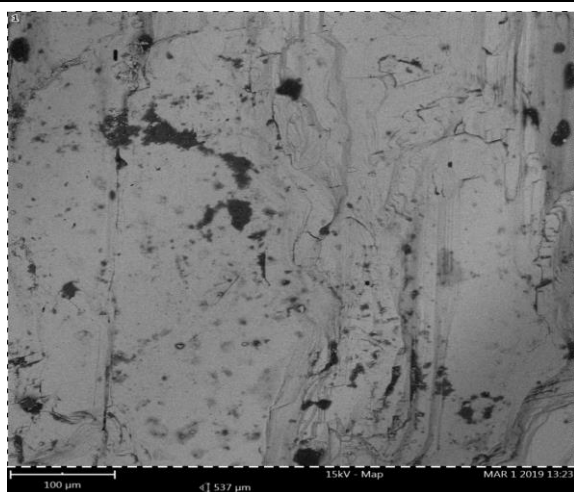
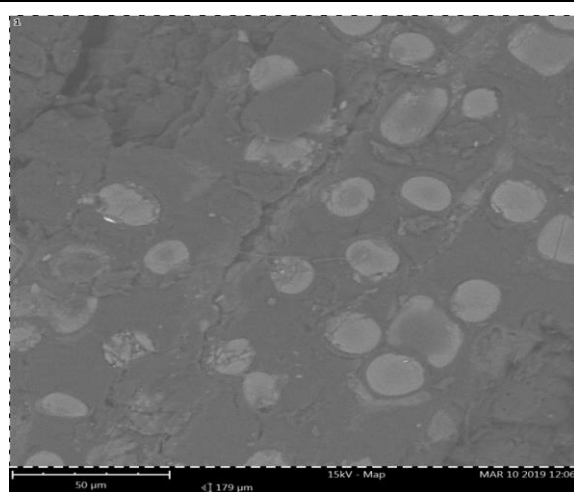


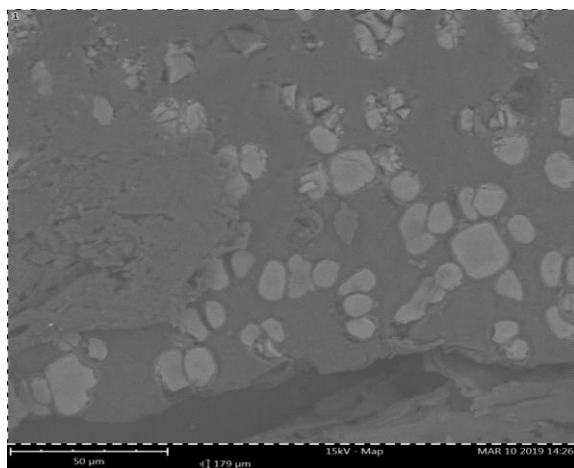
Figure D1: SEM of cassava peels biomass (Raw and pretreated)



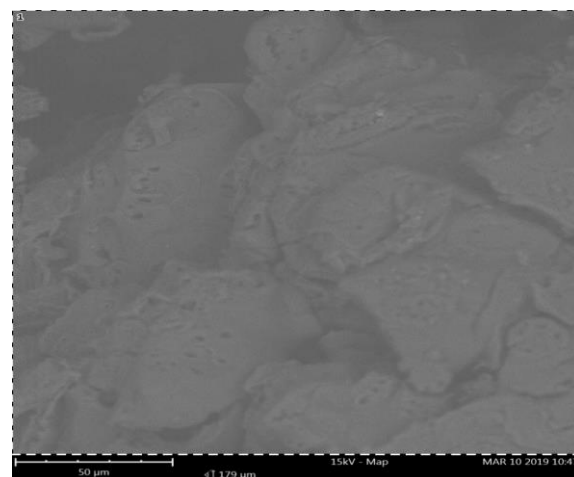
Raw corn cobs biomass (300 microns)



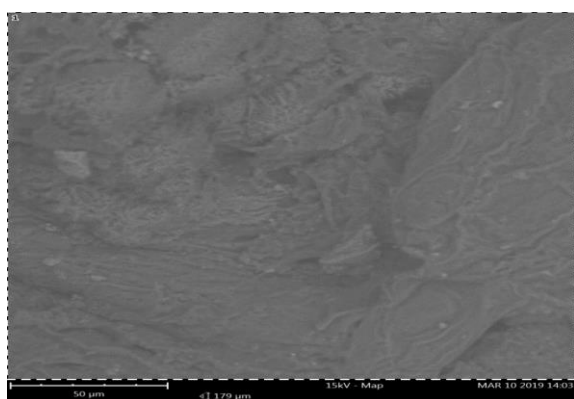
Raw corn cobs biomass (425 microns)



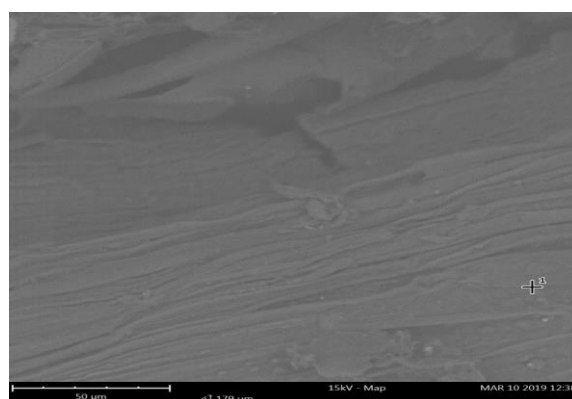
Acid Pretreated corn cobs biomass (300 microns)



Acid Pretreated corn cobs biomass (425 microns)

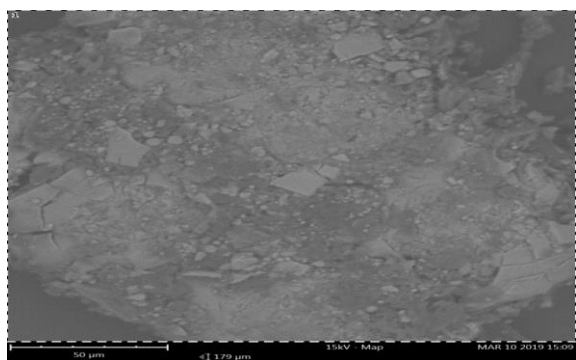


Alkali Pretreated corn cobs biomass (300 microns)

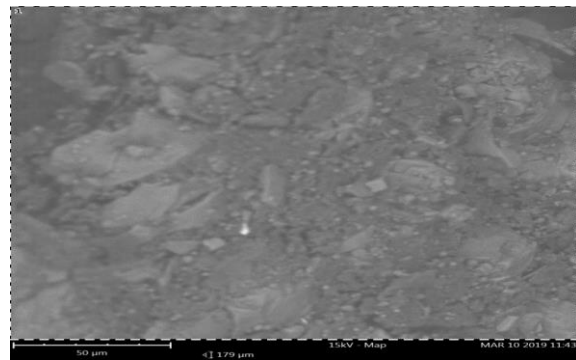


Hot water Pretreated corn cobs biomass (300 microns)

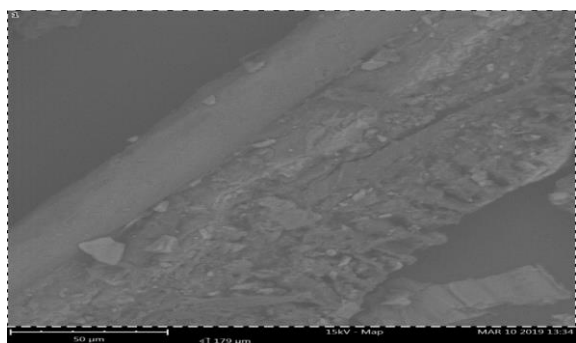
Figure D2: SEM of Corn cobs biomass (Raw and pretreated)



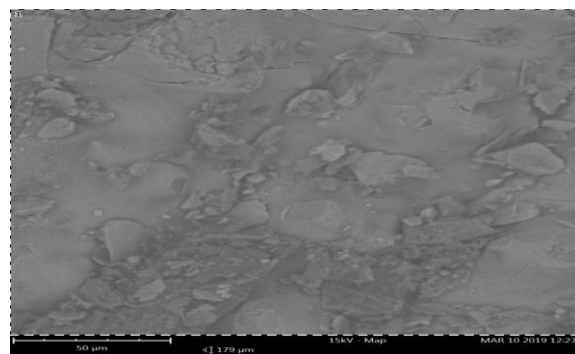
Raw rice husks biomass 300 microns



Raw rice husks biomass 425 microns



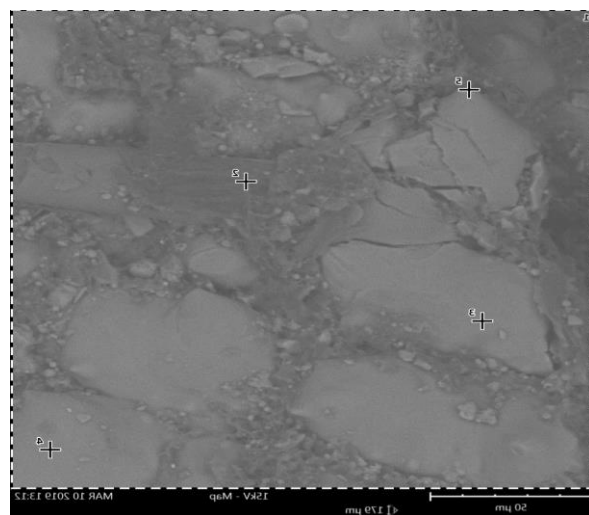
Acid pretreated 300 microns rice husks



Acid pretreated 425 microns rice husks

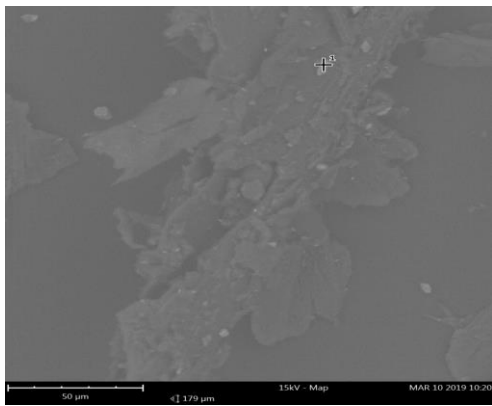


Hot water pretreated 300 microns rice husks

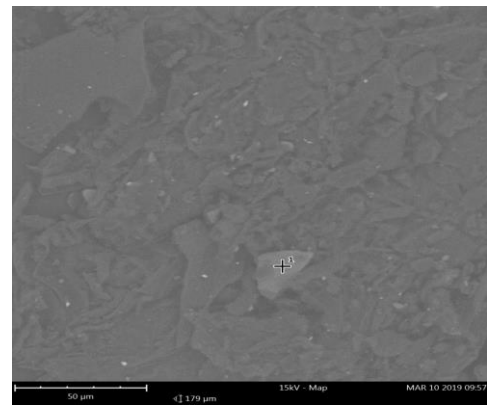


Hot water pretreated 425 microns rice husks

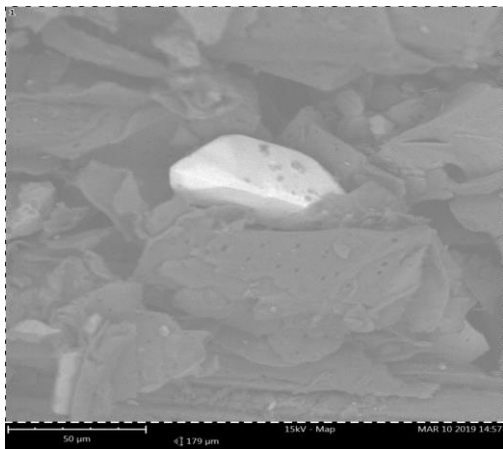
Figure D3: SEM of raw and pretreated Rice husks biomass



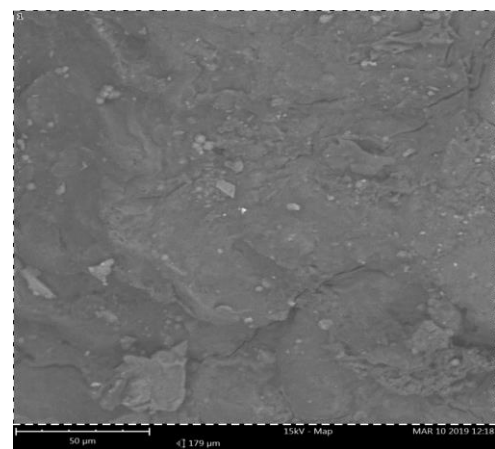
Raw sugar cane bagasse 300 microns



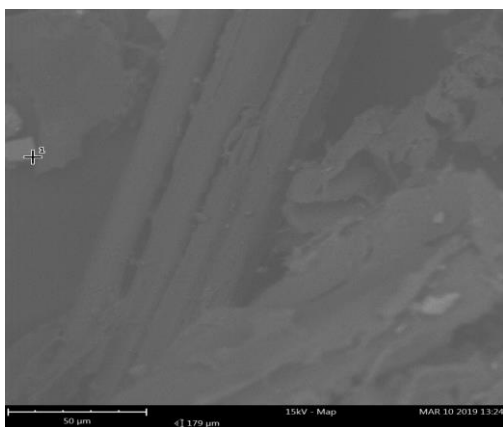
Raw sugar cane bagasse 425 microns



Acid Pretreated 300 microns



Alkali Pretreated 300 microns



Hot water pretreated 300 microns

Figure D4: SEM of raw and pretreated sugar cane bagasse biomass

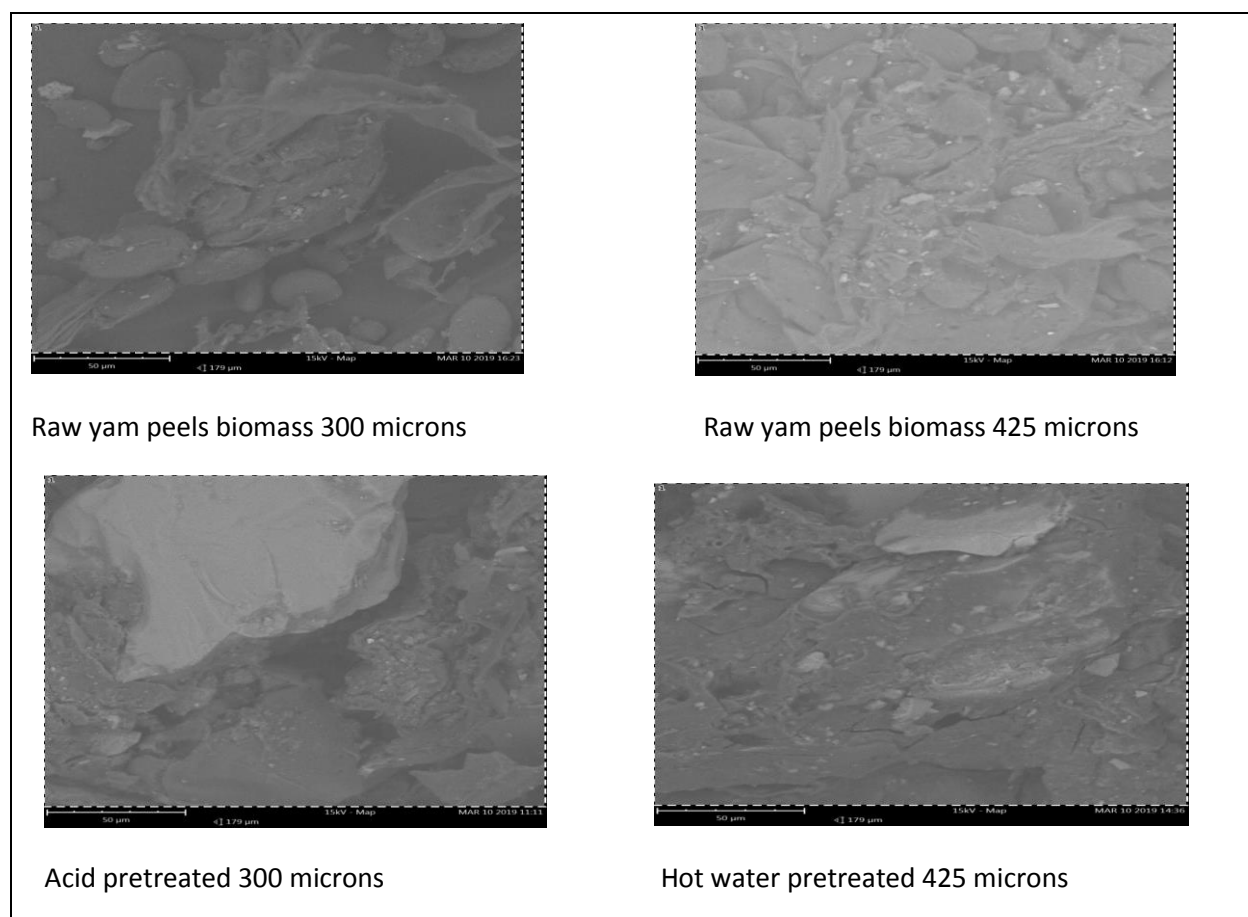


Figure D5: SEM of raw and pretreated yam peels biomass

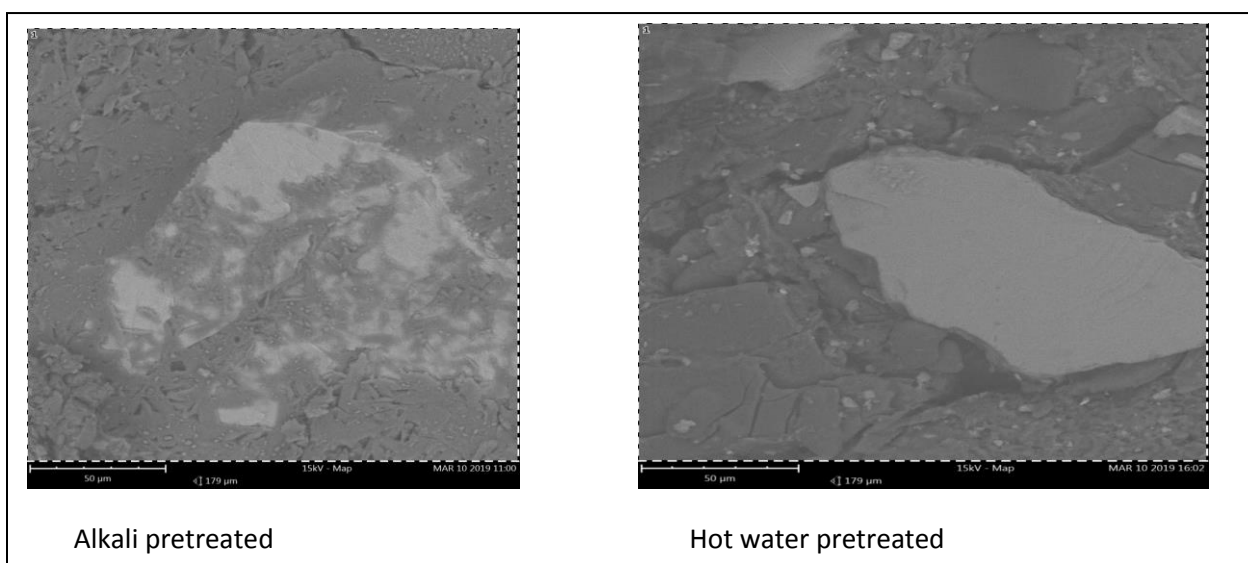
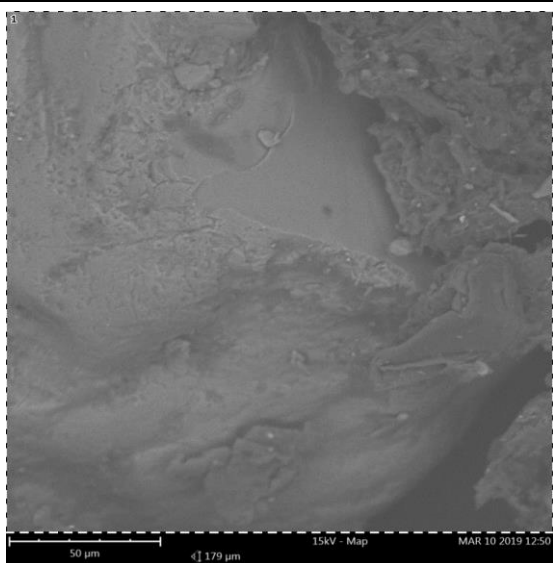
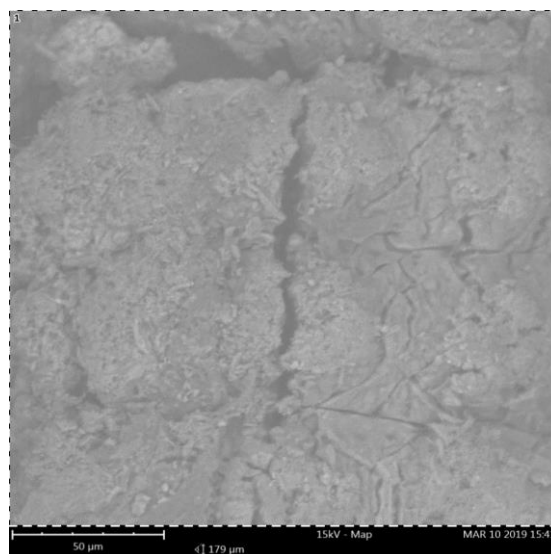


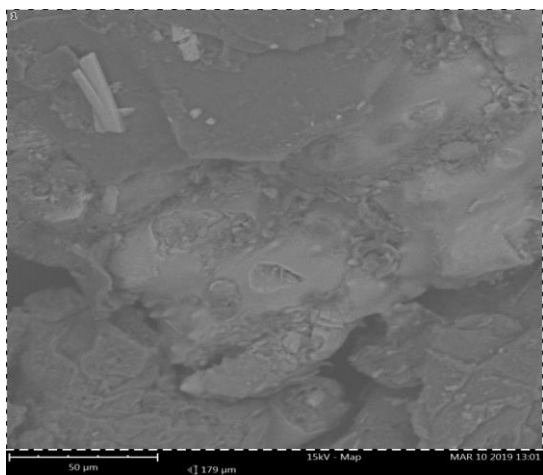
Figure D6: SEM of pretreated cassava peels plus yam peels biomass (300 microns)



Acid pretreated



Alkali pretreated



Hot water pretreated

Figure D7: SEM of pretreated all biomasses mixed (300 microns)

Appendix E: XRD patterns of raw and pretreated biomass

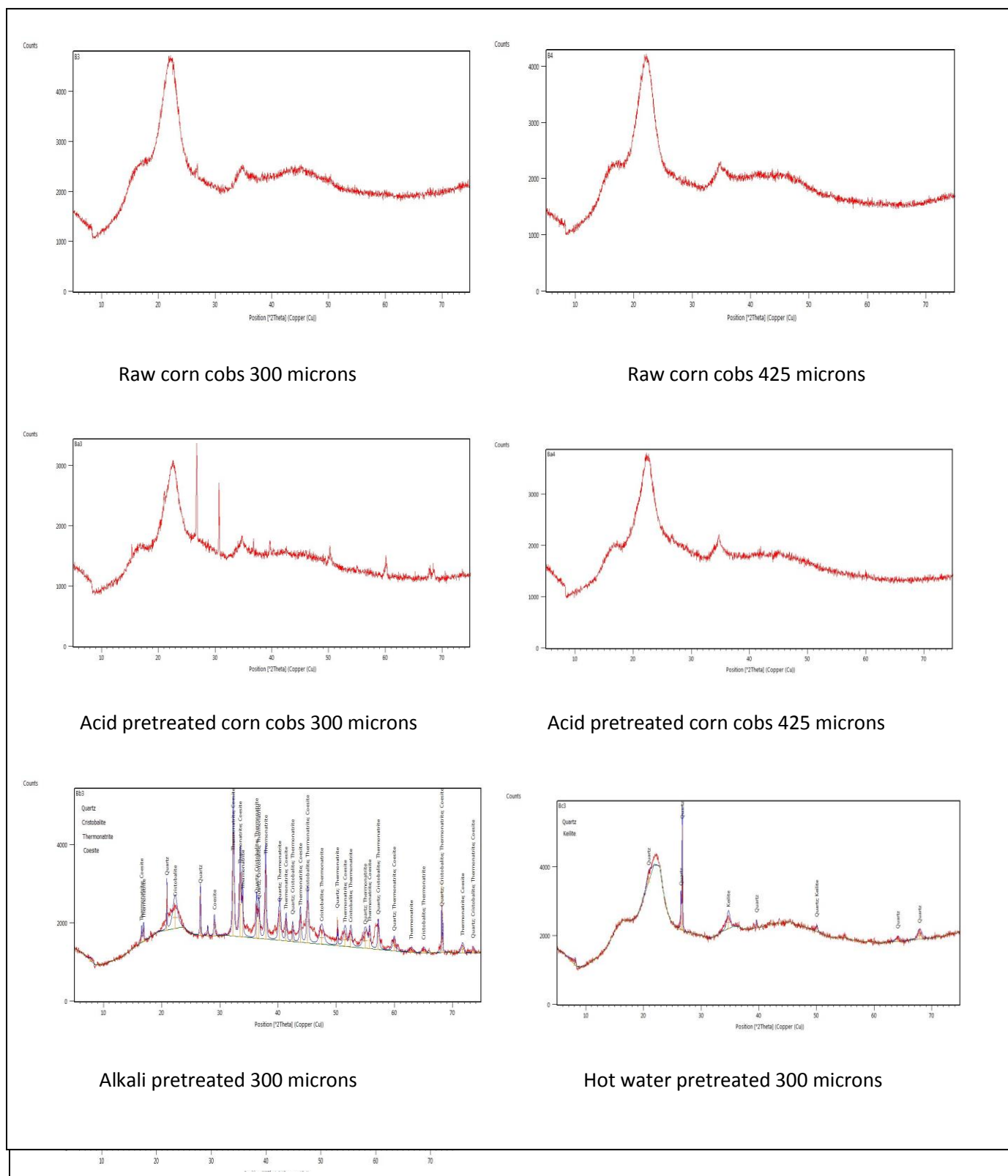
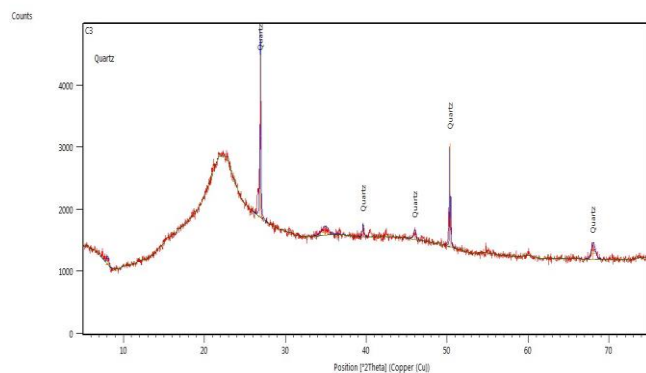
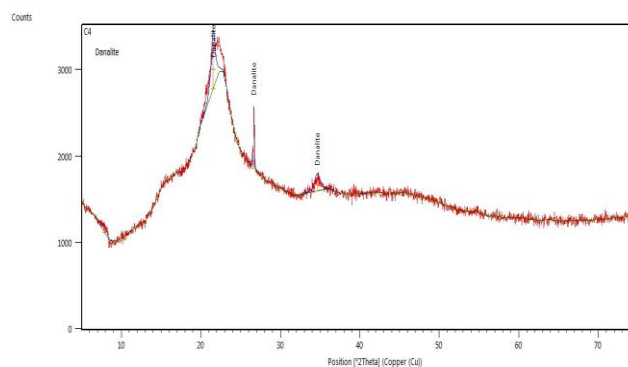


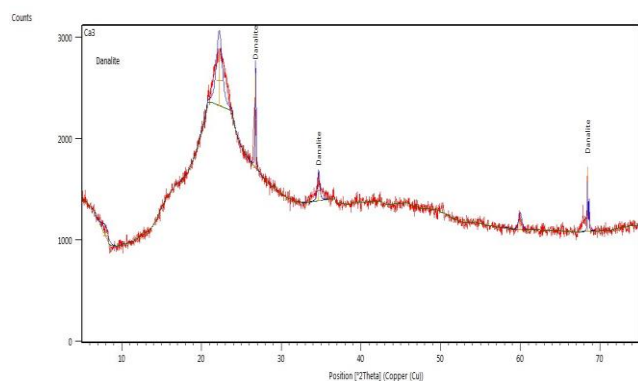
Figure E1: XRD patterns of raw and pretreated corn cobs biomass



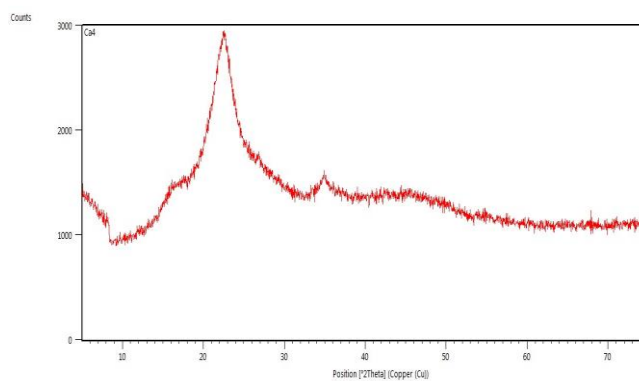
Raw rice husks biomass 300 microns



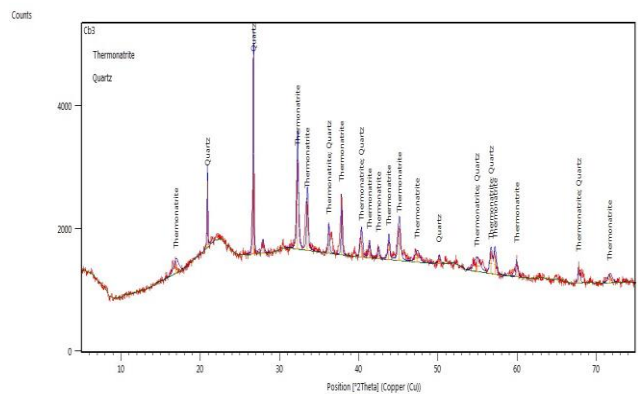
Raw rice husks biomass 425 microns



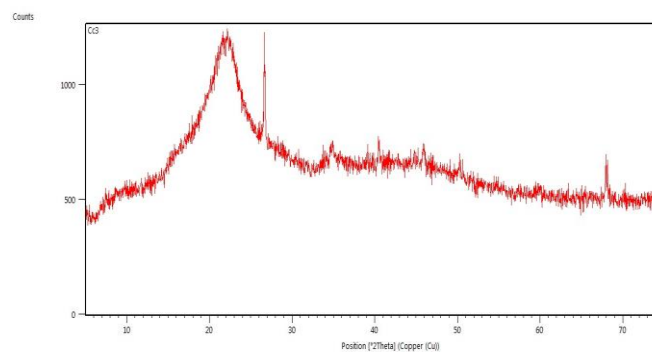
Acid pretreated rice husks biomass 300 microns



Acid pretreated rice husks biomass 425 microns

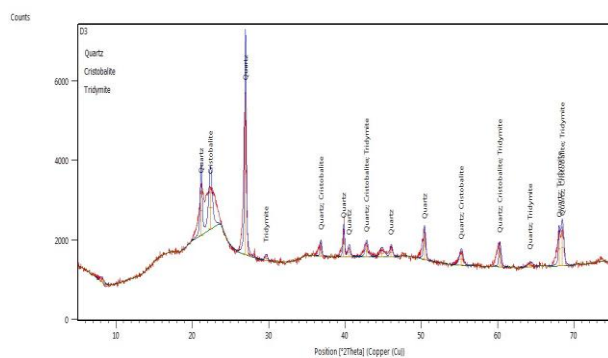


Alkali pretreated rice husks biomass 300 microns

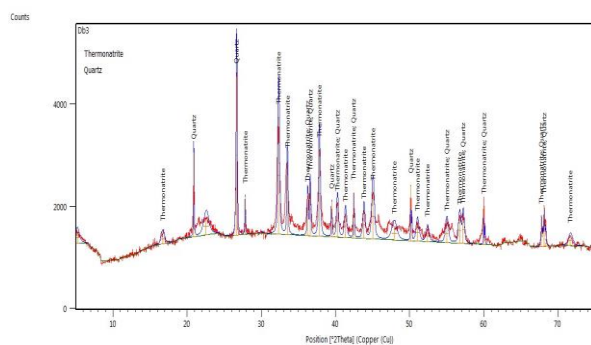


Hot water pretreated rice husks biomass 300 microns

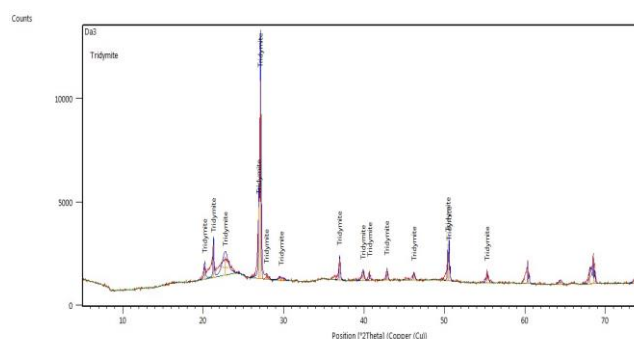
Figure E2: XRD of raw and pretreated rice husks



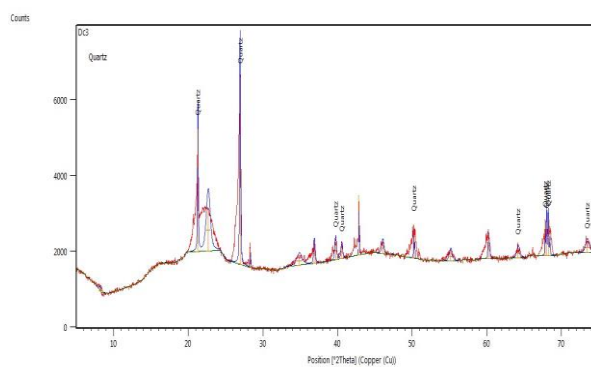
Raw sugar cane biomass 300 microns



Alkali pretreated sugar cane biomass 300 microns

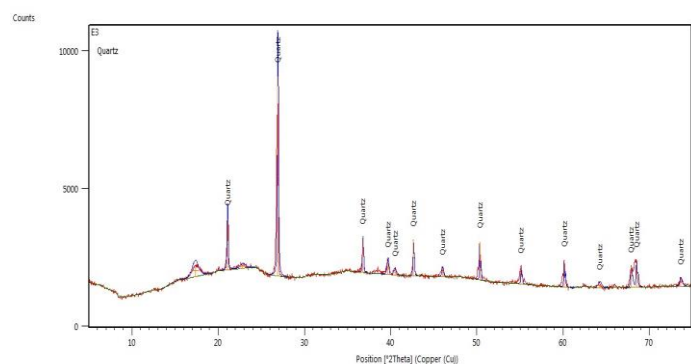


Acid pretreated sugar cane biomass 300 microns

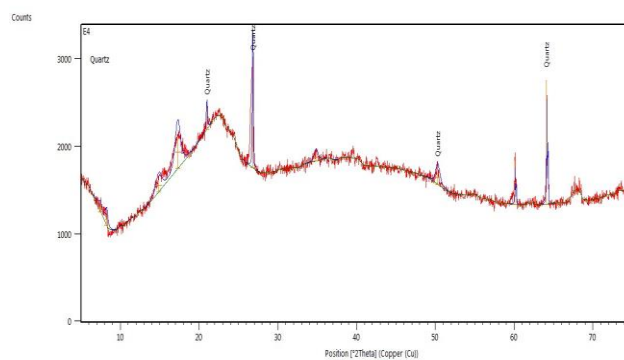


Hot water pretreated 300 microns

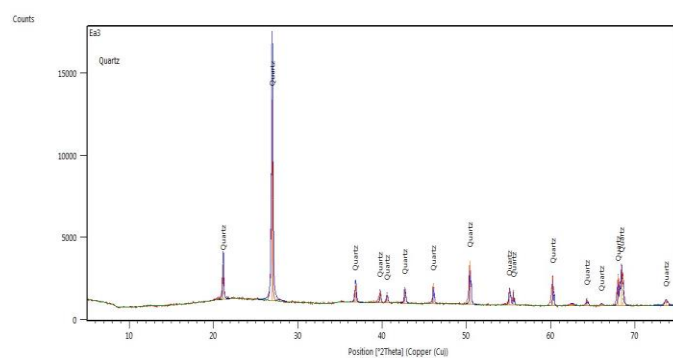
Figure E3: XRD of raw and pretreated sugar cane bagasse



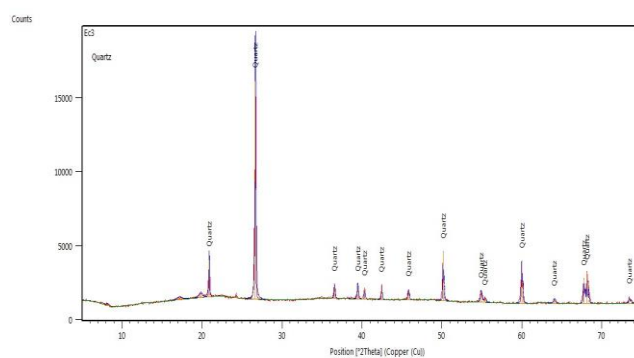
Raw yam peels biomass 300 microns



Raw yam peels biomass 425 microns

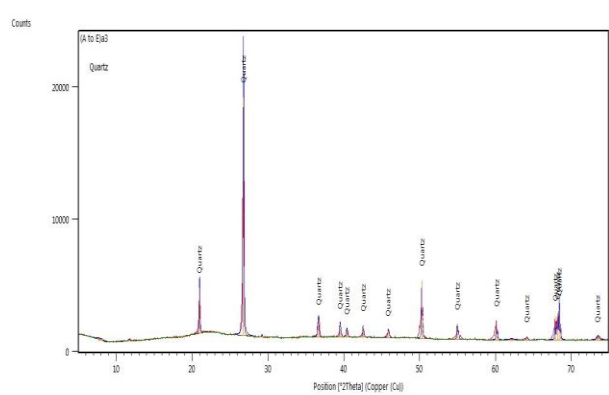


Acid pretreated yam peels biomass 300 microns

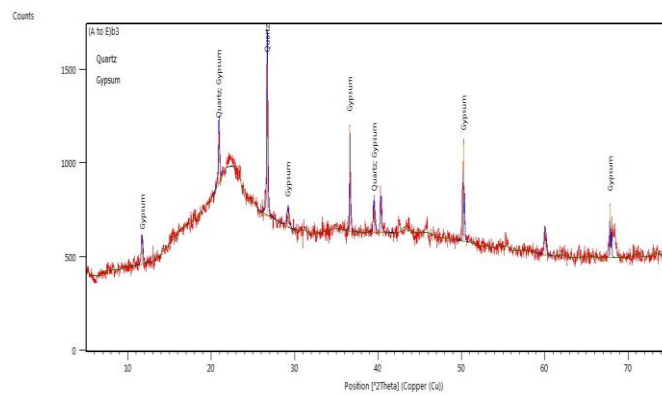


Hot water pretreated yam peels biomass 300 microns

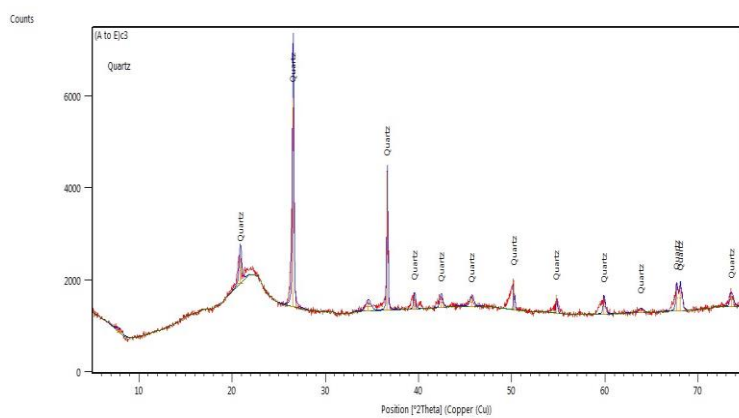
Figure E4: XRD of raw and pretreated Yam peels biomass



All biomasses mixed acid pretreated



All biomasses mixed alkali pretreated



All biomasses mixed hot water pretreated

Figure E5: XRD diffractograms of All biomasses

Appendix F: UOPCC distillation column



Appendix F Contd: UOPCC distillation column used



Appendix G: Acceptance letter for Paper 5

https://mail.yahoo.com/d/folders/1/messages/467547guc4_

Yahoo Mail - Journal of Oleo Science - Decision on Manuscript

Journal of Oleo Science - Decision on Manuscript ID ESS-21-038.R1

From: Journal of Oleo Science (onbehalfof@manuscriptcentral.com)

To: oluyale@yahoo.com

Date: Tuesday, March 23, 2021, 6:22 AM GMT+I

23-Mar-2021

Dear Mr. Awoyale.

It is a pleasure to accept your manuscript entitled "Hybridization of selected Nigerian lignocellulosic biomass feedstocks for bioethanol production: modeling and optimization of pretreatment and fermentation process parameters using response surface methodology" in its current form for publication in the Journal of Oleo Science.

Thank you for your fine contribution. On behalf of the Editors of the Journal of Oleo Science, we look forward to your continued contributions to the Journal.

Sincerely,

Osamu Shibata

Executive Editor, Journal of Oleo Science

wosamu@niu.ac.jp