MANGANESE (II)-ANDERSON POLYOXOMETALATE MEDIATED BIOETHANOL PRODUCTION FROM SUGARCANE CAKE

CLEOPA TIEMA

A Thesis Submitted to the Board of Postgraduate Studies in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in Chemistry.

Department of Physical sciences

SCHOOL OF BIOLOGICAL, PHYSICAL, MATHEMATICS AND ACTUARIAL SCIENCES

JARAMOGI OGINGA ODINGA UNIVERSITY OF SCIENCE AND TECHNOLOGY

DECLARATION

Student:

The work in this thesis is my original work and has not been presented for an award for conferment of a degree in any other university.

CLEOPA TIEMA

Reg. No. S252/4023/2019

Signature……………...……………………… Date……………………………

Supervisors:

This thesis has been submitted for examination with our approval as supervisors.

Dr. Solomon Omwoma Lugasi

Department of Physical Sciences

Jaramogi Oginga Odinga University of Science and Technology

Signature………………………………. Date……………………………

Dr. Angeline Ochung'-Orwa

Department of Physical Sciences

Jaramogi Oginga Odinga University of Science and Technology

Signature………………………….................. Date………………………

DEDICATION

To my son Israel MacDowell Alela.

ACKNOWLEDGEMENT

I express my deepest gratitude to Almighty God, for granting me this opportunity, His immeasurable love and favor upon my life which enabled me through the entire study. My gratitude and appreciation go to my able supervisors, Dr. Solomon Omwoma Lugasi and Dr. Angeline Ochung'-Orwa for guiding, inspiring and dedicating their time to read my work during the entire period of the study. The whole study was successful because of their commitment which encouraged and made me believe in myself to work towards my aspirations and dreams. May God bless them abundantly and supply them more grace in their lives. My gratitude also goes to Dr. Fred Okumu, JOOUST one of the people who encouraged me to advance and become a scholar in the field of science. The Transforming Climate Innovation Ecosystems through Inclusive Transdisciplinary project and Innovation for African Universities team who brought in the aspect of industrial student matching and linked me to entrepreneurial partners and showcased the knowledge learnt in class to real life situations, I'm grateful. To Members of the department of physical sciences of Jaramogi Oginga Odinga University of Science and Technology, I felt more encouraged from your discussion which gave me an insight, I say thank you. Special thanks to my mum, Jenipher Tiema and to my dad Jackson Tiema Munala for their prayers and encouragement. Finally, I appreciate all the persons who contributed towards the success of this work and has not been mentioned herein. May God bless you. Thank you.

TABLE OF CONTENTS

List of figures

List of tables

List of Acronyms and Abbreviations

MOE……………………………Ministry of Energy

POMs………………………….. Polyoxometalates

E10……………………………. 10% ethanol blend

COD…………………………… Chemical Oxygen Demand

BOD…………………………… Biological Oxygen Demand

WHO…………………….….…World Health Organization

ARM…………….……………… Athi River Mining

KEBS……………………………Kenya Bureau of Standards

ACFC ……………………..…. Agro Chemicals and Food Corporation

MoALF…………………………Ministry of Agriculture, Livestock, And Fisheries

MSTAT or MSTAT-C ……. A computer based Statistical software package by the Department of Crop and Soil Sciences, Michigan State University. USA.

KEPHIS………………………. Kenya Plant Health Inspectorate Service

UNEP…………………….…. United Nations Environmental Program

KPLC…………………….…. Kenya Power and Lighting Company

E.E……………………………. Energy Equivalent

KNBS……………………………Kenya National Bureau of Standards

LSD……………….……………Lower Significant Difference

KSB…………………………… Kenya Sugar Boars

HAP…………………………... Household Air Pollution

AgGDP ………………………... Agricultural Gross Domestic Product

HPLC…………………………. Higher Performance Liquid Chromatography

GHG………………………………Green House Gases

SEM……………………………... Scanning Electron Microscope

TMA………………………………Tetramethylammonium

ABSTRACT

Recalcitrant lignin compounds inhibit bioethanol production from biomass. The lignin bind cellulose and hemicellulose in biomass suppressing the fermentation process. Current delignification techniques have an average efficiency of 30% hence affecting the economics of bioethanol production from biomass. In the current work, we report a Manganese(II)-Anderson Polyoxometalate salt (Mn(II)-Anderson POM) , one of the discrete anionic metal oxide salts having a unique Lewis base and Lewis acid properties, to degrade lignin in sugar cane cake root stalk biomass. The results show the Mn(II)-Anderson POM salt to degrade lignin up to 46.97% effectively. Furthermore, upon fermentation of the pretreated cake, bioethanol concentration was 42.12% whereas the positive control (NaOH delignification) had bioethanol concentration of 26.9% using a salt concentration of 3% at 50℃ treating it for a duration of 4 hours. An increase in salt concentration from 1%, 2% and 3% gave significant increase in extracted lignin for both NaOH and POM salts pretreated samples. Similar results were obtained when temperature was varied from 30℃, 40℃, 50℃ and 60℃; and also, time from 2hrs, 3hrs and 4hrs. The quantity and quality of bioethanol obtained followed a similar trend. However, it is noted that in all cases, Mn(II)-Anderson Polyoxometalate salts performed better than NaOH pretreated samples. Specifically, an increase in bioethanol quality (46.13%) after pretreatment with Mn(II)-Anderson POM salt at a concentration of 3%, time 4hrs and temperature 60℃ as compared to NaOH (41.3%) at the same pretreatment parameters is noted

CHAPTER ONE

1.1 Background Information

Renewable energy is environment friendly; clean energy and may be regarded as the best energy in the entire globe due to its zero-carbon emission. The consumption of renewable energy depends on the total rate of production and the quantity produced. Apart from solar energy, wind energy, hydropower and geothermal energy, renewable energy can also be obtained from biomass commonly referred to as bioenergy (UNEP 2020). Bioenergy diversity arises from the need of exploring different feedstock with higher cellulose and hemicellulose compounds. This form of energy is a good replacement for fossil fuels.

The major biofuel used as a renewable energy is bioethanol (AFRINOL 2020). There are two types of ethanol: hydrous (10% water molecules also known as E10) only for special designed running engines and anhydrous (Without water molecules) for ordinary vehicle engines (Ndegwa *et al*,. 2011). The Spectre International Company located in Kisumu majorly focuses on Molasses produced from sugar companies in Western Kenya region produce anhydrous ethanol which is estimated to be 30M^3/year and it is exported to Tanzania (AFRINOL: The Kenya Market 2018).

The Agro Chemicals and Food Corporation (ACFC) in Muhoroni is estimated to produce 18M^3/Year. Mumias sugar company co-produces ethanol from sugar production process and capacity estimated to be 16M^3/year (AFRINOL, 2018). A report from MOE/GTZ 2008, stated that ACFCL and Spectre International Limited can produce 125,000 litres of ethanol per month which translates to 1.5 million liters per year. There are a number of biomass highly susceptible for bioethanol production. The major feedstock used in the production of bioethanol include; sugarcane bagasse, wheat, sorghum, corn Stover, rice Husk, Sugar beet roots, sweet potato peels and cassava (Jiuping Xu *et al.* 2015)

In the production value chain, the feedstock typical of bioethanol production should have high cellulose and hemicellulose compounds. These compounds are converted to simple sugars in order to enhance the effective fermentation process. However, the level of compounds necessary for bioethanol vary within the respective feedstock. In Kenya, the major biomass used in the bioethanol production value chain is Sugarcane bagasse from sugarcane. Sugarcane bagasse has sufficient cellulose and hemicellulose necessary for effective bioethanol production. It mainly contains 32-36% cellulose, 27-32% hemicellulose and 19-24% lignin and small extracts and mineral salts (Saleh and Halim 2018; Omwoma *et al.* 2017). The bagasse is the remains of sugarcane after sucrose extraction. The remains are then used in bioethanol production and any other industrial purposes like manufacture of fertilizers (Lopes et *al.*, 2021).

Sugarcane is one of the polysaccharide cellulose plants with high cellulose and hemicellulose compounds. The canes are grown purposively for the production of sugar (Kebede 2021). Regions growing sugarcane in western region of Kenya include: Mumias, Nzoia, Muhoroni and Migori. Sugarcane being a tropical plant may require moderate warm temperatures of about 21℃ to 26℃ and average rainfall of about 15000 mm (Bancy *et al*, 2019). High temperature is required especially at the end of growing season to enhance activation of sugar content in the plant. Apart from sugar and the bioethanol production, the cane can be used as animal feeds (Kebede 2021).

Sugarcane mainly contain 32-36% cellulose, 27-32% hemicellulose and 19-24% lignin and small extracts and mineral salts (Saleh 2018; Omwoma *et al.* 2017). It is grown in many parts of the country for large scale production of sugar. The sugarcane cake is the last ratoon that remains in the soil after harvesting the cane. It is either uprooted and burnt or used in fertilizer production (Lopes *et al.* 2021). According to Edwin Oseko, 2015 only 10,000 MT of Single Super Phosphate (SSP) are manufactured in Kenya by KEL chemicals company based in Thika Town.

The ratooned sugarcane cakes can be used in bioethanol production only if recalcitrant lignin are removed from the biomass. Lignin are complex biopolymer compounds that bind the cellulose and hemicellulos making them unavailable for the fermentation process (Liu *et al.* 2018). **Figure 1a shows** the upper section of the cane root stalk while **Figure 1b** shows the uprooted sugarcane cake (roots).

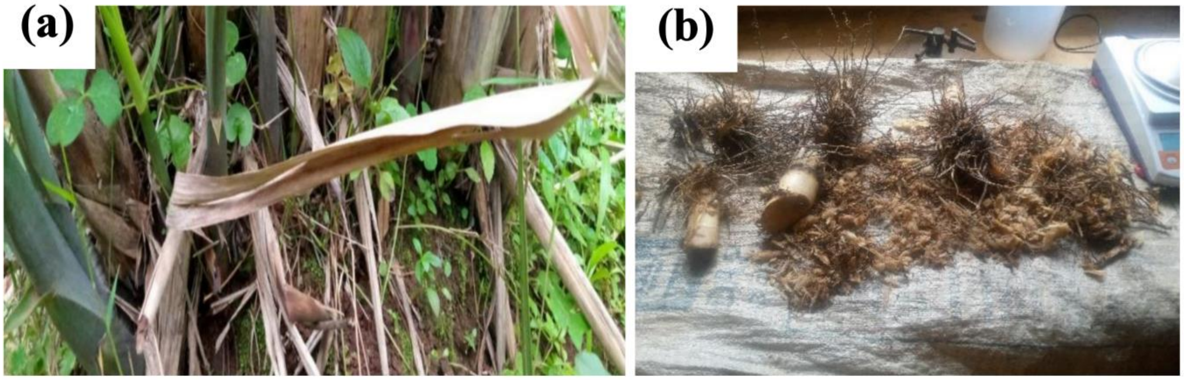


Fig 1: a) A photograph of a growing sugarcane; b) A photograph of sugarcane cake (roots) *Images taken by Tiema Cleopa on 17th July 2023 at 2:00 pm*.

The lignin can be removed by delignification process using various salts. According to (Liu *et al.* 2018), reducing the accumulation of lignin in energy plants can improve the production of the bioethanol. The de-lignifying salts remove the lignin making cellulose and hemicellulose available for fermentation process for bioethanol production (Omwoma *et al*,.2014, Saratale *et al*,. 2022*)*. Therefore, sugarcane cake is a potential feedstock in bioethanol production value chain. Using sugarcane cake feedstock to produce bioethanol can be a lucrative business for boosting Kenya's economy and evaluating the future prospect of energy from fossil fuel dependency to renewable energy which is enviro-friendly.There are various traditional techniques used to de-lignify biomass. The techniques can be grouped into two; Solvent and chemical processes (Malhotra and Suman 2021; Saratale *et al*,.2022). According to Wool 2005, Chemical treatment involving lignin modification targets the degradation of functional groups of lignin which include; acetylation, sulfonating, esterification etc. The processes involve soaking the feedstock in prepared salt solutions (Wool 2005; Saratale *et al*,.2022).

Traditional delignification methods are associated with a number of limitations. One of the methods employed is the use of steam explosion apart from use of alkaline solutions such as sodium hydroxide. Steam explosion involves heating the feedstock in water at high temperature in order to remove lignin. However, the efficacy of this method is not reliable if not well handled in regulating the conditions of temperature and time. Conventionally, sodium hydroxide is majorly used in delignification (Liu *et al.* 2018). However, since fermentation takes place effectively at a neutral pH and/or pH slightly below 7.0, the natural acids used to neutralize the broth makes the whole process ineffective and expensive. Similarly, there is less quantity of bioethanol produced when these techniques are employed. The feed stock is then fermented using *Saccharomyces cerevisiae* to speed up the rate of fermentation (Ndegwa *et al*. 2011). There are other delignifying salts with high delignification properties. Therefore, apart from use of steam explosion, acids and alkalis in pretreatment of biomass, Polyoxometalate salts can be employed in bioethanol production (Omwoma 2017).

Polyoxometalate (POMs) salts are described as discrete anionic metal oxides generally found in group 5 and 6 of the periodic table and have Lewis basic and acidic properties (Omwoma 2017; Gore *et al*. 2014). Examples of POM salts are Kegging, Dawson, Mn-Anderson, Lindquist, Waugh and Silverton. Its acidic properties find its way in bioenergy applications. Fermentation takes place effectively at a neutral pH, the use of natural acids to neutralize the broth makes the whole process expensive and less effective when alkali salts are used. In the alternative, Polyoxometalate can be employed in the delignification process since POM salts possesses high delignification properties exposing cellulose and hemicellulose for the fermentation process (Omwoma *et al*. 2017). POM salts have the ability to gain electrons during a chemical reaction. This ability is brought about by their redox nature (Lopez *et al*,. 2006). Therefore, POMs can be incorporated into the bioethanol production value chain. Polyoxometalate precursors such as Mn (II)-Anderson salt can be synthesized in the laboratory using routine procedures. Bioethanol produced from Mn (II)-Anderson pretreatment technique have similar characteristics with one prepared by conventional pretreatment methods.

In Kenya, the blended ethanol is used in pharmaceutical industry, alcoholic beverages and as a solvent (AFRINOL, 2018). According to a report given by United Nations Environmental Program (UNEP) 2022, 140 billion metric tons of biomass is generated every year from agriculture globally and equivalent to approximately 50 billion tons of oil. Agricultural biomass waste converted to energy can substantially displace fossil fuel, reduce emission of greenhouse gases and provide renewable energy to almost 1.6 billion people in developing countries (UNEP 2022).

1.2 Problem statement

Biofuel is a major target for industries producing energy. Research on more and suitable lignocellulose polysaccharide materials is required for a susceptible biomass that will give maximum energy (Kebede 2021). The feed stocks used (Sugarcane bagasse) has not been sufficient enough to produce bioethanol required in the market due to high cost of production. The focus is on feedstock which may require less successive pretreatment techniques to obtain sufficient bioethanol. The use of sodium hydroxide and natural acids as a neutralizing agent has been in use. However, it is less effective in de-lignifying biomass. Therefore, there is a need to employ other de-lignifying methods so as to improve the bioethanol production (Omwoma *et al*. 2017; Lopez 2006). Unutilized sugarcane cake feed stock and use of Polyoxometalate salts in delignification can be employed in bioethanol value chain (Omwoma *et al*. 2017).

1.3 Objective of the study

1.3.1 Main Objective

To assess the effectiveness of Polyoxometalate delignification on sugarcane cake root stalks for bioethanol production.

1.3.2 Specific Objectives

I. To optimize the delignification parameters for sugarcane cake (time, temperature and salt: Sodium hydroxide and Manganese(II)-Anderson Polyoxometalates).

II. To determine the quantity of bioethanol produced from sugarcane cake after pretreatment by sodium hydroxide and Manganese(II)-Anderson Polyoxometalates salt.

III. To assess bioethanol quality produced from sugarcane cake using Sodium hydroxide and Manganese(II)-Anderson Polyoxometalates salt pretreatment.

1.5 Justification of the study

Currently, sugarcane farmers depend on sugar for their investment returns. This makes farming unproductive and expensive. Diversifying products from the cane will improve on returns on investments in the sugar industry, government legal policies and enhance green energy diversity. Use of sugarcane cake root stalk in bioethanol production can stimulate an interest in sugarcane plantation. This can be as a result of the need to gain more from both sectors of bio-economy: Energy and Agriculture. The use of POMs pretreatment method can be employed in the bioethanol production value chain to improve and increase the yield and quality of bioethanol produced.

1.6 Significance of the study and anticipated outcomes

The contribution of this study to the economy finds its way in enhancing the exploitation of the unutilized sugarcane cake in the bioethanol production value chain. The sugarcane can also be used in bioethanol production other than sugar. Just like sugarcane bagasse, unutilized sugarcane cake root stalk can be used in the bioethanol production. This will favor the increase in metric tons of bioethanol produced per year. The need to increase the alternative pretreatment methods is also necessary in bioethanol production value chain. Mn (II)-Anderson salt will be employed in degradation of lignin apart from the convention salts used. Overdependence on fossil fuels will be reduced to the lowest need possible in order to raise the use of clean energy. Fossil fuels are associated with various disadvantages of climate change, greenhouse effect and other environmental pollution.

2.0 LITERATURE REVIEW

The section reviews some of the major research breakthroughs in sugarcane delignification processes. It also explores the chemistry of Polyoxometalate and its possible utilization in biomass pretreatment.

2.1 Lignin in biomass

Lignocellulosic biomass is a suitable source of renewable energy for sustainable development in green energy (Kathahira and Bekham, 2018). However, the vast research on feedstock typical of bioethanol production has been of need in this sector. The limiting factor to this contribution has been attached to lignin. Lignin, derived from Latin Name Lignum meaning wood, is a class of complex organic biopolymers that form key structural materials in the support tissues of vascular plants and some algae (Liu *et al.* 2018). It can be described as a polyphenolic material in plant cell walls (Kathahira *et al*. 2018). According to Katahira *et al.* 2018, lignin biosynthesis occurs through successive enzymatic dehydrogenation of three propanoid monomers.

The monomers include *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (National Center for Biotechnology Information 2023). They form cell walls in wood and encapsulate the seeds so as to prevent them from rotting and other damages. Although Lignin is of value to plants in terms of defence against cell wall degradation, water permeation to cells and other functions in terms of protecting xylems which transport water and nutrients to the whole plant, it has been undesirable to bioethanol production plants.

Lignin has complex repeating units which take the amorphous structure especially in solid state which binds the cellulose and hemicellulose in a plant (Hatakeyama, 2009; Penkina *et al*,.2012; Lathan *et al*,.2021). The cellulose and hemicellulose are key components in the bioethanol production value chain. They are reduced to simple sugars during the fermentation process. The polysaccharide lignocellulosic plants with high recalcitrant lignin require more advanced pretreatment methods (Kathahira *et al.* 2018). **Fig 2.0** shows the repeating complex units of lignin in lignocellulosic plants.

The radicals are randomly polymerized to produce biopolymers forming a three dimensional network (Kathahira *et al*. 2018). Klason method is used to measure the lignin contents in various plants and was found to be 15-25% in herbaceous, 20-25% in hardwood, and 25-35% in softwood plants (Rui Kathahira *et al.* 2018).

The perennial cellulosic plants like sugarcane have lignin which support the plant stem and the underlying tissues. The lignin in the process binds the cellulose and the hemicellulose compounds.

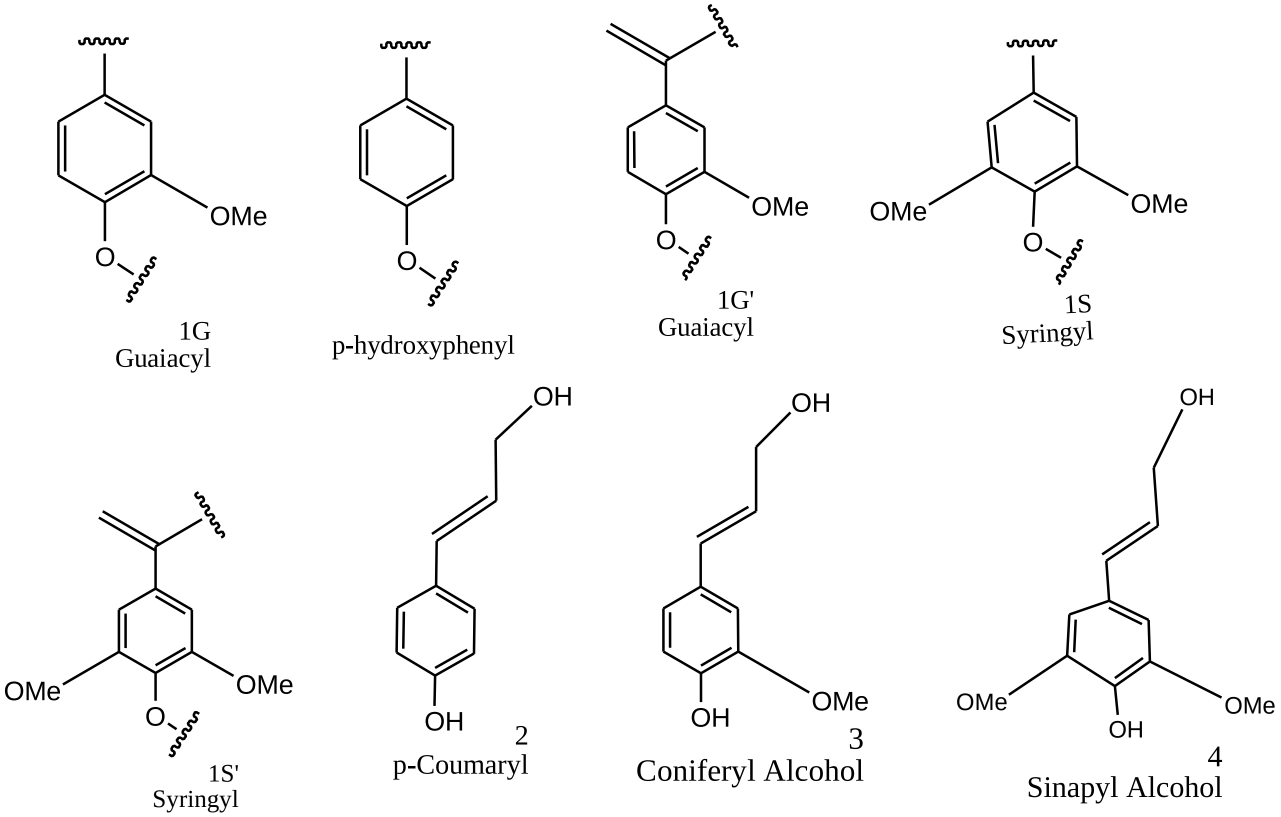
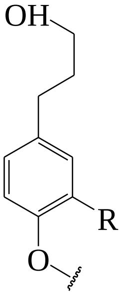
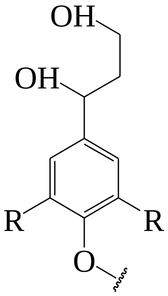


Fig 2.0: Repeating complex units of lignin in lignocellulosic plants (Rui Kathahira *et al*. 2018)

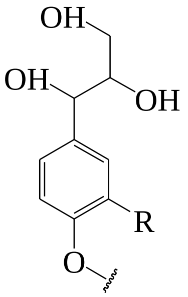
The structure of lignin can also be understood based on lignin degradation products. The analysis has been done and characterization carried out by various techniques (Linganiso *et al*,. 2019). The products formed end unit structures. Some of the methods include: FT-IR, UV-Vis, NMR, GC-MS, LC-MS, SEM and TEM (Stark *et al*,. 2016). According to kathahira *et al*. 2018, end units in **Fig. 2.1**: *p*-coumarate, ferulate, hydroxycinnamyl alcohol, hydroxycinnaldehyde and arylglycerol have been identified in lignin during the characterization using the NMR and FT-IR.



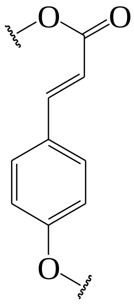
9
Dihydrocinnamyl alcohol end units



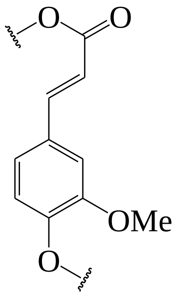
10
Arylpropane 1,3-diol end units



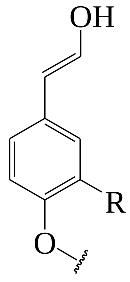
11
Arylglycerol end units



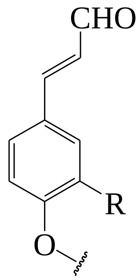
5
P-Coumarate



6
Ferulate



7
Hydroxycinnamyl alcohol end units



8
Hydroxycinnam-aldehyde end units

Fig 2.1: Side chain structures in end groups of lignin (Kathahira *et al*., 2018)

The degradation of the functional group in lignin leads to exposure of cellulose and hemicellulose making them available for fermentation process. The lignin if not removed from a biomass, fermentation process is hindered, and hence not effective (Omwoma *et al*. 2017). The parameters used during delignification process, should be evaluated carefully to enhance effective fermentation process. The lignin forms complex compounds with the polysaccharide leading to lignin-carbohydrates complex (LCC) (Feng *et al*,.2023).

2.2 Lignin content in polysaccharide lignocellulosic plants in Softwood and hardwood

The amount of lignin varies within polysaccharide cellulosic plants. The perennial plants with cellulose and hemicellulose compounds can be subjected to delignifying salts and acids. The suitability of methods employed depends on the quantity of lignin in the feedstock. Klason method is used to measure the lignin contents in various plants and was found to be 15-25% in herbaceous, 20-25% in hardwood, and 25-35% in softwood plants (Rui Kathahira et al., 2018).

The coniferous species (softwoods) have a higher lignin (26–34%), and higher cellulose content (40–45%) as compared to deciduous species (hardwoods) where lignin ranges 23–30% and cellulose 38–49% (Rowell *et al*,.2012). The dry mass of feed stock contains simple sugars, mainly the carbohydrates (QiYe *et al*,.2018). ***Table 2.0*** shows a summary of the lignin, carbohydrates, and ash content of softwoods and hardwoods (Pettersen, 1984; Suota *et al*,.2021).

Table 2.0. Lignin, carbohydrates, and ash content of softwoods and hardwoods in the United States (Pettersen, 1984; Suota *et al*,.2021). Source: (Rowell *et al*. 2012).

Species	**Holocellulose**	**α-cellulose**	**Klason Lignin**	**Ash**
Hardwoods	71.7 ± 5.7	45.4 ± 3.5	23.0 ± 3.0	0.5 ± 0.3
Softwoods	64.5 ± 4.6	43.7 ± 2.6	28.8 ± 2.6	0.3 ± 0.1

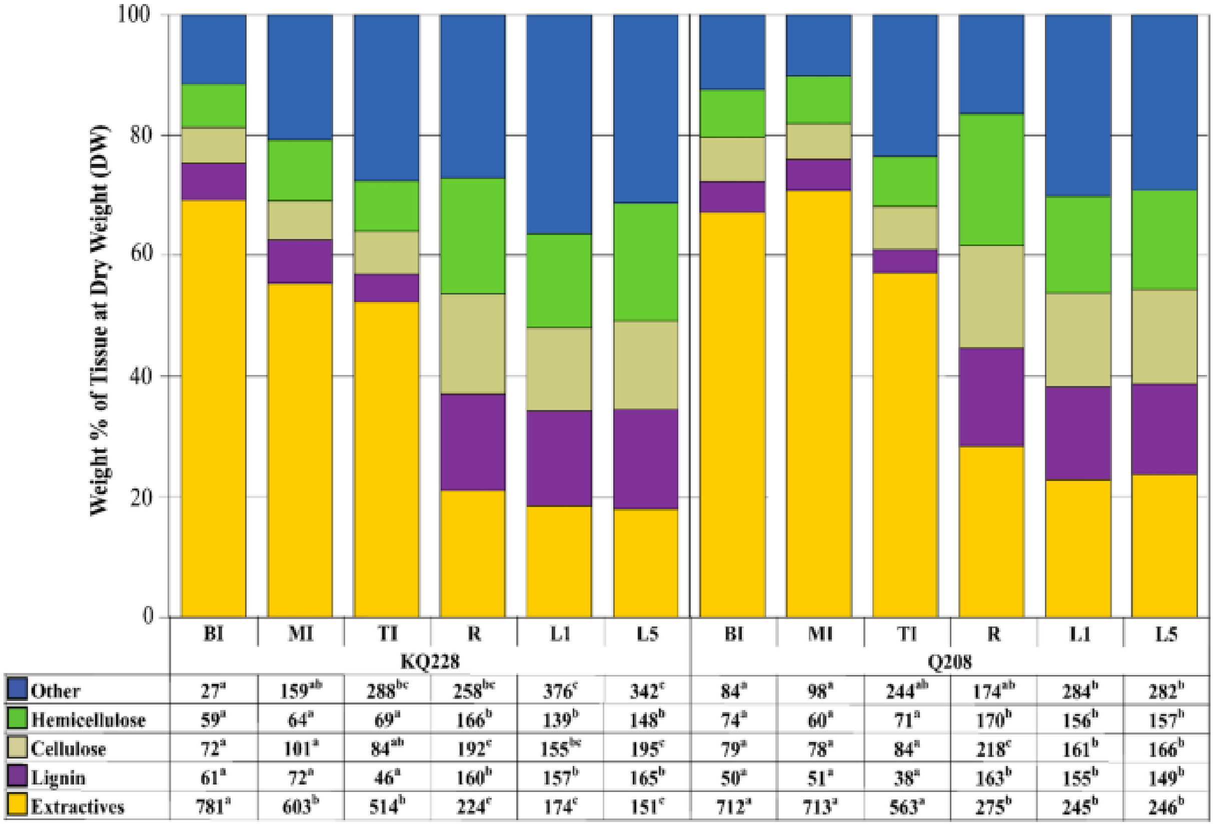
Holocellulose indicates the fraction of biomass including total polysaccharides obtained after lignin have been removed from a feedstock (Fernando *et al*. 2014).

2.3 Lignin in sugarcane bagasse

Sugarcane bagasse mainly contain 34-36% cellulose, 28-30% hemicellulose and 16-24% lignin and this is not exceptional to sugarcane cake root stalk (Sabiha-Hanim and Halim 2018). Sugarcane bagasse are biomass that remains after sucrose extraction. The biomass is effectively used in bioethanol production due to its availability and quantity. About 2.4 million metric tons of Sugarcane bagasse are generated annually by 12 sugar mills (UNEP, 2019). According to a report given by UNEP 2019, sugarcane bagasse is the main feedstock used in bioethanol production in Kenya. Generally, this can significantly be translated to give the approximate amounts of underutilized sugarcane cake root stalk. In relation to sugarcane bagasse, sugarcane cake root stalks have cellulose, hemicellulose and lignin which have varied quantities (Patrick J. Mason *et al*. 2020). This can be done through an integrated farming approach into those areas that grow sugarcane.

A research done by (Mason *et al*. 2020) indicated that, there was significant variation of lignin, cellulose and hemicellulose in different parts of the sugarcane plant: leaves, stalk, and root stalks (**Figure 2.2**). The weight of tissue evaluation was obtained when the feedstock was dry.

According to Mason *et al*. 2020, Hemicellulose and lignin fractions were significantly higher in root (*R*) and leaf (L1 and L5) tissues compared to internode (TI, MI and BI) tissues. Mason *et al*. 2020, reported that Cellulose content was also significantly higher in root (*R*) and leaf (L1 and L5) compared to internode (TI, MI and BI) tissues within the Q208 genotype.



	KQ228						Q208					
	BI	MI	TI	R	L1	L5	BI	MI	TI	R	L1	L5
🟦 Other	27^{a}	159^{ab}	288^{bc}	258^{bc}	376^{c}	342^{c}	84^{a}	98^{a}	244^{ab}	174^{ab}	284^{b}	282^{b}
🟩 Hemicellulose	59^{a}	64^{a}	69^{a}	166^{b}	139^{b}	148^{b}	74^{a}	60^{a}	71^{a}	170^{b}	156^{b}	157^{b}
▨ Cellulose	72^{a}	101^{a}	84^{ab}	192^{c}	155^{bc}	195^{c}	79^{a}	78^{a}	84^{a}	218^{c}	161^{b}	166^{b}
🟪 Lignin	61^{a}	72^{a}	46^{a}	160^{b}	157^{b}	165^{b}	50^{a}	51^{a}	38^{a}	163^{b}	155^{b}	149^{b}
🟨 Extractives	781^{a}	603^{b}	514^{b}	224^{c}	174^{c}	151^{c}	712^{a}	713^{a}	563^{a}	275^{b}	245^{b}	246^{b}

Fig 2.2: Cellulose, hemicellulose, lignin, extractive and other compounds in sugarcane plants. Source: (Mason *et al.* 2020).

2.4 Traditional Delignification method

The efficacy of all the traditional pretreatment techniques employed should be an insight to commercialization of the given biomass. There are various traditional techniques used to de-lignify biomass. The techniques can be grouped into two; Solvent and chemical processes (Malhotra and Suman, 2021; Saratale *et al*,.2022). Chemical treatment involving lignin modification targets the degradation of functional groups of lignin which include; acetylation, sulfonating, esterification etc. (Wool 2005; Nda-Umar *et al*,.2020). The processes involve soaking the feedstock in prepared salt solutions (Malhotra and Suman 2021).

Physical pretreatment method, hydrolysis, mechanical method and physicochemical pretreatment involves steam explosion, alkali pretreatment, biological pretreatment, acid pretreatment and microbiological pretreatment methods typical of delignification process (Amin *et al*. 2017). The thermochemical conversion process involves adding heat and chemicals to a biomass feedstock to produce syngas, which is a mixture of carbon (ii) oxide and hydrogen. Syngas is mixed with a catalyst such as copper-based catalysts and reformed into bioethanol and other liquid co-products (Wengi Li *et al*. 2018; Gupta *et al*,.2011).

According to Barahona *et al*. 2020, there are various pretreatment techniques which are essential for the fractionation of lignocellulosic biomass. Therefore, it contributes to removal of lignin, reduction of cellulose crystallinity, and rise in the material's porosity, hence resulting in the increase of fermentable sugars released into the liquid medium. Steam explosion involves heating the feedstock in water at high temperature in order to remove lignin. Traditional delignification methods are associated with a number of problems (Malhotra and Suman 2021). One of the methods employed is the use of steam explosion apart from use of alkali salts (Marques *et al*. 2021). However, the efficacy of this method is not reliable if not well handled in regulating the conditions of temperature and time. Conventionally, sodium hydroxide is also used in delignification (Liu *et al* 2018). However, since fermentation takes place effectively at a neutral pH, the natural acids used to neutralize the broth makes the whole process expensive and ineffective. Moreover, there is less quantity of bioethanol produced when these techniques are employed. The feed stock is then fermented using *Saccharomyces cerevisiae* to speed up the rate of fermentation (Ndegwa et al., 2011).

2.5 Feedstock delignification Methods

Delignification process involves removing and/or degrading the lignin from lignocellulosic polysaccharide plants (Wool, 2005). The process can take place when the biomass is soaked in alkaline or acidic solutions and adjusted to different conditions of temperature and time (Omwoma 2017; Chen *et al*,.2014). According to Wool 2005, Chemical treatment involving lignin modification targets the degradation of functional groups of lignin. Delignification method such as; Physical pretreatment method, hydrolysis, mechanical method, physicochemical pretreatment which involves steam explosion, alkali pretreatment, biological pretreatment, acid pretreatment and microbiological pretreatment are methods typical of lignin degradation (Amin *et al*. 2017). Liu *et al.* 2018 found that reducing the accumulation of lignin in energy plants can improve the production of bioethanol. The de-lignifying salts and acids remove the lignin making cellulose and hemicellulose available for fermentation process to produce bioethanol (Omwoma 2017; Gore *et al*., 2014).

2.6 **Delignification of feedstock**

2.6.1 **Microbiological delignification.**

Microbiological delignification framework involves using microorganisms such as fungi and enzymes to act on a biomass to degrade the lignin compounds (Tsegaye *et al*. 2019). Fungi degrade lignin components through the production of enzymes such as laccase. The enzymes degrade lignin making cellulose and hemicellulose available for the fermentation process as shown in ***Figure 2.3*** (Rajak & Banerjee 2015).

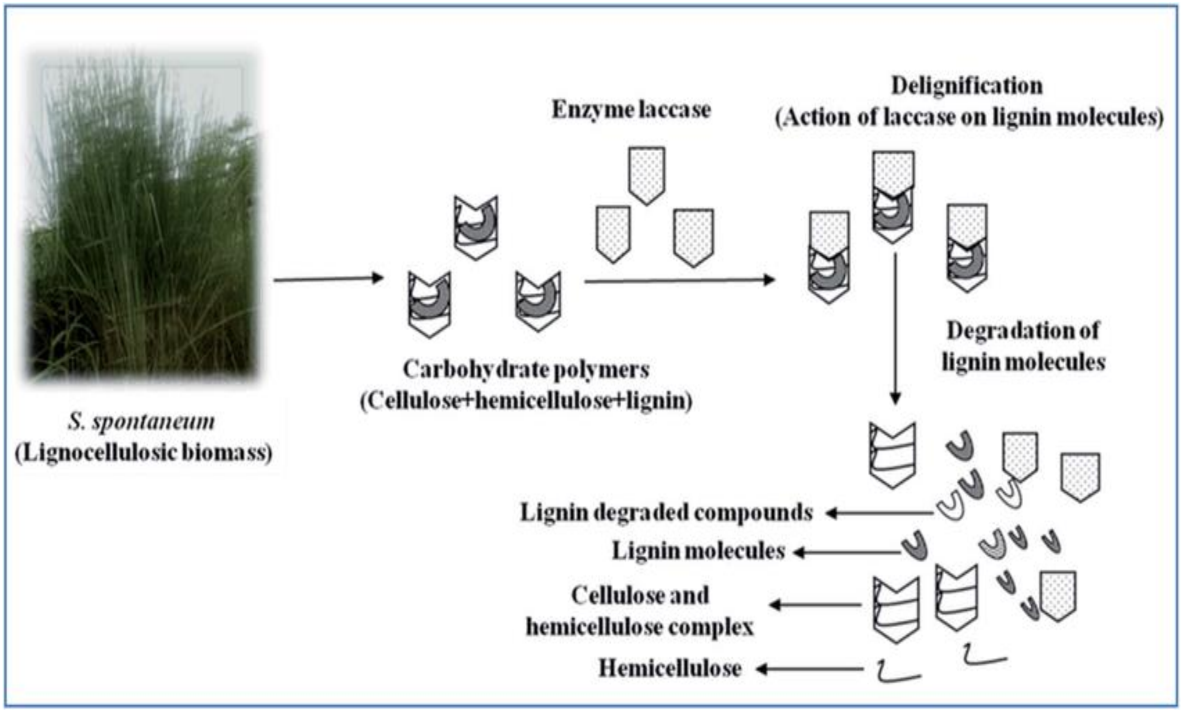


Fig 2.3: Microbiological delignification process on feedstock (switch grass) (Rajak & Banerjee, 2015)

Microbiological delignification process is summarized in F**igure 2.4** (Tsegaye *et al*,.2019). However, during the delignification using enzyme pretreatment method, pH should be regulated and adjusted to 4.0 to 5.0 to enhance functionality of enzyme (Tsegaye *et al*., 2019). pH can destroy the three-dimensional structure of enzymes which can affect the effectiveness of the enzyme. Even though the microbiological pretreatment method is promising, pH adjustment procedures maybe involving and the enzymes does not take longer time.

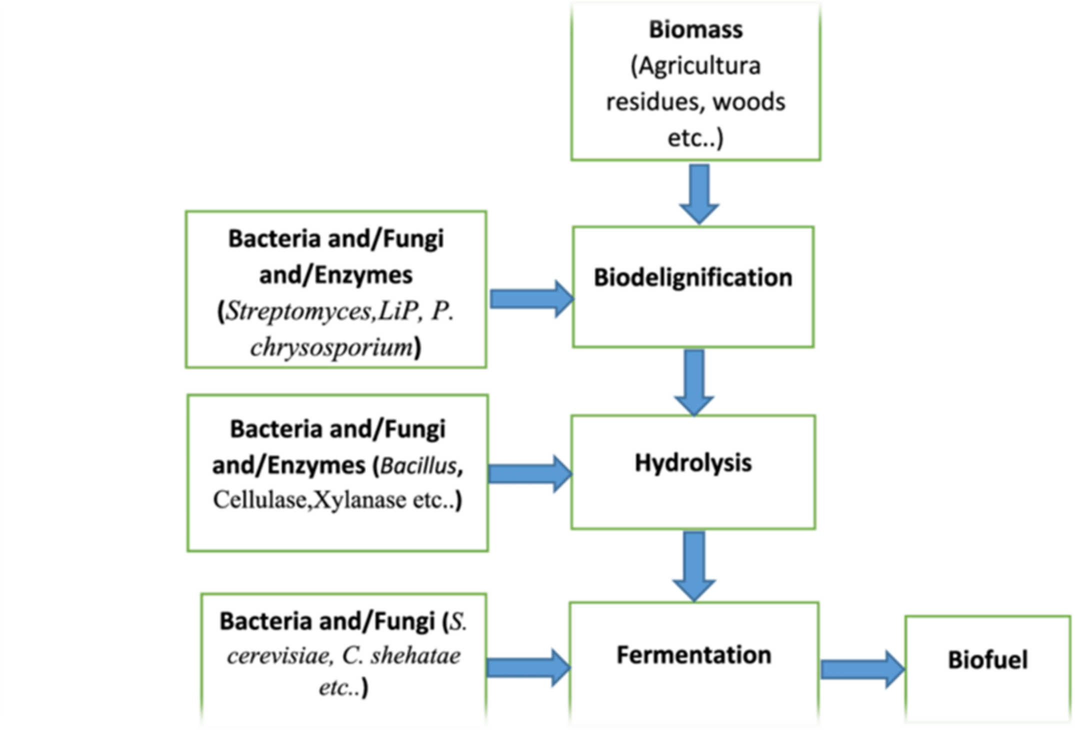


Fig 2.4: Microbiological delignification method using enzyme laccase. (Tsegaye et al., 2019).

According to (Sindhu *et al*,.2016), there are varied limitations when using enzyme pretreatment methods, for example the long incubation time for effective delignification and resistance to enzymatic hydrolysis. Therefore, the use of enzymes may not be much effective in delignification.

2.7. Physicochemical pretreatment

2.7.1 Steam explosion

Steam explosion pretreatment method involves using a high temperature of about 180°C to 285°C to open up the fibers and make the biopolymer more accessible for the subsequent process of fermentation (Stelte, 2013; Bandyopadhyay-Ghosh *et al*,.2015; Ahmad *et al*,.2018).

Steam pretreatment is commonly used in delignifying biomass due to its advantage of cost-effectiveness and environ-friendly (Keskin *et al*,.2019). However, according to (Keskin *et al*., 2019), the process has some short-coming as a result of inhibitory by-product formation and incomplete disruption of lignin. Moreover, the acid medium is used to sustain and improve the degradation of lignin and enhance the hydrolysis process.

2.7.2 Alkali pretreatment

Physicochemical pretreatment of biomass using Alkali salts such as NaOH and $Ca(OH)_2$ has been used over decades to sustain the production of bioethanol in the market. Bioethanol can be obtained from various lignocellulosic polysaccharide material with sufficient cellulose and hemicellulose using alkali salts (Chen *et al*., 2014). The method involves soaking the biomass in an alkaline prepared solution then adjusting to different conditions of temperature and time shown in ***Fig 2.5***

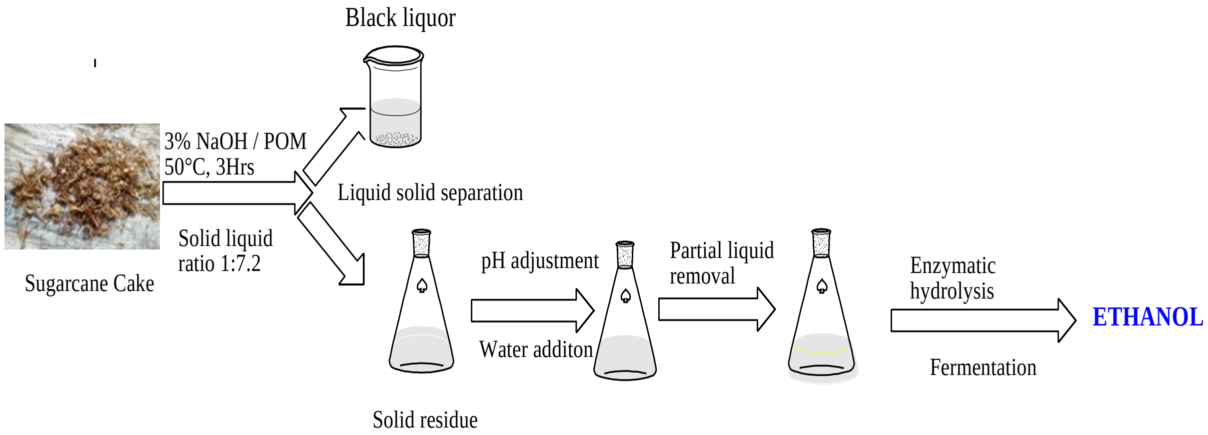


Fig 2.5: Delignification, fermentation and distillation of ethanol (Wang & Zhuang, 2019)

Alkali pretreatment method has limitations; large amounts of water is required for the washing of the pretreated biomass which is not economically viable (Sindhu *et al*., 2016). According to Mosier *et al*. 2005, during alkali pretreatment routine, inhibitors are generated which affect enzymatic hydrolysis and fermentation process. Recent studies indicate that Sodium hydroxides as a reagent has been found to be more effective although it has a number of limitations (Kim 2016). The broth involving the pretreatment requires dilute acids for neutralization (Omwoma 2014; Chen, *et al*., 2014). Therefore, it can be noted that use of alkali salts in bioethanol production may be inefficient in delignification of feedstock.

2.7.3 Acid Pretreatment

Dilute acid pretreatment of lignocellulosic biomass involves using dilute acids at different temperatures to remove and/or solubilize hemicellulose and acid soluble lignin (Chiranjeevi *et al*., 2018). However, according to Chiranjeevi *et al*. 2018, Removal of the biopolymers occurs at higher temperatures of about 160 (>160 °C) but it does not remove acid-insoluble lignin.

During acid pretreatment, coalesced lignin during condensation and re-deposition, cellulose fibers reduces the access of cellulose to cellulase thereby inhibiting the process (Chiranjeevi *et al*., 2018). Although single step pretreatment is preferred, the broth require multistage steps that are time consuming and high temperatures may cause vaporization of volatile acids (Kundu *et al,*. 2021). According to Kundu *et al*. 2021, Crystalline structure of acid pretreated biomass increases significantly with an increase in temperature and time (Kundu et al., 2021). Temperature and time are key aspects of the acid Pretreatment methods.

Research study done by Kundu *et al*. 2021 revealed a significant change in lignin removed as the band related to hemicellulose and lignin from 1 to 5h. He reported that there was a significant decrease at 3h and 5h transmittance bands related to hemicellulose and lignin compared to 1h pretreatment in hardwood and softwood.

The efficacy of traditional delignification pretreatment methods has a number of limitations as discussed previously in delignification methods. This hinders the total bioethanol produced to the market. Therefore, more delignification salts can be employed in the bioethanol production value chain. Polyoxometalate (POM) salts are other salts with delignification properties and can be coupled in bioethanol production (Omwoma *et al.* 2017).

2.8 Polyoxometalate Salts

Polyoxometalate salts typical of discrete anionic metal oxides of group 5 and 6 of the periodic table can be of significance in bioethanol production value chain (Omwoma 2021: Chen, *et al*., 2014). According to Nadii and Rompel 2018 and Wang 2015, POMs salts form a large group of anionic polynuclear Metal-Oxo-cluster with chemically modifiable structures has been greatly used in catalysis reaction in green energy. In POMs, the basic construction units are Oxo-metal polyhedra of MOx (x= 5 and 6) where the cations and polyanions cluster 1 has structural diversity (Annette Rompel 2018; Maksimov 2000).

The M in POMs can be substituted by transition metals such as Fe, Cu, Pb, Cd and Cr in Poly-Oxo anions and this include Mo, W, V, and Nb Poly-Oxo anions (Annette Rompel, 2018). In this case the transition metals are in their high oxidation state. POMs salt structures are constructed from condensation of metal oxide polyhedra with each other through corner-, edge (Omwoma *et al*. 2017).

In POM structures, the metal atoms form addenda atoms (Omwoma *et al.*2017). In POM structures, atoms (Addenda) change their coordination with oxygen as MOx polyhedra condenses in solution upon acidification (Bardin *at al*,. 2000). The atoms are able to change upon acidification as shown in **Figure 2.6**.

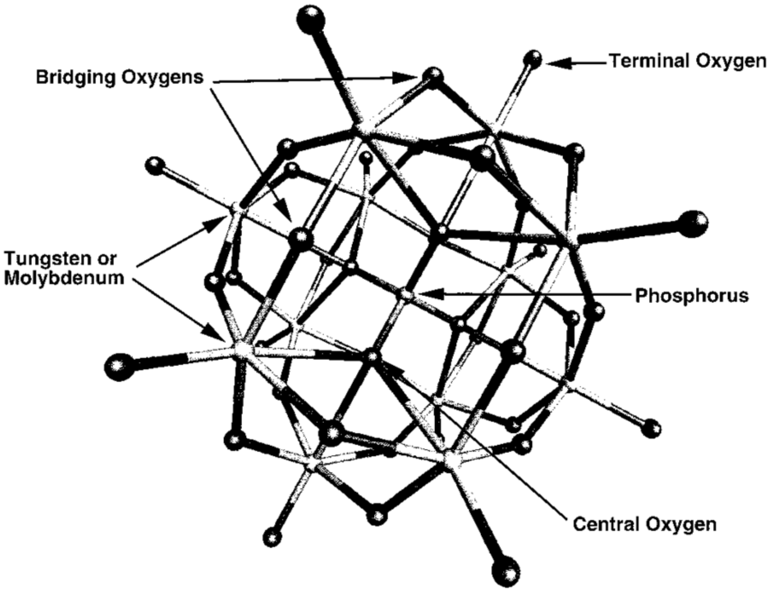


Fig 2.6: The Keggin unit contains a central atom (phosphorus), addenda atoms (tungsten or molybdenum), and oxygen atoms. (Bardin *et al*,.2000).

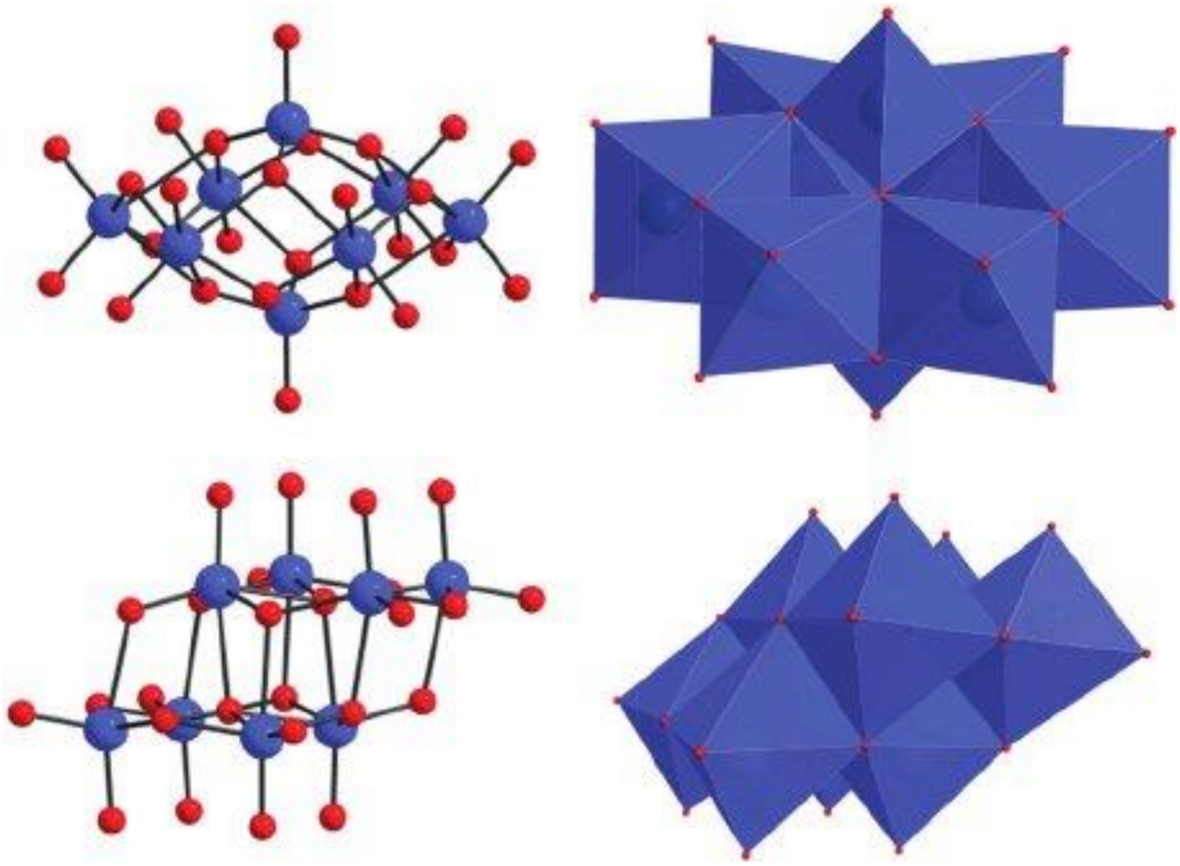


Fig 2.7: Mn(II)-Anderson POM salt structure as determined by X-ray analysis (Omwoma 2014).

POM salts have oxidative properties in nature and for this reason, they can be used in catalysis reactions in bioethanol production. POMs function by oxidizing the lignin compounds thus degrading and removing the lignin from the respective biomass (Bujanovic *et al*,.2014). According to Bujanovic *et al*,.2014, POM salts are reduced while lignin oxidizes during the delignification process.

$$\text{Lignin} + \text{POM}^{n+} \longrightarrow \text{Oxidized Lignin} + \text{POM}^{(n-2)+}.$$

The POM^{n+} is the oxidized form of the Polyoxometalate and $\text{POM}^{(n-2)+}$ is the reduced form after it has accepted electrons from lignin. POM oxidizes lignin by abstracting electrons leading to the cleavage of C-C and C-O bonds within the lignin structure. The result is the formation of lower molecular weight fragments, which are more soluble and easier to remove from the biomass. On the other hand, NaOH releases the hydroxide ions that attack the lignin structure resulting in oxidation breakdown of lignin. The robust oxidative nature of POMs can be regained by using ozone, Hydrogen Peroxide or Oxygen (Bujanovic *et al*,.2014). Similarly, POM's salts ionic nature make them soluble in polar solvents, such solvents may include; alcohols, ketones, water, dimethyl sulfoxide (DMSO) and many more (M. Piotr Putaj 2012). Therefore, the redox nature of Polyoxometalate salts finds its way in green energy application. According to Omwoma *et al*. 2017, most POMs are highly soluble in a variety of polar and polar-organic solvents.

The property of POMs to interact with most solutions via electrostatic forces, hydrogen bonding, and covalent and non-covalent interactions give it ability to be used in green energy production such as bioethanol production process (Nadii and Annette 2018; Omwoma *et al.* 2017). Vast studies done on POMs in recent years, reveals that POMs are able to form solutions in their varied dynamics (Xavier Lopez *et al*., 2006). According to Lopez 2015, the coagulation depends on the ratio between the metal and Poly-Oxo anion in solution and the solvent property.

The conventional method of alkali pretreatment methodologies requires dilute acids for neutralization. There are various solutions that form when POMs are used. Examples of POMs are Keggin, Wells-Dawson Structures, Mn (II)-Anderson and Lindquist (Deshlahra 2018). Keggin anions form spontaneously in aqueous solutions when alkali salt precursors of metal and heteroatom Oxo anions are mixed, and the solution is acidified to very low pH conditions according to Deshlahra 2018. The separation can be done through crystallization. All these POM form solutions which can be used in place of Sodium hydroxide in bioethanol production. Note that some POMs anions can also efficiently function as Lewis acids, which can happen to neutralize any basic properties during bioethanol processing (Omwoma *et al.* 2017).

2.9 Cost comparability of the salts used

POM salts are highly selective and can effectively breakdown lignin without significantly degrading cellulose. However, as compared to NaOH, POM salt may require more sophisticated equipment and controls due to the complexity of the chemical reactions involved. Even though routine production and use of NaOH is less in terms of cost, POM salt is more efficient in delignification as compared to NaOH. 1g Of POM salt can produce 12ml of ethanol while the same amount could require 8g of NaOH.

3.0 CHAPTER THREE: MATERIAL AND METHODS

3.1 Material and Equipment

Chemicals used in the study including Sodium Hydroxide, Sulphuric (vi) acid, Ethanol, acetic acid, molydate dehydrate ($Na_2MoO_4.2H_2O$), Tetra-butyl ammonium bromide, acetone, Diethyl ether, Acetonitrile, Hydrochloric acid, Commercial yeast (*Saccharomyces cerevisiae*) and distilled water were purchased from Sigma Aldrich Corporation from Nairobi (Kenya). The chemicals used were laboratory analar and required no purification. Laboratory protective gears, beakers, sample holders, fractional distillatory, hot plate, CJJ78-1 magnetic heating stirrer, FTIR, Erlenmeyer flask, thermometer and stopwatch were obtained from Jaramogi Oginga Odinga University of Science and Technology chemistry research laboratory. The Refractometer (RFM330 Refractometer) used was conducted at Maseno University chemistry laboratory.

3.1.1 Quality Assurance

Throughout the study, strict quality assurance protocols were adhered to, including the calibration of all measurement instruments before use. The refractometer used for determining bioethanol concentration was calibrated using a blank water sample and validated against a standard curve of ethanol-water mixtures, ensuring accuracy and reliability of the results. Temperature control was carefully monitored during all experimental procedures to minimize errors and ensure consistency. Cross-contamination risks were mitigated by thoroughly cleaning all equipment, especially the refractometer prism, between measurements, as outlined by DePalma (2017). Statistical analysis was performed using appropriate tools, and confidence limits were applied to validate the significance of the findings

3.2 Methods

3.2.1 Sugarcane Cake

Simple random sampling method was used to collect the sugarcane cake rootstalk for analysis. The ratooned sugarcane Cake root stalks (10 stalks) were uprooted from the soil 5 meters away from each other in Mumias sugarcane farm and mass of soil measured without removing and cleaning the Cakes. Mumias farm is located on latitude 0 º 20′ 6.9″

N and on Longitude 34 º 29′ 11.00″ E. The Cakes were then washed and the mass measured again (**Figure 3.0**). This was to determine the mass of soil attached to the sugarcane root stalks.

3.2.2 Cake preparation

The sugarcane cake was shredded into small pieces and dried at a temperature of 28.0 ℃ for 1 day to speed up the rate of change of cellulose to simple sugars according to (S. Niju & Swathika, 2019) (**Figure 3.0**). The feedstock was monitored daily to check the change in mass as water molecules reduce according to (Novo, 2011).

Fig 3.0: Shredded sugarcane cake root stalk

3.2.3 Preparation of Sodium hydroxide percentage concentration.

A stock solution of 10000 ppm, 20000 ppm and 30000 ppm was prepared according to (Deepak, 2014; Ilyas 2020). The stock solutions by calculation (w/v) represented 1%, 2% and 3% NaOH from the calculation according to Ilyas 2020.

$$Concentration\ (ppm)\ NaOH \ = \frac{mg\ of\ solute}{Volume\ of\ solution(L)}$$

mg of solute = concentration (ppm) x Volume of solution (L)……….. i

→ mg of NaOH =10000 ppm x 0.1L = 1000mg = 1g

Similarly, 1g of NaOH was dissolved in 100ml of distilled water in a 250ml beaker to make 1% NaOH according to (I. C. Montano & inti@ufg.br, 2020).

$$\% \text{ NaOH Concentration } (w/w) = \frac{\text{Mass of solute (NaOH)}}{Mass\ of\ water} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots ii$$

$$1\% \text{ NaOH Concentration } (w/v) = \frac{1\text{g}}{100mL}\ x100\%$$

3.2.4 Delignification

Delignification process involved removing the recalcitrant lignin biopolymers from the biomass. The sugarcane biomass was milled in order to shred it to 2.0 mm to improve the sugar release and bioethanol yield in the subsequent processes (Ming-Hsun Cheng *et al.* 2019).

During the Delignification process, the total impurities were removed by using a magnetic separator (Feng *et al*. 2022). The shredded and thoroughly cleaned biomass was then de-lignified by soaking in 1%, 2% and 3% prepared stock solution of sodium hydroxide solution according to Wang *et al.* 2019. The solid was loaded for 30℃, 40℃, 50 ℃ and 60℃ for 2,3 and 4 hours and agitating it at 65 rpm according to (Wang *et al*. 2019).

The solid-liquid ratio of 1:7.2 was maintained after delignification process before the fermentation step. This process enhances removal of recalcitrant lignin biopolymers that bind celluloses, hemicelluloses in sugarcane cake root stalk making them unavailable for the fermentation process. The sugarcane root stalks were also soaked in water and loaded for 30℃, 40℃, 50℃ and 60℃ for 2, 3 and 4 hours agitating it at 65rpm according to (Wang *et al*.2019).

3.3 Fermentation.

The delignified biomass was placed in a 250ml conical flask and 100ml distilled water added. The pH was adjusted to 4.7 at 25.0℃ to improve the efficacy of yeast during fermentation according to (Persson *et al*., 2002). Yeast functioned by lowering the activation energy for the reaction to start by changing metabolic pathways of the feedstock according to Recek *et al*,.2018. 2g of yeast (*Saccharomyces cerevisiae* W303-1A) was then added at room temperature and shaken thoroughly for 3 minutes (Kim 2018). The content was allowed to ferment for 3 days according to (El-Mekkawi *et al*. 2019). Fractional distillation was done 3 times to obtain and improve the concentration of bioethanol (Fan *et al*,. 2019). The concentration of the 3rd distillate was determined using the Refractometer according to (Owuama, 1993). The refractometer was used to determine the refractive Index of the bioethanol produced. The volume of the bioethanol was also recorded.

3.4 Preparations of POM precursors

3.4.1 Synthesis of Mn-Anderson

Starting material; Octamolybdate [Mo_8O_{26}]

$8Na_2MoO_4 + 12HCl + 4(n\text{-}C_4H_9)_4NBr \rightarrow [(n\text{-}C_4H_9)_4N]_4[\alpha\text{-}Mo_8O_{26}] + 12NaCl + 4NaBr + 6H_2O$

Thirty grams (30.0g equivalent to 1240 mmol) of commercial ACS reagent grade molybdate dihydrate ($Na_2MoO_4.2H_2O$) was mixed with 72 Ml of water and acidified with 31.0Ml of 6.0 N aqueous HCl (150.0m mol) in a 300 ml Erienmeyer flask with vigorous stirring over a period of 2 min at room temperature (24℃). 10ml hydrogen peroxide was added then left to settle. A solution of 3.34 × 6g of commercial 99% pure Tetrabutyl ammonium bromide (10.4m mol × 6) in 60 ml of water will then be added with vigorous stirring to cause immediate formation of a white precipitate. After stirring for 10 min, the precipitate was collected on a medium-porosity filter with sanction and washed successively with 20 ml of water, 20 ml of ethanol, 20 ml of acetone and 20 ml of diethyl ether. It was then dissolved in 35 mL of acetonitrile and stored for 24 hours at -10℃. The clear courless block-shaped crystals that form were collected by sanction filtrations and then dried for 12 hours (Martinetto et al. 2021; Ikegami and Yagasaki 2009: Rosnes and Long 2012).

Synthesis of (TMA)2Na_2[α-$M0_8O_{26}$] was from literature according to Rosnes *et al.* 2012. $(TMA)_2Na_2$[X-Mo_8O_{26}] was used to synthesize Mn-Anderson type. (TMA)2Na2[X-M08O26] (0.430g equivalent to 0.000031 mol) was dissolved in 50ml of DMF at room temperature and stirred for 20 min. $Mn(OAc)_3{\cdot}2H_2O$ (0.1268g equivalent to 0.0000465 mol) and 0.132g equivalent to 0.000011 mol of $(HOCH_2)_3CNH_2$ were added and the resulting solution was heated up to 80℃ for 18h. A brown precipitate formed was cooled at room temperature and the precipitate removed. The clear orange solution after removing the precipitate was crystallized at 4℃. The brown precipitate $(TMA)_3[MnMo_6O_{18}((OCH_2)_3CNH_2)_2]$ was obtained by re-dissolving the precipitate in DMF and crystallized by ether diffusion (Rosnes *et al*. 2012)

3.5 Manganese(II)-Anderson pretreatment method

Manganese (II)-Anderson Polyoxometalates pretreatment procedure was done similar to that of alkali (NaOH). The pretreatment process was evaluated and carried out using the Manganese(II)-Anderson in place of sodium hydroxide. The sugarcane cake root stalk biomass was cleaned and dried for about three days. The process was done at least 1 hour a day to enhance successive conversion of starch to simple sugars for fermentation according to (Hanim and Halim 2018). This involves breaking the complex compounds to simple ones according to Teeter and Compton 2023.

3.6 Bioethanol quality determination after Fermentation of delignified Biomass

The quality of the obtained bioethanol after fermentation was measured using a refractometer (RFM33). A blank experiment using water sample was used to calibrate the machine. The refractive Index (RI) of the samples were determined in accordance to (Malik *et al*. 2021). A standard curve was obtained using pure ethanol diluted with water to form percentages of 10%, 15%, 20%, 25%, 30%, 35% and 40%. The analysis temperature was 26.5℃. Replicate samples were then measured and compared to the established standard curve.

3.6.1 Blank experiment

To ensure the reliability and accuracy of the experimental results, a blank experiment was conducted to confirm that delignification and subsequent bioethanol production do not occur in the absence of POM or NaOH. This control experiment was essential for verifying that the observed effects were attributable to the active chemical agents rather than any inherent properties of the sugarcane cake biomass or the experimental conditions.

For the blank experiment, a separate batch of sugarcane cake root stalk biomass was prepared following the procedures outlined in the earlier sections. The biomass was divided into three portions: one portion was soaked in distilled water without the addition of NaOH or POM (Blank Control A), another portion was left completely untreated (Blank Control

B), and the final portion was soaked in distilled water and subjected to the same temperature and agitation conditions as the POM and NaOH treatments, but without any chemical intervention (Blank Control C).

After completing the blank experiments, the biomass from all three controls was processed through the same fermentation steps, including pH adjustment, yeast addition, and a 3-day fermentation period. Fractional distillation was then performed, and the bioethanol concentration was measured using a refractometer.

3.7 Errors and Uncertainties

Temperature control and cross-contamination errors were identified during the experimental work. These was as a result of the effect of temperature change during the experiment leading to incorrect Brix (DePalma 2017). Continuous change in temperature of the surrounding environment led to erroneous R.I values of the replicate. Cross-contamination as a result of improper cleaning of the prism which also led to retention of the residual samples of the previous measurements also was detected. The errors were eliminated by cooling and switching on to allow refractometer heat for about 10 minutes according to DePalma 2017.

3.8 Statistical Analysis

The means and ranges of the data values collected were determined in this study. Confidence limit of 5% was applied in order to test the significance of the analytical results. Analysis of variance (ANOVA) ($p \leq 0.05$) (three factor experiment) and students T-test values at ($p \leq 0.05$) were used to check the variations. Statistical analysis was performed using MSTATC three factor completely randomized design. With factorial ANOVA for the factors such that Replication was with values from 1 to 3, Factor A (Temperature) with values from 1 to 4 (30°C, 40°C, 50°C and 60°C), Factor B (Time) with values from 1 to 3 (2 hours, 3 hours and 4 hours) and Factor C (Concentration) with values from 1 to 3 for delignification, volume and concentration of bioethanol. The LSD values were calculated using the formula: $\sqrt{2} \times s/y \times t$ whereby s/y value was given by the ANOVA program results and t is the significant level ($P \leq 0.05$) obtained from the t-distribution table. Percentage delignification efficiency was determined according to Ashghar. 2013.

This was done as follows;

$$\textbf{\% efficiency} = \frac{\mathbf{30\%} \times \boldsymbol{m_2}}{\boldsymbol{m_1}},$$

Where $\boldsymbol{m_1}$ represents the mass of lignin using 3% NaOH at 50℃ treating it for 4 hours. $\boldsymbol{m_2}$ represents the average mass of lignin at contact time of 2, 3 and 4 hours respectively.

3.9 Ethical Consideration

Before proceeding to sample collection, a proposal was written and presented to a panel of experts at Jaramogi Oginga Odinga University of Science and Technology (JOOUST). Upon approval of content therein, the proposal was subjected to ethical review consideration at JOOUST ethical review committee. After a thorough scrutiny, the ethical review committee gave compliance certificate (**Appendix 1**).

4.0 CHAPTER FOUR:

RESULTS AND DISCUSSION

Analysis of means for lignin obtained after delignification of sugarcane cake, the quality and quantity of bioethanol obtained after the delignification process are presented and discussed below. For specific standard deviation of the analyzed means, they are presented as A**ppendix 2**. And the raw data from the MSTAT C program is presented in the **Appendix 3** for verification purposes.

4.1 Blank experiment and discussion

The expected outcome of this experiment was that the biomass in Blank Control A and Blank Control B would not undergo significant delignification, as evidenced by the absence of substantial lignin reduction or structural changes in the biomass. Consequently, these samples were anticipated to yield minimal or no bioethanol during fermentation, demonstrating that lignin removal is crucial for effective bioethanol production. Similarly, Blank Control C was expected to show that temperature and agitation alone, without chemical agents, do not significantly impact the delignification process or bioethanol yield. The refractometer readings for all three controls were predicted to be near zero or significantly lower than those from the POM and NaOH-treated samples, reinforcing the necessity of these reagents for the delignification and bioethanol production processes.

This blank experiment thus served as a vital control, providing clear evidence that the delignification and bioethanol production processes observed in the active treatments were indeed due to the presence of POM or NaOH, thereby validating the experimental results.

Table 4.0: Expected Results from treatment salt and Control Treatments

Sample	Treatment	Expected Lignin reduction	Bioethanol Yield	Refractometer reading /R.I
NaOH	Soaked in NaOH solution	Significant	High	High
Mn(II)-Anderson	Soaked in POM solution	Significant	High	High
Blank control A	Soaked in distilled water	Negligible	Low/None	1.3330
Blank control B	No chemical treatment	Negligible	Low/None	1.3330
Blank control C	Soaked in water, temperature control only	Negligible	Low/None	1.3330

The efficiency of lignin reduction in the active samples can be calculated using the following equation: Lignin Reduction Efficiency (%) $=\frac{Mi-Mf}{Mi}\ x100\%$

Where:

Mi -initial is the initial mass of lignin in the biomass before treatment.

Mf -final is the mass of lignin in the biomass after treatment.

4.2 Delignification using Alkali NaOH salt pretreatment

The delignification process using alkali salt was carried out at different parameters: concentration (1%, 2%, 3%), temperature (30℃, 40℃, 50℃, 60℃) and time (2hrs, 3hrs, 4hrs). There was a statistically significant change in mass of lignin obtained when the aforementioned parameters were varied (**Table 4.1 and 4.2**). In **Table 4.1**, the means were obtained from an average of means of replicated samples. The means were separated using Least Significant Difference (LSD) values obtained from MSTAT C statistical program (**Table 4.1**). Therefore, from the study, the mass of lignin removed from the sugarcane cake root stalk increased with increase in concentration of NaOH salt used. As such, there was no optimal concentration from the stock solution of 1%, 2% and 3% NaOH salt used in the study. It indicates that an optimal NaOH concentration was not obtained as an increase in concentration resulted into an increase in the delignified amounts (**Table 4.1**). As compared to a study done by Gomes *et al.* 2020, the same results were obtained when sodium hydroxide was used during the extraction of lignin. However, use of high concentration of NaOH is discouraged due to high amount of acid that will be required for neutralization during the fermentation step (Tsegave$_2$ *et al*. 2019).

When temperature was varied (at 30°C, 40°C, 50°C, 60°C), again all the means separated by LSD were statistically different (**Table 4.2**). From the study, increasing temperature increases the mass of lignin removed (**Table 4.2**). This is not surprising as steam explosion is an industrial lignin pretreatment method (Omwoma *et al.* 2017). In addition, a study done by Mohamad and Jai 2022, indicated that approximately 23.2% of lignin was removed from banana stem when heated at 170°C. Therefore, industrially, the temperature chosen should be guided by process economics and not yield.

Furthermore, time variation from 2hrs, 3hrs to 4hrs yielded statistically significant means (**Table 4.2**). It is therefore important to note that the time used for delignification using NaOH should be determined based on other economic factors and not lignin yield. Therefore, other parameters were more statistically viable compared to time even though time was still on incline from 4 hours. Although we did not determine alkali-stable lignin complex compounds, a study done by Mohamad and Jai 2022, indicated that approximately 8% of the un-extracted lignin forms stable lignin complex compounds after 2 hours. There was no mass of lignin obtained when distilled water was use. The water in this case was used as a blank experiment indicating that there was no interaction of the delignifying solvent with the feedstock used. Therefore, varying the aforementioned parameters did not degrade the lignin in the feedstock .

Table 4.1: A three factor analysis table for the means of mass of lignin obtained after sugarcane cake was treated with NaOH salt

Temp	**30** °C					**40** °C					**50** °C					**60** °C				
NaOH Conc. Time	1%	2%	3%	Av. Time		1%	2%	3%	Av. Time		1%	2%	3%	Av. Time		1%	2%	3%	Av Time	Av. Time
	Mass (g)	Mass (g)	Mass (g)	Mass (g)		Mass (g)	Mass (g)	Mass (g)	Mass (g)		Mass (g)	Mass (g)	Mass (g)	Mass (g)		Mass (g)	Mass (g)	Mass (g)	Mass (g)	Mass (g)
2 Hour	0.113	0.117	0.194	0.141		0.184	0.191	0.191	0.189		0.215	0.204	0.221	0.213		0.205	0.214	0.231	0.217	**0.190**
3 Hours	0.182	0.209	0.219	0.203		0.213	0.215	0.218	0.216		0.221	0.230	0.252	0.234		0.251	0.251	0.246	0.249	**0.226**
4 Hours	0.220	0.224	0.248	0.231		0.243	0.251	0.249	0.248		0.256	0.289	0.289	0.278		0.287	0.307	0.304	0.299	**0.264**
Av. Conc.	0.172	0.183	0.220	**0.192**		0.213	0.219	0.219	**0.217**		0.231	0.241	0.254	**0.242**		0.247	0.257	0.260	**0.255**	
Av. Temp.	**0.192g**					**0.217g**					**0.242g**					**0.255g**				

1.	LSD Temp.	0.0014
2.	LSD Time.	0.0018
3.	LSD Conc.	0.0018
4.	Coefficient of variation	0.730%
5.	Significant levels	**All means are statistically significant**

Key

⬛	**Factor A**: Temp averages
🟦	**Factor B**: Time averages
🟧	**Factor C**: Concentration averages

Table 4.2 Analysis of variance at 95% confidence level using t test on a three factor completely randomized design in MSTAT C program for the mass of lignin obtained from sugarcane cake after treatment with NaOH salt.

K Value	Source	Degrees of freedom	Squares	Mean Square	F value	Prob.	s/y	Two tail T value @0.05	LSD = √2 * s/y * t
2	Temp A	3	0.063	0.021	7704.3839	0.0000	0.0003	3.182	0.0014
4	Time B	2	0.009	0.005	1722.6508	0.0000	0.0003	4.303	0.0018
6	AB	6	0.006	0.001	351.1327	0.0000	0.0006	2.447	0.0021
8	NaOH Conc. C	2	0.099	0.049	18064.1192	0.0000	0.0003	4.303	0.0018
10	AC	6	0.006	0.001	352.8344	0.0000	0.0006	2.447	0.0021
12	BC	4	0.002	0.001	183.0539	0.0000	0.0005	2.776	0.0020
14	ABC	12	0.005	0.000	153.6754	0.0000	0.0010	2.179	0.0031
15	Error	72	0.000	0.000					

4.3 Delignification using POMs Salt (Mn(ii)-Anderson) pretreatment

The synthesized POM (Manganese(II)-Anderson) salt was verified using FT-IR (**Appendix 4**) which is comparable to literature (Rosnes *et al*. 2012). The POM salt at different concentrations (1%, 2%, 3%) was used to extract lignin from sugarcane cake (root). The results are recorded in **Table 4.3** and analysis of variance in **Table 4.4.** Statistically, all the obtained means for all the parameters (time: 2hr, 3hrs and 4 hrs.; Temp: 30°C, 40°C, 50°C, 60°C and Concentration 1%, 2% and 3%) were significantly different. The results are therefore similar in trend to delignification using NaOH pretreatment method discussed earlier. However, we noted the high levels of lignin yields obtained when POM salt is used as compared to the NaOH salt (**Fig. 4.1, 4.2 and 4.3**). The lignin levels obtained are also similar to those obtained by Bujanovic and Attala in 2011 when they used the Kegging-type POM in delignification of Birch Kraft Pulp with a 17.5% efficiency. Balakshin *et al*. 2001, also reports similar results in the trend of varying the POM salt concentration. Their variation of the Keggin type POMs from 0.1mMolar to 4.2mMolar showed an increase in the total degree of delignification. In their work, however, it was noted that an increase of temperature beyond 70°C was uneconomical with statistically insignificant yield increase.

Table 4.3: A three factor analysis table for the means of mass of lignin obtained after sugarcane cake was treated with Mn(II)-Anderson salt.

Temp	**30** ℃					**40** ℃					**50** ℃				**e**	**60** ℃				
POM Conc. Time	1%	2%	3%	Av. Time		1%	2%	3%	Av. Time		1%	2%	3%	Av. Time		1%	2%	3%	Av Time	Av. Time
	Mass (g)	Mass (g)	Mass (g)	Mass (g)		Mass (g)	Mass (g)	Mass (g)	Mass (g)		Mass (g)	Mass (g)	Mass (g)	Mass (g)		Mass (g)	Mass (g)	Mass (g)	Mass (g)	Mass (g)
2 Hour	0.137	0.144	0.215	0.165		0.195	0.193	0.231	0.206		0.231	0.234	0.265	0.243		0.218	0.251	0.254	0.241	**0.214**
3 Hours	0.187	0.233	0.230	0.217		0.235	0.228	0.239	0.234		0.240	0.386	0.262	0.296		0.248	0.276	0.276	0.267	**0.252**
4 Hours	0.186	0.194	0.204	0.195		0.221	0.225	0.257	0.234		0.275	0.283	0.291	0.283		0.321	0.287	0.314	0.307	**0.255**
Av. Conc.	0.170	0.190	0.216	**0.192**		0.217	0.213	0.242	**0.224**		0.249	0.301	0.273	**0.274**		0.262	0.271	0.281	**0.271**	
Av. Temp.	**0.192g**					**0.224g**					**0.274g**					**0.271g**				

1. LSD Temp.	0.0315
2. LSD Time.	0.0365
3. LSD Conc.	0.0365
4. Coefficient of variation	15.02%
5. Significant levels	**All means are statistically significant**

Key

▨	**Factor A:** Temp averages
🟦	**Factor B:** Time averages
🟧	**Factor C**: Concentration averages

Table 4.4: Analysis of variance at 95% confidence level using t test on a three factor completely randomized design in MSTAT C program for the mass of lignin obtained from sugarcane cake after treatment with Mn-Anderson salt.

K Value	Source	Degrees of freedom	Squares	Mean Square	F value	Probability	s/y	Two tail T value @0.05	LSD = √2 * x/y * t
2	Temp A	3	0.126	0.042	32.1480	0.0000	0.007	3.182	0.0315
4	Time B	2	0.016	0.008	5.9349	0.0041	0.006	4.303	0.0365
6	AB	6	0.012	0.002	1.5586	0.1718	0.0121	2.447	0.0419
8	POM Conc. C	2	0.039	0.019	14.7567	0.0000	0.006	4.303	0.0365
10	AC	6	0.012	0.002	1.4794	0.1974	0.0121	2.447	0.0419
12	BC	4	0.018	0.005	3.4406	0.0125	0.0104	2.776	0.0408
14	ABC	12	0.021	0.002	1.3229	0.2249	0.0209	2.179	0.0644
15	Error	72	0.094	0.001					

In addition to significantly higher lignin yields obtained from Mn(II)-Anderson delignified samples, an observation of the delignified sample in a Scanning Electron Microscope (SEM) show a clear difference between delignified samples using NaOH and Mn(II)-Anderson salts (**Fig 4**). In the POM delignified sample, clear exposed grains are observed as opposed to the control samples and NaOH salt pretreated samples.

In contrast to our findings, whereby delignified amount increased significantly with increase in POM concentration, Wang *et al*. 2019 reported that 3% POM concentration at 50℃ and 4 hours as the optimal delignification conditions. The main difference is in the mixing ratio wherein they used a ratio of 1:9 and we used 1:7.2. This therefore shows that the mixing ratio could play a great role in determining optimal delignification conditions.

It is noted that the Mn(II)-Anderson POM salt is redox in nature and also has the ability to form hydrogen bonds with polar and non-polar substances (Omwoma 2014). Probably these two properties make it a good delignification substrate as well as being reusable hence reducing production costs in the bioethanol production value chain.

The percentage efficiency was determined and recorded in tables 4.4 and 4.5

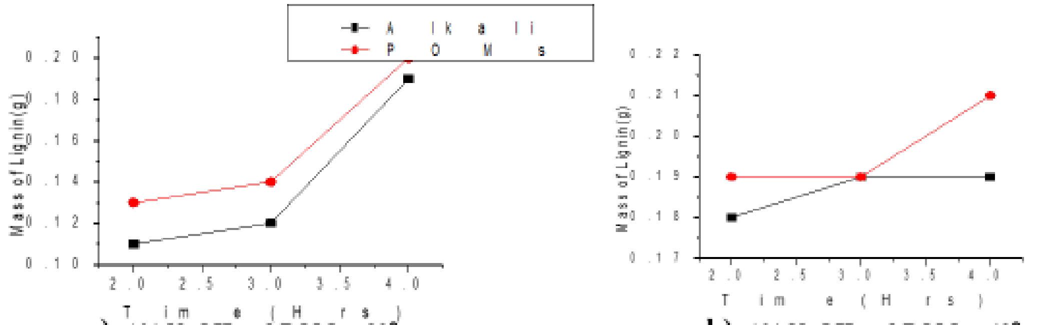
Table 4.5: Percentage efficiency delignification of average mass of lignin obtained from sugarcane root stalk at 2, 3 and 4 hours.

Average mass of lignin for,	**2 hours**	**3 hours**	**4 hours**
% efficiency for NaOH treatment	19.72	23.46	27.40
% efficiency for POMs treatment	22.21	26.16	26.47

Table 4.6: Percentage efficiency delignification of average mass of lignin obtained from sugarcane root stalk at 30℃, 40℃, 50℃ and 60℃.

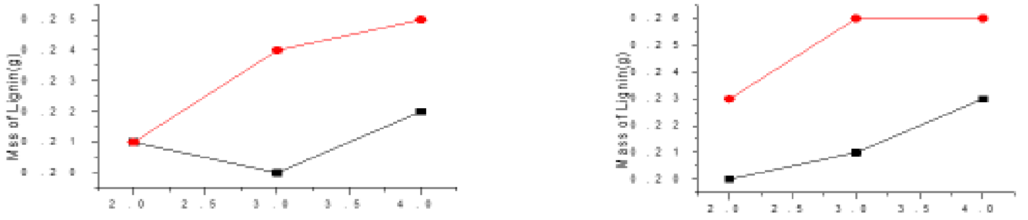
Average mass of lignin at,	**30℃**	**40℃**	**50℃**	**60℃**
% efficiency for NaOH	19.93	22.53	25.12	26.47
% efficiency for POMs	19.93	23.25	28.44	28.11

In t**able 4.5**, increasing contact time led to an increase in the percentage efficiency of delignification of the sugarcane cake root stalk. The percentage efficiency increased in both cases. However, the delignified amounts recorded was higher for the case of Mn(II)-Anderson salt. Similar trend was observed when temperature was increased steadily as shown in **table 4.6**. Temperature is a viable factor that could be evaluated due to economies of scale unlike contact time which should be for other economic factors of the bioethanol producing companies. Treatment at 30℃ was similar for both salts. Percentage efficiency trend when concentration of salt used is increased, gives a broader insight on the commercialization of the optimal concentration used for delignification of feedstock. The total amounts of lignin removed from the feedstock was compared when two salts were used as shown in the Figures 4.0, 4.1 and 4.2 respectively.



a). 1% NaOH and POM at 30°C

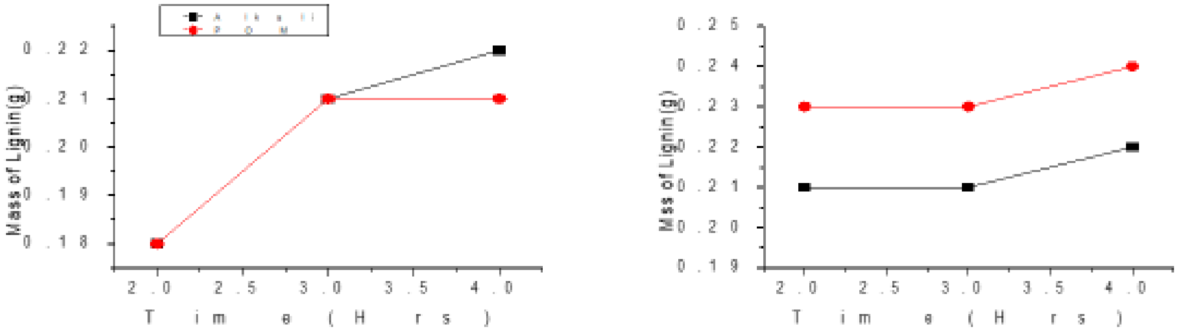
b). 1% NaOH and POM at 40°C



c). 1% NaOH and POM at 50°C

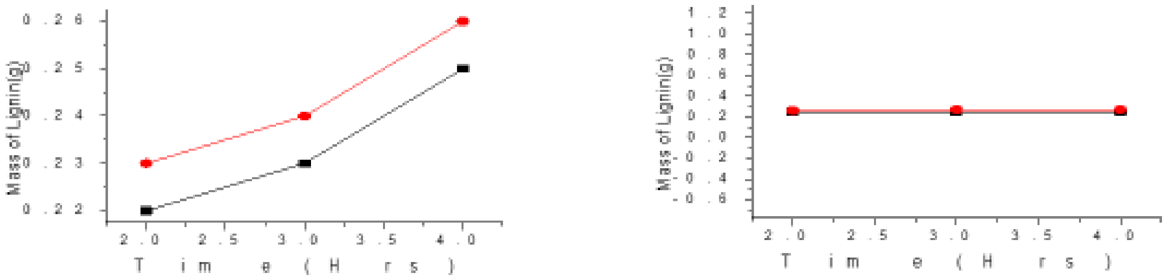
d). 1% NaOH and POM at 60°C

Fig 4.0: Comparison of delignification at 1% concentration of NaOH and Mn-Anderson POM salt pretreatment.



e). 2% NaOH and POM at 30°C

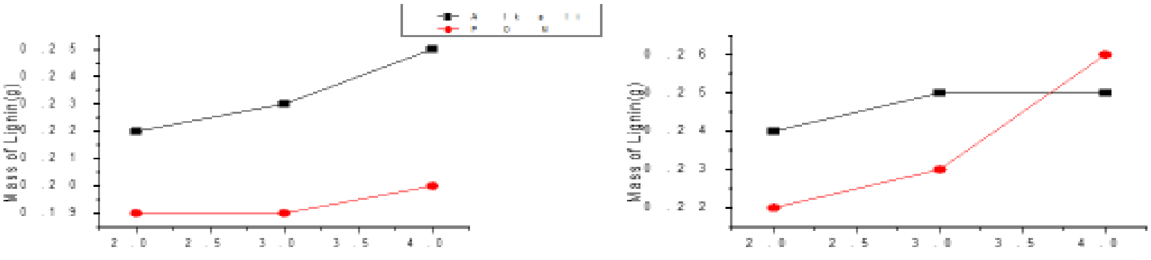
f). 2% NaOH and POM at 40°C



g). 2% NaOH and POM at 50°C

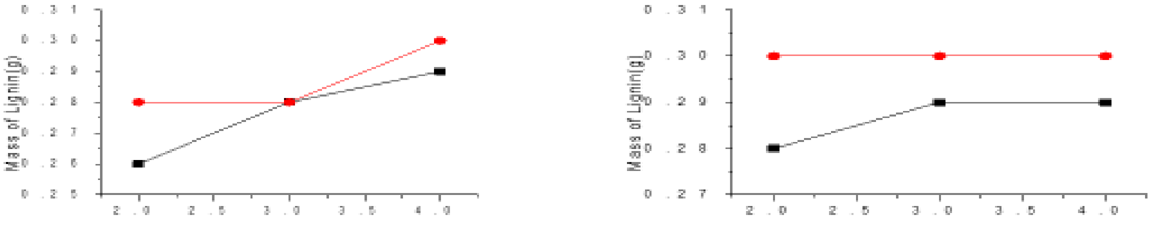
h). 2% NaOH and POM at 60°C

Fig 4.1: Comparison of delignification at 2% concentration of NaOH and Mn(II)-Anderson POM salt pretreatment.



i). 3% NaOH and POM at 30°C

j). 3% NaOH and POM at 40°C



k). 3% NaOH and POM at 50°C

L).3% NaOH and POM at 60°C

Fig 4.2: Comparison of delignification at 3% concentration of NaOH and Mn(II)-Anderson POM salt pretreatment.

Scanning electron microscope was used to magnify the nature of the delignified sugarcane root stalk pores when distilled water, NaOH and Mn(II)-Anderson salt was used during delignification (**fig 4.**3).

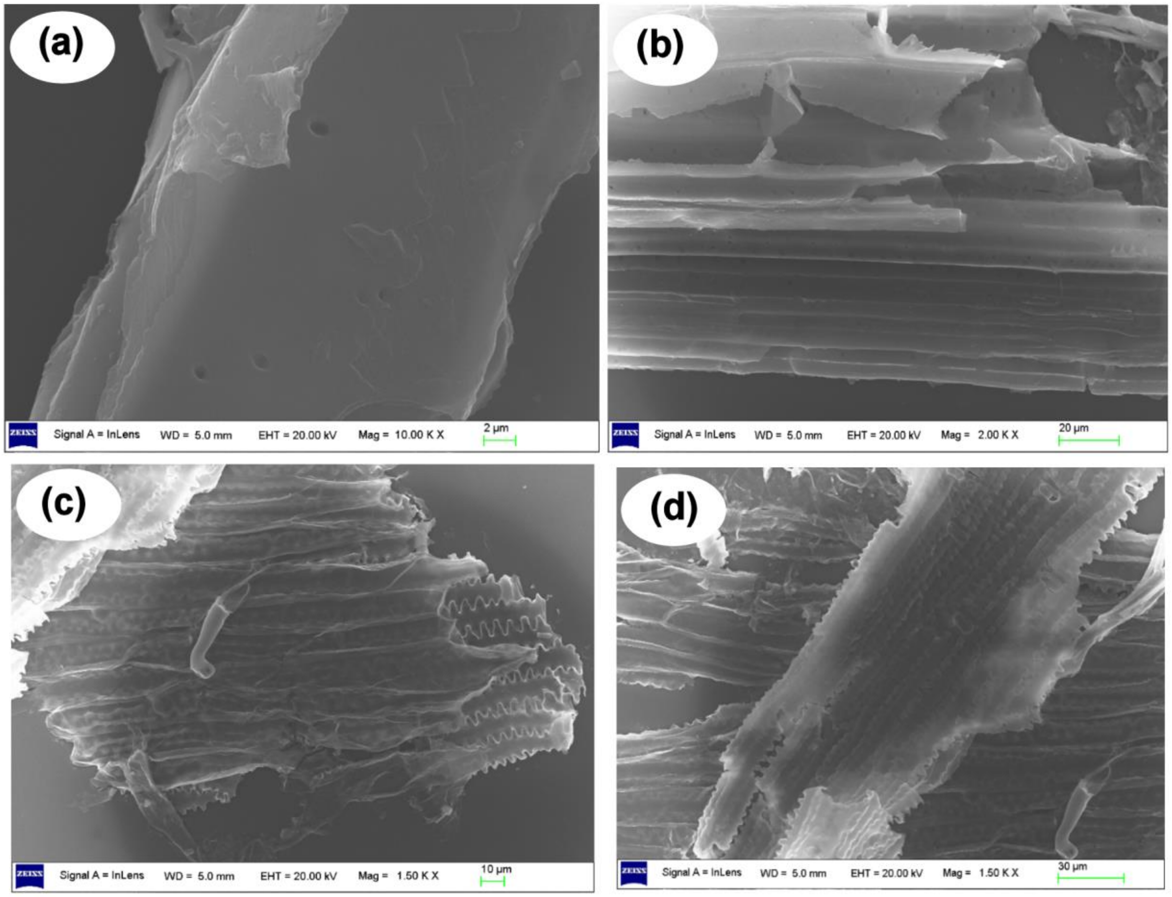


Fig 4.3: Scanning Electron Microscope (SEM) results of the sugarcane cake. a) Soaked in water, b) lignin degradation with NaOH (standard method in industry), c) and d) lignin degradation using Mn(II)-Anderson.

4.4 Quantity of bioethanol produced from sugarcane cake under NaOH and POM salt pretreatment methods

Analysis of quantities for the resultant bioethanol after fermentation by NaOH pretreatment of samples is recorded in **Tables 4.7** and **4.8**. Once again, all the means obtained are statistically significant. The results are in line with the delignification results showing that the more lignin is removed from the sugarcane cake, the more bioethanol is produced. The current study is also similar to other bioethanol production from different feed stocks. For instance, a study conducted by Kim 2018 revealed that in a batch fermentation process, with alkali-pretreatment (NaOH) of empty palm fruit bunch fiber, using concentrations from 0.5 to 2M NaOH, lignin reduction led to production of 21g/L ethanol within 28 hours when *Saccharomyces cerevisiae* was used during the fermentation step. Similarly, a study conducted by Trevorah and Othman 2015 revealed a notable increase in bioethanol yields with increase in the concentration (10%) of NaOH producing 1.92 - 2.07 times more than those treated using 3% NaOH.

Additionally, in a study conducted by Trevorah and Othman 2015, using alkali pretreatment and enzymatic hydrolysis of Australian timber mill sawdust for biofuel production, showed that maximum yields of bioethanol obtained were at 121℃ using 7% NaOH salt. According to them, this produced 29.3% and 30.6% ethanol yield after treating it for 0.5h and 24h respectively. Therefore, treating biomass at higher temperatures potentially results to significant higher yields of bioethanol. Furthermore, they demonstrated that an increase in bioethanol production is obtained if the fermentation process takes a longer time. For instance, there was an increase of 29.3% to 30.6% ethanol yields when treatment time was increased from 0.5h to 24h. Similarly, analysis of means for quantities of bioethanol produced after Mn(II)-Anderson POM salt pretreatment method are recorded in **Table 4.9** and **4.10**. All the means are statistically significant and the yields are higher than those obtained when NaOH was used as a pretreatment salt (**Table 4.7 and 4.8**). To the best of our knowledge, POM salts have not been employed in the sugarcane bioethanol production value chain. Therefore, the current work serves as a new knowledge that can be utilized to increase yields of bioethanol from sugarcane value chain. The cake of sugarcane is definitely very high in lignin amounts and therefore the use of POMs to remove the lignin would be very beneficial in the production value chain of bioethanol. SEM images (**Fig. 4.3**) show a significant decomposition of the lignin exposing celluloses and hemicelluloses for the fermentation enzymes. Furthermore, the performance of POMs salts in delignification of other biomass and production of organic solvents has been previously explored. For instance, Deng *et al*. 2023, used Keggin type POM as a catalyst to obtain significant increase in alkanes and aromatics of up to 30%. According to his findings, <5 wt.% required for biofuel conversion had a higher oxygen content and calorific valued of approximately 41MJ/kg. There was no bioethanol obtained from the fermented sugarcane root stalk which was delignified by distilled water.

Table 4.7: A three factor analysis table for the means of bioethanol quantity obtained after sugarcane cake was treated with NaOH salt.

Temp	**30** ℃					**40** ℃					**50** ℃					**60** ℃				
NaOH Conc. Time	1%	2%	3%	Av. Time		1%	2%	3%	Av. Time		1%	2%	3%	Av. Time		1%	2%	3%	Av Time	Av. Time
	Vol (ml)	Vol (ml)	Vol (ml)	Vol (ml)		Vol (ml)	Vol (ml)	Vol (ml)	Vol (ml)		Vol (ml)	Vol (ml)	Vol (ml)	Vol (ml)		Vol (ml)	Vol (ml)	Vol (ml)	Vol (ml)	Vol (ml)
2 Hour	0.0	0.3	0.3	0.2		1.5	1.7	2.1	1.8		2.1	1.5	1.8	1.8		1.8	1.9	2.5	2.1	**1.5**
3 Hours	0.03	0.4	0.6	0.34		2.4	2.2	2.3	2.3		2.2	2.8	2.7	2.6		2.4	2.5	2.8	2.6	**2.0**
4 Hours	0.4	0.7	0.7	0.6		2.4	2.4	3.3	2.7		2.3	2.9	3.8	2.7		3.6	3.2	3.5	3.4	**2.4**
Av. Conc.	0.23	0.47	0.53	**0.41**		2.1	2.1	2.6	**2.3**		2.2	2.4	2.8	**2.5**		2.6	2.5	2.9	**2.7**	
Av. Temp.	**0.38ml**					**2.3ml**					**2.4ml**					**2.7ml**				

a. LSD Temp.	0.0585
b. LSD Time.	0.0682
c. LSD Conc.	0.0682
d. Coefficient of variation	3.43%
e. Significant levels	**All means are statistically significant**

Key

▨	**Factor A:** Temp averages
🟦	**Factor B:** Time averages
🟧	**Factor C:** Concentration averages

Table 4.8: Analysis of variance at 95% confidence level using t test on a three factor completely randomized design in MSTAT C program for the quantity of bioethanol obtained from sugarcane cake after treatment with NaOH salt.

K Value	Source	Degrees of freedom	Squares	Mean Square	F value	Probability	s/y	Two tail T value @0.05	LSD = √2 * x/y * t
2	Temp A	3	91.116	30.372	6694.2233	0.0000	0.0130	3.182	0.0585
4	Time B	2	3.712	1.856	409.1223	0.0000	0.0112	4.303	0.0682
6	AB	6	0.646	0.108	23.7347	0.0000	0.0225	2.447	0.0779
8	NaOH Conc. C	2	16.436	8.218	1811.3467	0.0000	0.0112	4.303	0.0682
10	AC	6	2.738	0.456	100.5714	0.0000	0.0225	2.447	0.0779
12	BC	4	0.636	0.159	35.0714	0.0000	0.0194	2.776	0.0762
14	ABC	12	4.083	0.340	74.9898	0.0000	0.0389	2.179	0.1199
15	Error	72	0.327	0.005		0.0000			

Table 4.9: A three factor analysis table for the means of bioethanol quantity obtained after sugarcane cake was treated with Mn(II)-Anderson salt.

Temp	**30** °C					**40** °C					**50** °C					**60** °C				
POM Conc. Time	1%	2%	3%	Av. Time		1%	2%	3%	Av. Time		1%	2%	3%	Av. Time		1%	2%	3%	Av Time	Av. Time
	Vol (ml)	Vol (ml)	Vol (ml)	Vol (ml)		Vol (ml)	Vol (ml)	Vol (ml)	Vol (ml)		Vol (ml)	Vol (ml)	Vol (ml)	Vol (ml)		Vol (ml)	Vol (ml)	Vol (ml)	Vol (ml)	Vol (ml)
2 Hour	0.1	0.5	0.6	0.4		1.7	1.8	1.5	1.7		1.9	2.5	2.7	2.4		3.1	3.1	3.4	3.2	**2.6**
3 Hours	0.13	0.6	0.9	0.54		1.4	1.2	1.4	1.33		2.2	2.8	2.7	2.6		3.4	3.1	2.9	3.13	**1.9**
4 Hours	0.7	0.7	1.0	0.8		2.1	2.8	3.5	2.8		3.3	3.5	3.7	3.5		3.7	3.8	3.5	3.7	**2.7**
Av. Conc.	0.31	0.6	0.83	**0.58**		1.73	1.93	2.13	**1.93**		2.5	2.93	3.03	**2.81**		3.4	3.33	3.3	**3.34**	
Av. Temp.	**0.58ml**					**1.94ml**					**2.83ml**					**3.34ml**				

1. LSD Temp.	0.0571
2. LSD Time.	0.0670
3. LSD Conc.	0.0670
4. Coefficient of variation	3.04%
5. Significant levels	**All means are statistically significant**

Key

▒	**Factor A**: Temp averages
🟦	**Factor B:** Time averages
🟧	**Factor C:** Concentration averages

Table 4.10: Analysis of variance at 95% confidence level using t test on a three factor completely randomized design in MSTAT C program for the quantity of bioethanol obtained from sugarcane cake after treatment with Mn(II)-Anderson POM salt.

K Value	Source	Degrees of freedom	Squares	Mean Square	F value	Probability	s/y	Two tail T value @0.05	LSD = √2 * x/y * t
2	Temp A	3	119.592	39.864	9160.2534	0.0000	0.0127	3.182	0.0571
4	Time B	2	1.965	0.982	223.7234	0.0000	0.0110	4.303	0.0670
6	AB	6	1.794	0.299	68.7021	0.0000	0.0220	2.447	0.0761
8	POM Conc. C	2	15.430	7.715	1772.8294	0.0000	0.0110	4.303	0.0670
10	AC	6	4.566	0.761	174.8723	0.0000	0.0220	2.447	0.0761
12	BC	4	0.361	0.090	20.7660	0.0000	0.0190	2.776	0.0746
14	ABC	12	3.131	0.261	59.9574	0.0000	0.0381	2.179	0.1174
15	Error	72	0.313	0.004					

.

4.5 Quality of bioethanol produced from sugarcane cake under NaOH and POM salt pretreatment methods

The quality of bioethanol produced (means) from various pretreatment methods and monitored by refractive index (RFM330 at 26.5°C) are recorded in **Table 4.11, 4.12, 4.13** and **4.14.** As previously observed, all the means are statistically significant: meaning that increase in pretreatment time, temperature and salt concentration gave high quality yields. The most striking result is the increase in bioethanol quality (46.13%) after pretreatment with Mn(II)-Anderson POM salt at a concentration of 3%, at a temperature of 60°C and treating it for a duration of 4hrs (**Table 4.11)** as compared to NaOH (41.3%) at the same pretreatment parameters (**Table 4.13).**

Similar results are found in a study done by Wu *et al* 2011. They found out that quality of degraded xylan in sorghum bagasse, increased steadily when the parameters were increased. However, the glucan recovered from most runs was approximately 90% according to Wu *et al* 2011. According to Wu *et al*. 2011 and Ma *et al*, 2016, lignocellulosic biomass has poor yield of fermentable sugars for bioethanol production when the enzymes are not able to attack the complex recalcitrant lignin. In his study, the removal of the lignin using alkali pretreatment was affected by the conditions of temperature and the duration of delignification process. However, the current studies have not approximated the statistical data for the concentration of the bioethanol when POM salts are used in delignification process. In this study, it was found that, increase in the pretreatment parameter, increases the quality of the bioethanol obtained.

Table 4.11: A three factor analysis table for the means of bioethanol quality obtained after sugarcane cake was treated with NaOH salt.

Temp	**30** ℃					**40** ℃					**50** ℃					**60** ℃				
NaOH Conc. Time	1%	2%	3%	Av. Time		1%	2%	3%	Av. Time		1%	2%	3%	Av. Time		1%	2%	3%	Av Time	Av. Time
	Conc. (%)	Conc. (%)	Conc. (%)	Conc. (%)		Conc. (%)	Conc (%)	Conc. (%)	Conc. (%)		Conc. (%)	Conc. (%)	Conc. (%)	Conc. (%)		Conc. (%)	Conc. (%)	Conc. (%)	Conc. (%)	Conc. (%)
2 Hour	0.00	0.013	0.018	0.01		1.2	3.6	4.0	2.93		2.8	3.2	4.0	3.33		3.6	4.4	4.8	4.27	**2.64**
3 Hours	0.01	0.014	0.018	0.014		3.2	4.4	5.2	4.27		11.6	12.8	15.6	13.33		14.4	17.7	17.6	16.57	**8.55**
4 Hours	0.01	0.015	0.094	0.04		6.0	7.6	10.8	8.13		21.3	26.1	26.9	24.8		34.1	38.9	41.3	38.1	**17.77**
Av. Conc.	0.01	0.042	0.13	**0.061**		3.47	5.2	6.67	**5.1**		11.9	14.0	15.5	**13.8**		17.4	20.3	21.2	**19.63**	
Av. Temp.	**0.021%**					**5.1%**					**13.8%**					**19.65%**				
1. LSD Temp. 2. LSD Time. 3. LSD Conc. 4. Coefficient of variation 5. Significant levels	0.0099 0.0116 0.0116 0.12% **All means are statistically significant**																			

Key

▨	**Factor A:** Temp averages
🟦	**Factor B:** Time averages
🟧	**Factor C:** Concentration averages

Table 4.12 Analysis of variance at 95% confidence level using t test on a three factor completely randomized design in MSTAT C program for the quantity of bioethanol obtained from sugarcane cake after treatment with NaOH salt.

K Value	Source	Degrees of freedom	Squares	Mean Square	F value	Probability	s/y	Two tail T value @0.05	LSD = √2 * x/y * t
2	Temp A	3	6229.112	2076.371	16128085.0244	0.0000	0.0022	3.182	0.0099
4	Time B	2	132.691	66.346	515336.3122	0.0000	0.0019	4.303	0.0116
6	AB	6	46.849	7.808	60648.9695	0.0000	0.0038	2.447	0.0132
8	NaOH Conc. C	2	4176.121	2088.061	16218886.1710	0.0000	0.0019	4.303	0.0116
10	AC	6	3292.098	548.683	4261863.0778	0.0000	0.0038	2.447	0.0132
12	BC	4	30.975	7.744	60148.7532	0.0000	0.0033	2.776	0.0130
14	ABC	12	32.442	2.703	20999.168	0.0000	0.0066	2.179	0.0203
15	Error	72	0.009	0.000					

Table 4.13: A three factor analysis table for the means of bioethanol quality obtained after sugarcane cake was treated with Mn(II)-Anderson POM salt.

Temp	**30** °C					**40** °C					**50** °C					**60** °C				
POM Conc. Time	1%	2%	3%	Av. Time		1%	2%	3%	Av. Time		1%	2%	3%	Av. Time		1%	2%	3%	Av Time	Av. Time
	Conc. (%)	Conc. (%)	Conc. (%)	Conc. (%)		Conc. (%)	Conc. (%)	Conc. (%)	Conc. (%)		Conc. (%)	Conc. (%)	Conc. (%)	Conc. (%)		Conc. (%)	Conc. (%)	Conc. (%)	Conc. (%)	Conc. (%)
2 Hour	0.01	0.14	0.98	0.38		1.4	2.41	2.81	2.21		3.21	3.61	5.22	4.01		6.82	6.82	8.42	22.06	**7.17**
3 Hours	0.04	0.54	1.24	0.61		5.22	9.23	15.24	9.90		17.25	19.66	21.26	19.39		21.26	22.86	24.47	22.86	**13.19**
4 Hours	0.65	0.87	1.36	0.96		32.30	37.31	39.71	36.44		41.08	40.5	42.12	41.23		42.12	43.32	46.13	43.86	**30.62**
Av. Conc.	0.23	0.52	1.2	**0.65**		13.0	16.32	19.25	**16.19**		20.51	21.26	22.87	**21.55**		23.4	24.33	26.34	**24.69**	
Av. Temp.	**0.65%**					**16.18%**					**21.54%**					**29.59%**				

1. LSD Temp.	0.1616
2. LSD Time.	0.1886
3. LSD Conc.	0.1886
4. Coefficient of variation	1.18%
5. Significant levels	**All means are statistically significant**

Key

▨	**Factor A:** Temp averages
🟦	**Factor B:** Time averages
🟧	**Factor C:** Concentration averages

Table 4.14: Analysis of variance at 95% confidence level using t test on a three factor completely randomized design in MSTAT C program for the quality of bioethanol obtained from sugarcane cake after treatment with Mn(II)-Anderson POM salt.

K Value	Source	Degrees of freedom	Squares	Mean Square	F value	Probability	s/y	Two tail T value @0.05	LSD = √2 * x/y * t
2	Temp A	3	9250.903	3083.634	88846.9841	0.0000	0.0359	3.182	0.1616
4	Time B	2	186.174	93.087	2682.0563	0.0000	0.0310	4.303	0.1886
6	AB	6	67.332	11.222	332.3343	0.0000	0.0621	2.447	0.2149
8	POM Conc. C	2	13492	6746.267	194376.3086	0.0000	0.0310	4.303	0.1886
10	AC	6	4557.569	759.595	21885.7698	0.0000	0.0621	2.447	0.2149
12	BC	4	29.250	7.313	210 .6913	0.0000	0.538	2.776	2.1121
14	ABC	12	52.482	4.374	126.0121	0.0000	0.1076	2.179	0.4246
15	Error	72	2.499	0.035					

5.0 CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

In this study, NaOH and Mn(II)-Anderson Polyoxometalate salts significantly delignified sugarcane cake (roots). An increase in salt concentration from 1%, 2% and 3% gave significant increase in extracted lignin for both NaOH and POM salts pretreated samples. Similar results were obtained when temperature was varied from 30℃, 40℃, 50℃ and 60℃; and also, time from 2hrs, 3hrs and 4hrs. The quantity and quality of bioethanol obtained followed a similar trend. However, it is noted that in all cases, Mn(II)-Anderson Polyoxometalate salts performed better than NaOH pretreated samples. Specifically, an increase in bioethanol quality (46.13%) after pretreatment with Mn(II)-Anderson POM salt at a concentration of 3%, time 4hrs and temperature 60℃ as compared to NaOH (41.3%) at the same pretreatment parameters is noted.

5.2 Recommendation

Mn(ii)-Anderson POM salt is a good delignifying substrate as compared to NaOH. And that an increase in delignifying temperature, time, and salt concentration results into more lignin yields. Furthermore, the quality of bioethanol produced from Mn(ii)-Anderson POM salt pretreated biomass is better than that produced from NaOH pretreated samples, hence the POM salt is recommended in the bioethanol production value chain.

5.3 Recommendation for future studies

The ratio of feedstock (in Kg) to amount of pretreatment salt (in liters) should be investigated further as the current results differ from literature results due to the difference in treatment ratios. Similarly, other POM salts can be investigated in the reaction to determine the most suitable salt to be used during delignification process.

References

AFRINOL. (2018). The Kenya Market: Afrinol. www.https://afrinol.com, *accessed on 12/10/2021*.

Bardin, Billy B., Davis, Robert J., & Neurock, Matthew. (2000). Ammonia Adsorption on Keggin-Type Heteropolyacid Catalysts Explored by Density Functional Quantum Chemistry Calculations. *The Journal of Physical Chemistry B, 104*(15), 3556-3562. doi: 10.1021/jp993227t.

Bujanovic, Biljana, Ralph, Sally, Reiner, Richard, Hirth, Kolby, & Atalla, Rajai. (2014). Polyoxometalates in Oxidative Delignification of Chemical Pulps: Effect on Lignin. *Materials, 3*(3), 1888-1903. doi: 10.3390/ma3031888.

Chandra Rajak, Rajiv, & Banerjee, Rintu. (2015). Enzymatic delignification: an attempt for lignin degradation from lignocellulosic feedstock. *RSC Advances, 5*(92), 75281-75291. doi: 10.1039/c5ra09667g.

Chiranjeevi, Thulluri, Mattam, Anu Jose, Vishwakarma, Kusum K., Uma, Addepally, Peddy, V. C. Rao, Gandham, Sriganesh, & Ravindra Velankar, Harshad. (2018). Assisted Single-Step Acid Pretreatment Process for Enhanced Delignification of Rice Straw for Bioethanol Production. *ACS Sustainable Chemistry & Engineering, 6*(7), 8762-8774.doi: 10.1021/acssuschemeng.8b01113.

Cintia Masuco Lopes, Antonio Marcos Miranda Silva, German Andres Estrada-Bonilla, Risely Ferraz-Almeida, Jose Luis Vilela Vieira, Rafael Otto, Cardoso, Elke Jurandy Bran Nogueira. (2021). Improving the fertilizer value of sugarcane wastes through phosphate rock amendment and phosphate-solubilizing bacteria inoculation. *cleaner production, 298*(126821). doi: https://doi.org/10.1016/j.jclepro.2021.126821

Daniel Ciolkosz, P.E. (2020). Characteristics of Biomass as a Heating Fuel.

El-Mekkawi, Samar A., Abdo, Sayeda M., Samhan, Farag A., & Ali, Gamila H. (2019). Optimization of some fermentation conditions for bioethanol production from microalgae using response surface method. *Bulletin of the National Research Centre, 43*(1). doi: 10.1186/s42269-019-0205-8.

Fan, Senqing, Li, Jianfeng, Liu, Yicai, & Xiao, Zeyi. (2019). Bioethanol production in membrane distillation bioreactor with permeate fractional condensation and mechanical vapor compression. *Energy Procedia, 158*, 21-25. doi: 10.1016/j.egypro.2019.01.026.

Fernando Segato, Andre R. L. Damasio, Rosymar C. de Lucas, Fabio M. Squina, & Prade, Rolf A. (2014). Genomics Review of Holocellulose Deconstruction by Aspergilli. *Microbiol Mol Rev, 78*(4), 588-613. doi: 10.1128/MMBR.00019-14.

Geoffrey Ndegwa, Violet Moraa, & Iiyama, Miyuki. (2011). Potential for Biofuel Feedstock in Kenya.

Hatakeyama, H., Hatakeyama, T. (2009). Lignin Structure, Properties, and Applications. *Advances in Polymer Science, 232*. doi: https://doi.org/10.1007/12_2009_12

I. C. Montano, & inti@ufg.br. (2020). Effect of reaction time and sodium hydroxide

concentration on delignification and enzymatic hydrolysis of brewer's spent grain from two brazilian brewers.

Kundu, C., Samudrala, S. P., Kibria, M. A., & Bhattacharya, S. (2021). One-step peracetic acid pretreatment of hardwood and softwood biomass for platform chemicals production. *Sci Rep, 11*(1), 11183. doi: 10.1038/s41598-021-90667-9.

Linda Z. Linganiso, Thuli Buthelezi, & Motaung, Tshwafo E. (2019). A comparative study of sugarcane Bagasse and softwood. *Wood Research, 64*(2), 273-280.

Martinetto, Y., Basset, S., Pegot, B., Roch-Marchal, C., Camerel, F., Jeftic, J., . . . Floquet, S. (2021). Synthesis, Physical Properties and Application of a Series of New Polyoxometalate-Based Ionic Liquids. *Molecules, 26*(2). doi: 10.3390/molecules26020496.

Mali Husby Rosnes and De-Liang Long (2012). Mapping the synthesis of low nuclearity polyoxometalates from octamolybdates to Mn-Anderson clusters. doi: 10.1039/c2dt31008b.

National Center for Biotechnology Information (2023). Sinapyl alcohol. *PubChem Compound Summary for CID 5280507*. doi: https://pubchem.ncbi.nlm.nih.gov/compound/Sinapyl-alcoho.

Nicole M. Stark, Daniel J. Yelle, & Agarwal, Umesh P. (2016). Techniques for characterizing lignin. *Lignin in Polymer Composites*. doi: https://doi.org/10.1016/B978-0-323-35565-0.00004-7.

Novo, L. P., Gurgel, L. V., Marabezi, K., & Curvelo, A. A. (2011). Delignification of sugarcane bagasse using glycerol-water mixtures to produce pulps for saccharification. *Bioresour Technol, 102*(21), 10040-10046. Doi: 10.1016/j.biortech.2011.08.050.

Omwoma, Solomon, Chen, Wei, Tsunashima, Ryo, & Song, Yu-Fei. (2014). Recent advances on polyoxometalates intercalated layered double hydroxides: From synthetic approaches to functional material applications. *Coordination Chemistry Reviews, 258-259*, 58-71. doi: 10.1016/j.ccr.2013.08.039.

Omwoma, Solomon, Gore, Christopher T, Yuanchun Ji, Changwen Hu, & Song, Yu-Fei. (2014). Evironmentally benign polyoxometalate materials. doi: 10.1016/j.ccr.2014.11.013.

Owuama, C. (1993). Refractometric determination of ethanol concentration. *Food Chemistry, 48*(4), 415-417. doi: 10.1016/0308-8146(93)90327-c.

Patrick J. Mason, Agnelo Furtado, Annelie Marquardt, Katrina Hodgson-Kratky, Nam V. Haong, Frederik C. Botha, Henry, Robert J. (2020). Variation in sugarcane biomass composition and enzymatic saccharification of leaves, internodes and roots. *Biotechnology for Biofuels and Bioproducts*.

Persson, P., Andersson, J., Gorton, L., Larsson, S., Nilvebrant, N. O., & Jonsson, L. J. (2002). Effect of different forms of alkali treatment on specific fermentation inhibitors and on the fermentability of lignocellulose hydrolysates for production of fuel ethanol. *J Agric Food Chem, 50*(19), 5318-5325. doi: 10.1021/jf025565o.

Rezende, C. A., de Lima, M. A., Maziero, P., deAzevedo, E. R., Garcia, W., & Polikarpov, I. (2011). Chemical and morphological characterization of sugarcane bagasse submitted to a delignification process for enhanced enzymatic digestibility. *Biotechnol Biofuels, 4*(1), 54. doi: 10.1186/1754-6834-4-54.

Roger M. Rowell, Roger Pettersen, & Tshabalala, Mandla A. (2012). Cell Wall Chemistry. doi: 10.1201/b12487-5.

Rosnes, M. H., Yvon, C., Long, D. L., & Cronin, L. (2012). Mapping the synthesis of low nuclearity polyoxometalates from octamolybdates to Mn-Anderson clusters. *Dalton Trans, 41*(33), 10071-10079. doi: 10.1039/c2dt31008b.

Rui Kathahira, and, Thomas J. Elder, & Bekham, Geng T. (2018). A brief Introduction to Lignin Stucture. *Royal Society of Chemistry*.

S. Niju, & Swathika, M. (2019). Delignification of sugarcane bagasse using pretreatment strategies for bioethanol production. Doi: https://doi.org/10.1016/j.bcab.2019.101263

Sindhu, R., Binod, P., & Pandey, A. (2016). Biological pretreatment of lignocellulosic biomass--An overview. *Bioresour Technol, 199*, 76-82. doi: 10.1016/j.biortech.2015.08.030

Stelte, Wolfgang. (2013). Steam explosion for biomass pre-treatment. *Energy & Climate Centre for Renewable Energy and Transport Section for Biomass*.

Tsegaye, Bahiru, Balomajumder, Chandrajit, & Roy, Partha. (2019). Microbial delignification and hydrolysis of lignocellulosic biomass to enhance biofuel production: an overview and future prospect. *Bulletin of the National Research Centre, 43*(1). doi: 10.1186/s42269-019-0094-x.

Shusaku Ikegami and Atsushi Yagasaki (2009) Efficient Syntheses of $[(n\text{-}C_4H_9)_4N]_4[\alpha\text{-}Mo_8O_{26}]$ and $[(n\text{-}C_4H_9)_4N]_2[Mo_2O_7]$.

Tugba Keskin, Haris Nalakath Abubackar, Kubra Arslan, & Azbar, Nuri. (2019). Biohydrogen Production From Solid Wastes. *Biomass, Biofuels, Biochemical*, 321-346. Doi: https://doi.org/10.1016/B978-0-444-64203-5.00012-5.

UNEP. (2019). Sustainability of Sugarcane Bagasse Briquettes and Charcoal Value Chains in Kenya: Results and recommendations from implementation of the Global Bioenergy Partnership Indicators.

Wang, QingFeng, & Zhuang, Xinshu. (2019). Low-temperature sodium hydroxide pretreatment for ethanol production from sugarcane bagasse without washing process. *Bioresource Technology, 291*. Doi: https://doi.org/10.1016/j.biortech.2019.121844

Wool, Richard P. (2005). Lignin Polymers and Composites. *ScienceDirect*, 551-598. doi: https://doi.org/101016/B978-012763952-9/50017-4.

Xavier Lopez, Jorge A Fernandez, & Poblet, Joseph M. (2006). Redox Properties of Polyoxometalates: New insights on the aion charge effect. *1162-7*(6(9)). Doi: 10.1039/b507599h.

Adam Malik, D Mardianti, D Nurlutfiah, D W Izzah (2021). Determination of refractive index on three mediums based on the principle of refraction of light. DOI: 10.1088/1742-6596/1806/1/012003.

Xiao qi Ye, Bo Zeng, Jin Liu Meng, Ming Wu and Xiao ping Zhang. 2018. Responses in shoot elongation, carbohydrate utilization and growth recover of an invasive species to submergence at different water temperatures. Science Reports (2018) 8:306. DOI: 10.1038/s41598-017-18735-7.

Maria Juliane Suota, Thiago Alessandre da Silva, Sonia Faria Zawadzki, Guilherme Lanzi Sassaki, Fabricio Augusto Hansel, Michael Paleoloqou and Luiz Pereira Ramos. 2021.Chemical and structural characterization of hardwood and softwood LignoForceTM Lignins. https://doi.org/10.1016/j.indcrop.2021.114138.

Usman Idris Nda-Umar, Irmawati Ramli, Ernee Noryana Muhamad and Norsahida Azri. 2020. Optimization and Characterization of Mesoporous sulfonated Carbon Catalyst and Its Application in Modelling and Optimization of Acetin production. DOI:10.3390/molecules25225221.

Mayank Gupta, Miranda L. Smith and James J. Spivey. 2011. ACS Catal. 2011, 1, 641-656. https://doi.org/10.1021/cs2001048.

Ejaz Ahmad, Kamal K. Pant. 2018. Lignin conversion: Akey to the concept of Lignocellulosic Biomass-Based Integrated Biorefinery.https://doi.org/10.1016/B978-0-444-63992-9.00014-8.

S. Bandyopadhyay-Gosh, S.B. Ghosh, M. Sain.2015. Biofiber Reinforcements in Composite Materials. DOI: https://doi.org/10.1016/c2013-0-16470-7.

Anna Penkina, Maija Hakola, Urve Paaver, Sirpa Vuorinen, Kalle Kirsimae, Karin Kogermann, Peep Veski, Jouko Yliruusi, Timo Repo and Jyrki Heinamaki. 2012.. Solid-State properties of softwood lignin and cellulose isolated by a new acid precipitation method. International journal of Biological Macromolecules. 51(5):939-45. DOI:10.1016/j.ijbiomac.2012.07.024.

Kenneth G. Lathan, Leonidas Matsakas, Joao Figueira, Ulrika Rova, Paul Christakopoulos, Stina Jasson.2021. Examination of how variations in ligninn properties from Kraft and Organosolv extration influence the physicochemical characteristics of hydrothermal carbon. Journal of Analytical and Applied Pyrolysis 155(2021) 105095. https://creativecommons.org/licenses/by/4.0/.

Nianjie Feng, Shaowen She, Fei Tang, Xiangdonng Zhao, Jinggian Chen, Peng Wang, Qian Wu and Orlando J. Rojas. 2023. Formation and Identification of Lignin-Carbohydrate Complexes in Pre-hydrolysis Liquors. Biomacromolecules. 12; 24(6): 2541-2548. https://doi.org/10.1021/acs.biomac.3c00053

Rijuta Ganesh Saratale, Si-Kyung Cho, Ram Naraesh Bharagava, Anil Kumar Patel, Sunita Varjani, Sikandar I. Mulla, Dong S Kim, Shashi Kant Bhatia, Luiz Fernando Romanholo Ferreira, Han Seung Shin and Ganesh Dattatraya Saratale. 2022A critical review on biomass-based sustainable biorefineries using nanobiocatalysts: Opportunities, Challenges, and future perspectives. Bioresource Technology. https://doi.org/10.1016/j.biortech.2022.127926.

Raymond Trevorah and Othman Maazuza.2015. Hydrolysis of Australian Timber Mill Sawdust for Biofuel production. Doi:101155/2015/284250.

Haoyu Deng, Wenbiao Xu, Dan Zhang, Xiangyu Li and Jungou Shi.2023. Recent Advances in Application of Polyoxometalates in Lignocellulose pretreatment and Transformation. https://doi.org/10.3390/polym15102401.

Seonghu Kim.2018. Enhancing Bioethanol productivity using alkali - pretreated empty Palm Fruit Bunch Fiber Hydrolysate. Doi: 10.1155/2018/5272935.

Mikhail Balakshin, Dmitry V. Evtuguin, C. Pascoal Neto and Artur Caraco-Paulo.2001. Polyoxometalate as mediators in the laccase catalyzed delignification. Doi: 10.1016/s1381-1177(01) 00054-6.

F. Ma, N. Yang, C Xu, H. Yu, J. Wu, X. Zhang.2010. Combination of biological pretreatment with mild acid pretreatment for enzymatic hydrolysis and ethanol production from water hyacinth. Bioresource technology 101, no.24 (2010): 9600-9604. https://doi.org/10.1016/j.biortech.2010.07.084.

Long Wu, Mitsuhiro Arakane, Masakazu Ike, Masahisa Wada, Tomoyuki Takai, Mitsuru Gau, Ken Tokuyasu.2011. Low temperature alkali pretreatment for improving enzymatic digestibility of sweet sorghum bagasse for ethanol production. https://doi.org/10.1016/j.biortech.2011.01.023.

Umar Ashghar. 2013. Alkaline pretreatment: A potential Tool to Explore Kallar Grass (Leptochloa Fusca) as a Substrate for Bio-Fuel production.DOI: 10.5829/idosi.mejsr.2013.18.8.11848.

UNEP 2022. Converting Waste Agricultural Biomass into a Resource-Compendium of Technologies. https://stg-wedocs.unep.org/handle/20.500.11822/7614

KSB 2018. https://energypedia.info/wiki/Ethanol_Cooking_Fuel_in_Kenya

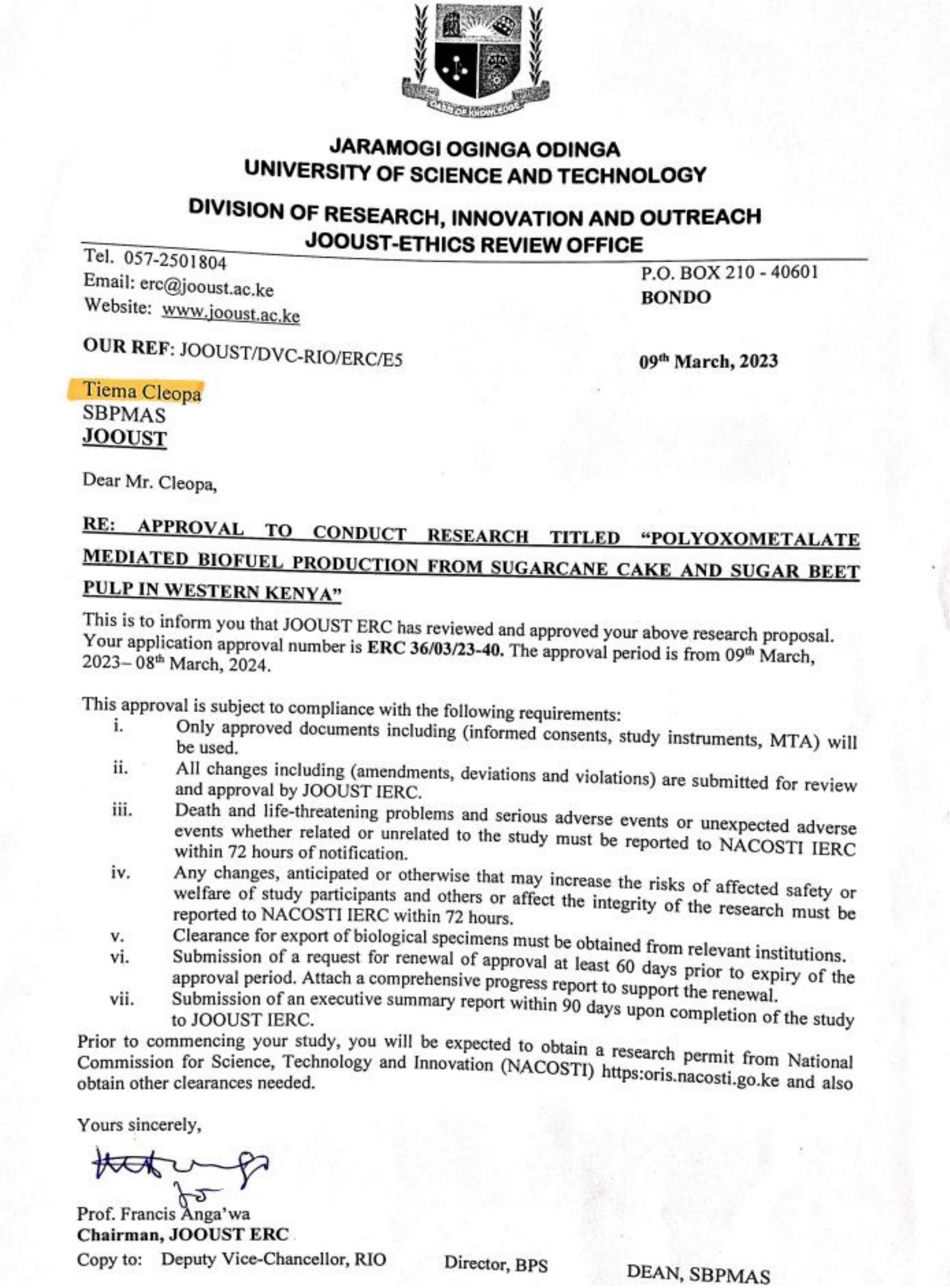
Recek, N., Zhou, R. *et al*. Improved fermentation efficiency of S. Cerevisiae by changing glycoltic metabolic pathway with plasma agitation. Sci Rep 8, 8252 (2018). https://doi.org/10.1038/s41598-018-26227-5

Nicole Teeter and Shannon Compton 2023. Simple sugar molecule| Overview, Formula and stgructure. *accessed on 20/11/2023*

Edwin Oseko and Dienya T. (2015). Fertilizer consumption and fertilizer use by Crop (FUBC) in Kenya: Technical report AfricaFertilizer.org (AFO) (2015), P.47

DePalma, D. (2017). Quality Assurance and Testing in a Global Economy. Journal of Software Testing, Verification & Reliability, 27(5), 345-360. https://doi.org/10.1002/stvr.1586

APPENDIX 1: Ethical Review permission to do research



JARAMOGI OGINGA ODINGA
UNIVERSITY OF SCIENCE AND TECHNOLOGY

DIVISION OF RESEARCH, INNOVATION AND OUTREACH
JOOUST-ETHICS REVIEW OFFICE

Tel. 057-2501804
Email: erc@jooust.ac.ke
Website: www.jooust.ac.ke

P.O. BOX 210 - 40601
BONDO

OUR REF: JOOUST/DVC-RIO/ERC/E5

09th March, 2023

Tiema Cleopa
SBPMAS
JOOUST

Dear Mr. Cleopa,

RE: APPROVAL TO CONDUCT RESEARCH TITLED "POLYOXOMETALATE MEDIATED BIOFUEL PRODUCTION FROM SUGARCANE CAKE AND SUGAR BEET PULP IN WESTERN KENYA"

This is to inform you that JOOUST ERC has reviewed and approved your above research proposal. Your application approval number is **ERC 36/03/23-40.** The approval period is from 09th March, 2023– 08th March, 2024.

This approval is subject to compliance with the following requirements:

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations and violations) are submitted for review and approval by JOOUST IERC.
iii. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to NACOSTI IERC within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks of affected safety or welfare of study participants and others or affect the integrity of the research must be reported to NACOSTI IERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to JOOUST IERC.

Prior to commencing your study, you will be expected to obtain a research permit from National Commission for Science, Technology and Innovation (NACOSTI) https:oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

Prof. Francis Anga'wa
Chairman, JOOUST ERC

Copy to: Deputy Vice-Chancellor, RIO Director, BPS DEAN, SBPMAS

Appendix 2: Standard deviation of analyzed means for various factors

Table Appendix 2a: Mass of lignin obtained using alkali pretreatment method.

Mass of biomass(g)	Temp (ºC)	Time (Hrs.)	1% NaOH treatment Mean ± Standard Mass(g) Deviation	2% NaOH treatment Mean ± Standard Mass(g) Deviation	3% NaOH treatment Mean ± Standard Mass(g) Deviation
10	30	2	0.113 ±0.00003	0.182 ±0.00071	0.219 ±0.00071
		3	0.117 ±0.00071	0.207 ±0.00071	0.224 ±0.00071
		4	0.194 ±0.00212	0.220 ±0.00071	0.247 ±0.00071
10	40	2	0.184 ±0.00071	0.213 ±0.00058	0.243 ±0.00000
		3	0.190 ±0.00071	0.214 ±0.00071	0.251 ±0.00071
		4	0.190 ±0.00990	0.217 ±0.00071	0.249 ±0.00071
10	50	2	0.214 ±0.00071	0.220 ±0.00071	0.256 ±0.00001
		3	0.202 ±0.00071	0.230 ±0.00071	0.287 ±0.00141
		4	0.220 ±0.00283	0.251 ±0.00071	0.289 ±0.00071
10	60	2	0.203 ±0.00071	0.250 ±0.00071	0.287 ±0.01910
		3	0.214 ±0.00071	0.249 ±0.00071	0.307 ±0.00071
		4	0.230 ±0.00212	0.249 ±0.02120	0.304 ±0.00110

Table Appendix 2b: Mass of lignin from POMs salt, M-Anderson pretreatment method.

Mass of biomass(g)	Temp(ºC)	Time (Hrs.)	1% Mn-Anderson treatment Mean ± Standard Mass(g) Deviation	2% Mn-Anderson treatment Mean ± Standard Mass(g) Deviation	3% Mn-Anderson treatment Mean ± Standard Mass(g) Deviation
10	30	2	0.136 ±0.00071	0.187 ±0.00000	0.186 ±0.00283
		3	0.144 ±0.00071	0.225 ±0.00071	0.194 ±0.00071
		4	0.215 ±0.00010	0.230 ±0.00071	0.204 ±0.00071
10	40	2	0.195 ±0.00000	0.235 ±0.00071	0.220 ±0.00071
		3	0.193 ±0.00071	0.228 ±0.00990	0.224 ±0.02263
		4	0.230 ±0.00778	0.239 ±0.00071	0.257 ±0.00071
10	50	2	0.231 ±0.00636	0.240 ±0.00010	0.275 ±0.00919
		3	0.234 ±0.00495	0.263 ±0.00000	0.283 ±0.00000
		4	0.227 ±0.00071	0.262 ±0.00071	0.291 ±0.00071
10	60	2	0.217 ±0.00071	0.248 ±0.00071	0.321 ±0.00071
		3	0.251 ±0.00010	0.276 ±0.00010	0.287 ±0.00071
		4	0.253 ±0.00071	0.276 ±0.00000	0.310 ±0.00071

Table appendix 2c: Mean volume obtained from distillation of the bioethanol produced from treatment using various percentage concentration of NaOH

Temp(ºC)	Time (Hrs.)	1% NaOH treatment Mean volume ± Standard Deviation	2% NaOH treatment Mean volume ± Standard Deviation	3% NaOH treatment Mean volume ± Standard Deviation
30	2	0.0 ±0.0000	0.0 ±0.0710	0.5 ±0.0710
	3	0.4 ±0.0710	0.5 ±0.0710	0.7 ±0.0710
	4	0.4 ±0.0710	0.6 ±0.0710	0.8 ±0.0710
40	2	1.5 ±0.0000	2.4 ±0.0710	2.4 ±0.0710
	3	1.7 ±0.0710	2.2 ±0.0710	2.4 ±0.0710
	4	2.1 ±0.0710	2.3 ±0.0710	3.3 ±0.0710
50	2	2.1 ±0.0710	2.1 ±0.0710	2.2 ±0.0710
	3	1.5 ±0.0710	2.8 ±0.0000	2.9 ±0.0000
	4	1.8 ±0.0710	2.6 ±0.0710	3.7 ±0.0710
60	2	1.8 ±0.0710	2.4 ±0.0000	3.6 ±0.0701
	3	1.9 ±0.0000	2.5 ±0.0710	3.2 ±0.0000
	4	2.5 ±0.0000	2.8 ±0.0710	3.5 ±0.0710

Table appendix 2d: Mean volume obtained from distillation of the bioethanol produced from treatment using various percentage concentration of Mn-Anderson POM salt.

Temp(ºC)	Time (Hrs.)	1% Mn-Anderson treatment Mean volume ± Standard Deviation	2% Mn-Anderson treatment Mean volume ± Standard Deviation	3% Mn-Anderson treatment Mean volume ± Standard Deviation
30	2	0.0 ±0.0000	0.2 ±0.0710	0.7 ±0.0710
	3	0.5 ±0.0710	0.6 ±0.0710	0.7 ±0.0710
	4	0.7 ±0.0710	0.9 ±0.0710	1.1 ±0.0710
40	2	1.7 ±0.0000	1.4 ±0.0710	2.1 ±0.0000
	3	1.7 ±0.0000	1.2 ±0.0710	2.7 ±0.0000
	4	1.5 ±0.0710	1.3 ±0.0000	3.5 ±0.0710
50	2	1.9 ±0.0710	2.1 ±0.0710	3.3 ±0.0710
	3	2.5 ±0.0710	2.8 ±0.0710	3.5 ±0.0000
	4	2.7 ±0.0710	2.6 ±0.0000	3.7 ±0.0000
60	2	3.1 ±0.0000	3.4 ±0.0000	3.7 ±0.0701
	3	3.1 ±0.0000	3.1 ±0.0710	3.8 ±0.0701
	4	3.3 ±0.07100	2.8 ±0.0000	3.5 ±0.0710

Table appendix 2e: Sample result of % NaOH, Temperature, Time, Refractive Index and Concentration determined using the calibration curve in ***Fig 4.4***. *The refractive Index was obtained using refractometer at 26.5^{o}C*

%NaOH	Temp (^{o}C)	Time (Hrs.)	R.I (n) ± SD	% concentration ± SD
1	30	3	1.3331 ± 0.0000	1.2034 ± 0.0000
2	50	3	1.3360 ± 0.00000	13.3333 ± 0.0001
3	50	3	1.3393 ± 0.00001	26.0728 ± 0.00001
3	60	3	1.3397 ± 0.00001	27.6773 ± 0.00001

Table appendix 2f: Refractive Index and concentration of bioethanol obtained for three successive distillations from a fermented Sugarcane cake after delignification using 1% and 2% NaOH.

Temp(^{o}C)	Time (Hrs.)	1% NaOH		2% NaOH	
		R. I (n) ± SD	%Conc. ± SD	R. I (n) ± SD	%Conc. ± SD
40	2	1.3332 ±0.00007	1.2034 ± 0.000071	1.3340 ±0.000212	3.2100 ±0.00000
	3	1.3335 ±0.00000	3.6101 ± 0.000071	1.3356 ±0.000000	4.4123 ±0.00000
	4	1.3338 ±0.00014	4.0112 ±0.000000	1.3364 ±0.000071	5.2156 ±0.00007
50	2	1.3335 ±0.00007	2.8078 ± 0.000071	1.3339 ±0.000000	11.6320 ±0.00007
	3	1.3336 ±0.00000	3.2090 ±0.000000	1.3359 ±0.000000	12.8360 ±0.00007
	4	1.3338 ±0.00000	4.0112 ±0.000000	1.3372 ±0.000071	15.6450 ±0.00007
60	2	1.3336 ±0.00000	3.6101 ±0.000000	1.3341 ±0.000071	14.4210 ±0.00007
	3	1.3338 ±0.00000	4.4123 ±0.000071	1.3367 ±0.000000	17.7400 ±0.00000
	4	1.3340 ±0.00000	4.8134 ±0.000000	1.3371 ±0.000000	17.6230±0.00000

Table appendix 2g: Refractive Index and concentration of bioethanol obtained for three successive distillations from a fermented cake after delignification using 3% NaOH

Temp(^{o}C)	Time (Hrs.)	3% NaOH R. I (n) ± SD	%Conc. ± SD
40	2	1.3343 ±0.00000	6.0168 ±0.000071
	3	1.3347 ±0.00007	7.6213 ±0.000000
	4	1.3355 ±0.00000	10.8302 ±0.000000
50	2	1.3381 ±0.00000	21.2594 ±0.000000
	3	1.3393 ±0.00007	26.0728 ±0.000071
	4	1.3395 ±0.00007	26.8750 ±0.000071
60	2	1.3413 ±0.00007	34.0952 ±0.000071
	3	1.3425 ±0.00000	38.9310 ±0.000000

	4	1.3431 ±0.00007	41.2725 ±0.000000

Table appendix 2h: Refractive Index and concentration of ethanol obtained for three successive distillations for 1% and 2% Mn-Anderson POM pretreated method

Temp(°C)	Time (Hrs.)	1%Mn-Anderson treatment R. I (n) ± SD	%Conc. ± SD	2% Mn-Anderson treatment R. I (n) ± SD	%Conc. ± SD
40	2	1.3333 ±0.00000	2.0056 ± 0.000000	1.3341 ±0.000071	5.2146 ±0.00007
	3	1.3334 ±0.00000	2.4067 ± 0.000071	1.3351 ±0.000000	9.2258 ±0.00000
	4	1.3335 ±0.00007	2.8078 ±0.000000	1.3366 ±0.000000	15.2426 ±0.00007
50	2	1.3336 ±0.00007	3.2090 ± 0.000071	1.3371 ±0.000000	17.2482 ±0.00007
	3	1.3337 ±0.00000	3.6101 ±0.000000	1.3377 ±0.000000	19.6549 ±0.00000
	4	1.3341 ±0.00000	5.2146 ±0.000071	1.3381 ±0.000071	21.2594 ±0.00000
60	2	1.3345 ±0.00000	6.8194 ±0.000000	1.3382 ±0.000071	21.6605 ±0.00007
	3	1.3345 ±0.00000	6.8194 ±0.000071	1.3385 ±0.000000	22.8638 ±0.00000
	4	1.3349 ±0.00000	8.4235 ±0.000071	1.3389 ±0.000000	24.4683 ±0.000071

Table appendix 2i: Refractive Index and concentration of bioethanol obtained after three successive distillations for 3% Mn-Anderson pretreated method

Temp(°C)	Time (Hrs.)	3% Mn-Anderson treatment R. I (n) ± SD	%Conc. ± SD
40	2	1.3411 ±0.00007	32.2929 ±0.000071
	3	1.3421 ±0.00000	37.3042 ±0.000000
	4	1.3427 ±0.00000	39.7109 ±0.000000
50	2	1.3425 ±0.00000	41.0788 ±0.000000
	3	1.3429 ±0.00007	40.5131 ±0.000071
	4	1.3433 ±0.00000	42.1176 ±0.000071
60	2	1.3431 ±0.00007	41.3154 ±0.000071
	3	1.3436 ±0.00000	43.3210 ±0.000000
	4	1.3443 ±0.00000	46.1287 ±0.000000

Appendix 3: Raw data obtained from MSTAT C program for 3 factor Analysis of variance

Appendix 3a: Mass of lignin obtained from sugarcane cake samples treated with NaOH Salt

Corresponding to Tables 4.0 and 4.1 in the main text

```
Function: FACTOR

Experiment Model Number 3:

Three Factor Completely Randomized Design

Data case no. 1 to 108.

Factorial ANOVA for the factors:

   Replication (Replicate) with values from 1 to 3

   Factor A (Temp) with values from 1 to 4

   Factor B (Time) with values from 1 to 3

   Factor C (Conc.) with values from 1 to 3

Variable 5: Lignin

Grand Mean = 0.227   Grand Sum = 24.464   Total Count = 108

          T A B L E   O F   M E A N S

 1  2  3  4           5          Total

-------------------------------------------------------------

 *  1  *  *         0.192         5.179

 *  2  *  *         0.217         5.868

 *  3  *  *         0.242         6.531

 *  4  *  *         0.255         6.886

-------------------------------------------------------------

 *  *  1  *         0.216         7.769

 *  *  2  *         0.225         8.107

 *  *  3  *         0.239         8.588

-------------------------------------------------------------

 *  1  1  *         0.172         1.546

 *  1  2  *         0.183         1.649

 *  1  3  *         0.220         1.984

 *  2  1  *         0.213         1.921
```

```
 *  2  2  *         0.219       1.973
 *  2  3  *         0.219       1.974
 *  3  1  *         0.231       2.075
 *  3  2  *         0.241       2.170
 *  3  3  *         0.254       2.286
 *  4  1  *         0.247       2.227
 *  4  2  *         0.257       2.315
 *  4  3  *         0.260       2.344
------------------------------------------------------------
 *  *  *  1         0.190       6.838
 *  *  *  2         0.226       8.123
 *  *  *  3         0.264       9.503
------------------------------------------------------------
 *  1  *  1         0.141       1.272
 *  1  *  2         0.203       1.831
 *  1  *  3         0.231       2.076
 *  2  *  1         0.189       1.698
 *  2  *  2         0.216       1.940
 *  2  *  3         0.248       2.230
 *  3  *  1         0.213       1.918
 *  3  *  2         0.234       2.108
 *  3  *  3         0.278       2.505
 *  4  *  1         0.217       1.950
 *  4  *  2         0.249       2.244
 *  4  *  3         0.299       2.692
------------------------------------------------------------
 *  *  1  1         0.179       2.150
 *  *  1  2         0.217       2.603
 *  *  1  3         0.251       3.016
 *  *  2  1         0.182       2.178
```

```
 *   *   2   2        0.226        2.715
 *   *   2   3        0.268        3.214
 *   *   3   1        0.209        2.510
 *   *   3   2        0.234        2.805
 *   *   3   3        0.273        3.273
----------------------------------------------------------------
 *   1   1   1        0.113        0.340
 *   1   1   2        0.182        0.547
 *   1   1   3        0.220        0.659
 *   1   2   1        0.117        0.351
 *   1   2   2        0.209        0.626
 *   1   2   3        0.224        0.672
 *   1   3   1        0.194        0.581
 *   1   3   2        0.219        0.658
 *   1   3   3        0.248        0.745
 *   2   1   1        0.184        0.552
 *   2   1   2        0.213        0.640
 *   2   1   3        0.243        0.729
 *   2   2   1        0.191        0.573
 *   2   2   2        0.215        0.646
 *   2   2   3        0.251        0.754
 *   2   3   1        0.191        0.573
 *   2   3   2        0.218        0.654
 *   2   3   3        0.249        0.747
 *   3   1   1        0.215        0.644
 *   3   1   2        0.221        0.662
 *   3   1   3        0.256        0.769
 *   3   2   1        0.204        0.611
 *   3   2   2        0.230        0.691
 *   3   2   3        0.289        0.868
```

```
     *  3  3  1          0.221        0.663
     *  3  3  2          0.252        0.755
     *  3  3  3          0.289        0.868
     *  4  1  1          0.205        0.614
     *  4  1  2          0.251        0.754
     *  4  1  3          0.286        0.859
     *  4  2  1          0.214        0.643
     *  4  2  2          0.251        0.752
     *  4  2  3          0.307        0.920
     *  4  3  1          0.231        0.693
     *  4  3  2          0.246        0.738
     *  4  3  3          0.304        0.913
    -----------------------------------------------------

      A N A L Y S I S   O F   V A R I A N C E   T A B L E

  K                    Degrees of   Sum of      Mean      F
Value    Source        Freedom      Squares     Square    Value          Prob
-------------------------------------------------------------------------------------------
  2      Factor A            3      0.063       0.021      7704.3839    0.0000
  4      Factor B            2      0.009       0.005      1722.6508    0.0000
  6      AB                  6      0.006       0.001       351.1327    0.0000
  8      Factor C            2      0.099       0.049     18064.1192    0.0000
 10      AC                  6      0.006       0.001       352.8344    0.0000
 12      BC                  4      0.002       0.001       183.0539    0.0000
 14      ABC                12      0.005       0.000       153.6754    0.0000
-15      Error              72      0.000       0.000
-------------------------------------------------------------------------------------------
         Total             107      0.190
-------------------------------------------------------------------------------------------
```

```
Coefficient of Variation: 0.73%

s_ for means group 2:     0.0003       Number of Observations: 27
 y
s_ for means group 4:     0.0003       Number of Observations: 36
 y
s_ for means group 6:     0.0006       Number of Observations: 9
 y
s_ for means group 8:     0.0003       Number of Observations: 36
 y
s_ for means group 10:     0.0006       Number of Observations: 9
 y
s_ for means group 12:     0.0005       Number of Observations: 12
 y
```

Appendix 3b: Mass of lignin obtained from sugarcane cake samples treated with POM Salt

Corresponding to Tables 4.2 and 4.3 in the main text

```
Function: FACTOR

Experiment Model Number 3:

Three Factor Completely Randomized Design

Data case no. 1 to 108.

Factorial ANOVA for the factors:

   Replication (Rep) with values from 1 to 3

   Factor A (Temp) with values from 1 to 4

   Factor B (Time) with values from 1 to 3

   Factor C (Conce) with values from 1 to 3

Variable 5: Lignin

Grand Mean = 0.241   Grand Sum = 26.020   Total Count = 108

          T A B L E   O F   M E A N S

 1  2  3  4          5          Total

------------------------------------------------------------

 *  1  *  *        0.192         5.197

 *  2  *  *        0.225         6.075

 *  3  *  *        0.274         7.407

 *  4  *  *        0.272         7.341

------------------------------------------------------------

 *  *  1  *        0.225         8.089

 *  *  2  *        0.245         8.812

 *  *  3  *        0.253         9.119

------------------------------------------------------------

 *  1  1  *        0.170         1.531

 *  1  2  *        0.191         1.716

 *  1  3  *        0.217         1.950

 *  2  1  *        0.217         1.954
```

*	2	2	*	0.216	1.940
*	2	3	*	0.242	2.181
*	3	1	*	0.249	2.241
*	3	2	*	0.301	2.711
*	3	3	*	0.273	2.455
*	4	1	*	0.263	2.363
*	4	2	*	0.272	2.445
*	4	3	*	0.281	2.533
*	*	*	1	0.214	7.711
*	*	*	2	0.254	9.127
*	*	*	3	0.255	9.182
*	1	*	1	0.165	1.489
*	1	*	2	0.217	1.953
*	1	*	3	0.195	1.755
*	2	*	1	0.207	1.859
*	2	*	2	0.234	2.107
*	2	*	3	0.234	2.109
*	3	*	1	0.244	2.193
*	3	*	2	0.296	2.664
*	3	*	3	0.283	2.550
*	4	*	1	0.241	2.170
*	4	*	2	0.267	2.403
*	4	*	3	0.308	2.768
*	*	1	1	0.195	2.344
*	*	1	2	0.228	2.734
*	*	1	3	0.251	3.011

```
 *  *  2  1        0.206       2.470

 *  *  2  2        0.281       3.372

 *  *  2  3        0.247       2.970

 *  *  3  1        0.241       2.897

 *  *  3  2        0.252       3.021

 *  *  3  3        0.267       3.201

---------------------------------------------------------------

 *  1  1  1        0.137       0.410

 *  1  1  2        0.187       0.562

 *  1  1  3        0.186       0.559

 *  1  2  1        0.144       0.433

 *  1  2  2        0.233       0.700

 *  1  2  3        0.194       0.583

 *  1  3  1        0.215       0.646

 *  1  3  2        0.230       0.691

 *  1  3  3        0.204       0.613

 *  2  1  1        0.195       0.586

 *  2  1  2        0.235       0.706

 *  2  1  3        0.221       0.662

 *  2  2  1        0.193       0.580

 *  2  2  2        0.228       0.685

 *  2  2  3        0.225       0.675

 *  2  3  1        0.231       0.693

 *  2  3  2        0.239       0.716

 *  2  3  3        0.257       0.772

 *  3  1  1        0.231       0.694

 *  3  1  2        0.240       0.721

 *  3  1  3        0.275       0.826

 *  3  2  1        0.234       0.703
```

```
     *  3   2   2           0.386          1.158
     *  3   2   3           0.283          0.850
     *  3   3   1           0.265          0.796
     *  3   3   2           0.262          0.785
     *  3   3   3           0.291          0.874
     *  4   1   1           0.218          0.654
     *  4   1   2           0.248          0.745
     *  4   1   3           0.321          0.964
     *  4   2   1           0.251          0.754
     *  4   2   2           0.276          0.829
     *  4   2   3           0.287          0.862
     *  4   3   1           0.254          0.762
     *  4   3   2           0.276          0.829
     *  4   3   3           0.314          0.942
    ------------------------------------------------------------

         A N A L Y S I S   O F   V A R I A N C E   T A B L E

 K                  Degrees of   Sum of      Mean        F
Value   Source       Freedom   Squares     Square     Value    Prob
------------------------------------------------------------------------------------------

 2       Factor A         3       0.126       0.042    32.1480  0.0000
 4    Factor B            2       0.016       0.008     5.9349  0.0041
 6    AB                  6       0.012       0.002     1.5586  0.1718
 8    Factor C            2       0.039       0.019    14.7567  0.0000
10     AC                 6      0.012       0.002     1.4794  0.1974
12     BC                 4      0.018       0.005     3.4406  0.0125
14     ABC               12       0.021       0.002     1.3229  0.2249
-15     Error            72       0.094       0.001
------------------------------------------------------------------------------------------
     Total          107       0.337
```

```
-----------------------------------------------------------------------------------------

Coefficient of Variation: 15.02%

s_ for means group 2:     0.0070       Number of Observations: 27

y

s_ for means group 4:     0.0060       Number of Observations: 36

y

s_ for means group 6:     0.0121       Number of Observations: 9

y

s_ for means group 8:     0.0060       Number of Observations: 36

y

s_ for means group 10:     0.0121       Number of Observations: 9

y

s_ for means group 12:     0.0104       Number of Observations: 12

y

s_ for means group 14:     0.0209       Number of Observations: 3

y
```

Appendix 3c: Quantity of bioethanol from sugarcane cake samples pretreated with NaOH salt

Corresponding to Tables 4.4 and 4.5 in the main text

```
Function: FACTOR

 Experiment Model Number 3:

 Three Factor Completely Randomized Design

Data case no. 1 to 108.

 Factorial ANOVA for the factors:

    Replication (Replication) with values from 1 to 3

    Factor A (Temp) with values from 1 to 4

    Factor B (Time) with values from 1 to 3

    Factor C (Conce) with values from 1 to 3

 Variable 5: Volume

 Grand Mean = 1.962   Grand Sum = 211.900   Total Count = 108

            T A B L E   O F   M E A N S

  1   2   3   4            5           Total

------------------------------------------------------------

  *   1   *   *           0.393          10.600

  *   2   *   *           2.289          61.800

  *   3   *   *           2.456          66.300

  *   4   *   *           2.711          73.200

------------------------------------------------------------

  *   *   1   *           1.781          64.100

  *   *   2   *           1.889          68.000

  *   *   3   *           2.217          79.800

------------------------------------------------------------

  *   1   1   *           0.156           1.400

  *   1   2   *           0.467           4.200
```

```
 *  1  3  *        0.556        5.000

 *  2  1  *        2.133       19.200

 *  2  2  *        2.133       19.200

 *  2  3  *        2.600       23.400

 *  3  1  *        2.200       19.800

 *  3  2  *        2.411       21.700

 *  3  3  *        2.756       24.800

 *  4  1  *        2.633       23.700

 *  4  2  *        2.544       22.900

 *  4  3  *        2.956       26.600

---------------------------------------------------------------

 *  *  *  1        1.486       53.500

 *  *  *  2        1.958       70.500

 *  *  *  3        2.442       87.900

---------------------------------------------------------------

 *  1  *  1        0.211        1.900

 *  1  *  2        0.367        3.300

 *  1  *  3        0.600        5.400

 *  2  *  1        1.800       16.200

 *  2  *  2        2.333       21.000

 *  2  *  3        2.733       24.600

 *  3  *  1        1.833       16.500

 *  3  *  2        2.556       23.000

 *  3  *  3        2.978       26.800

 *  4  *  1        2.100       18.900

 *  4  *  2        2.578       23.200

 *  4  *  3        3.456       31.100

---------------------------------------------------------------

 *  *  1  1        1.375       16.500
```

```
 *  *  1  2          1.767        21.200

 *  *  1  3          2.200        26.400

 *  *  2  1          1.375        16.500

 *  *  2  2          1.992        23.900

 *  *  2  3          2.300        27.600

 *  *  3  1          1.708        20.500

 *  *  3  2          2.117        25.400

 *  *  3  3          2.825        33.900

-------------------------------------------------------------

 *  1  1  1          0.000         0.000

 *  1  1  2          0.033         0.100

 *  1  1  3          0.433         1.300

 *  1  2  1          0.300         0.900

 *  1  2  2          0.433         1.300

 *  1  2  3          0.667         2.000

 *  1  3  1          0.333         1.000

 *  1  3  2          0.633         1.900

 *  1  3  3          0.700         2.100

 *  2  1  1          1.533         4.600

 *  2  1  2          2.433         7.300

 *  2  1  3          2.433         7.300

 *  2  2  1          1.733         5.200

 *  2  2  2          2.233         6.700

 *  2  2  3          2.433         7.300

 *  2  3  1          2.133         6.400

 *  2  3  2          2.333         7.000

 *  2  3  3          3.333        10.000

 *  3  1  1          2.133         6.400

 *  3  1  2          2.167         6.500
```

```
   *  3  1  3         2.300         6.900
   *  3  2  1         1.533         4.600
   *  3  2  2         2.833         8.500
   *  3  2  3         2.867         8.600
   *  3  3  1         1.833         5.500
   *  3  3  2         2.667         8.000
   *  3  3  3         3.767        11.300
   *  4  1  1         1.833         5.500
   *  4  1  2         2.433         7.300
   *  4  1  3         3.633        10.900
   *  4  2  1         1.933         5.800
   *  4  2  2         2.467         7.400
   *  4  2  3         3.233         9.700
   *  4  3  1         2.533         7.600
   *  4  3  2         2.833         8.500
   *  4  3  3         3.500        10.500
  -----------------------------------------------------------

      A N A L Y S I S   O F   V A R I A N C E   T A B L E

  K                   Degrees of   Sum of       Mean        F
Value   Source        Freedom      Squares      Square      Value       Prob
------------------------------------------------------------------------------------------

  2    Factor A          3       91.116       30.372    6694.2233   0.0000
  4    Factor B          2        3.712        1.856     409.1223   0.0000
  6    AB                6        0.646        0.108      23.7347   0.0000
  8    Factor C          2       16.436        8.218    1811.3467   0.0000
 10    AC                6        2.738        0.456     100.5714   0.0000
 12    BC                4        0.636        0.159      35.0714   0.0000
 14    ABC              12        4.083        0.340      74.9898   0.0000
-15    Error            72        0.327        0.005
```

```
--------------------------------------------------------------------------------------------
      Total        107      119.694
---------------------------------------------------------------------------
     Coefficient of Variation: 3.43%

     s_ for means group 2:     0.0130       Number of Observations: 27
     y

     s_ for means group 4:     0.0112       Number of Observations: 36
     y

     s_ for means group 6:     0.0225       Number of Observations: 9
     y

     s_ for means group 8:     0.0112       Number of Observations: 36
     y

    s_ for means group 10:     0.0225       Number of Observations: 9
     y

     s_ for means group 12:     0.0194       Number of Observations: 12
     y

     s_ for means group 14:     0.0389       Number of Observations: 3
     y
```

Appendix 3d: Quantity of bioethanol from sugarcane cake samples pretreated with POM salt

Corresponding to Tables 4.6 and 4.7 in the main text

```
Function: FACTOR

Experiment Model Number 3:

Three Factor Completely Randomized Design

Data case no. 1 to 108.

Factorial ANOVA for the factors:

   Replication (Replication) with values from 1 to 3

   Factor A (Temp) with values from 1 to 4

   Factor B (Time) with values from 1 to 3

   Factor C (Conc) with values from 1 to 3

Variable 5: Vol

Grand Mean = 2.169   Grand Sum = 234.200   Total Count = 108

         T A B L E   O F   M E A N S

 1  2  3  4          5          Total

----------------------------------------------------------

 *  1  *  *        0.567       15.300

 *  2  *  *        1.944       52.500

 *  3  *  *        2.811       75.900

 *  4  *  *        3.352       90.500

----------------------------------------------------------

 *  *  1  *        1.989       71.600

 *  *  2  *        2.203       79.300

 *  *  3  *        2.314       83.300

----------------------------------------------------------

 *  1  1  *        0.289        2.600

 *  1  2  *        0.578        5.200
```

```
 *  1  3  *          0.833         7.500
 *  2  1  *          1.767        15.900
 *  2  2  *          1.922        17.300
 *  2  3  *          2.144        19.300
 *  3  1  *          2.467        22.200
 *  3  2  *          2.944        26.500
 *  3  3  *          3.022        27.200
 *  4  1  *          3.433        30.900
 *  4  2  *          3.367        30.300
 *  4  3  *          3.256        29.300
-----------------------------------------------------------
 *  *  *  1          1.917        69.000
 *  *  *  2          1.886        67.900
 *  *  *  3          2.703        97.300
-----------------------------------------------------------
 *  1  *  1          0.389         3.500
 *  1  *  2          0.533         4.800
 *  1  *  3          0.778         7.000
 *  2  *  1          1.678        15.100
 *  2  *  2          1.333        12.000
 *  2  *  3          2.822        25.400
 *  3  *  1          2.389        21.500
 *  3  *  2          2.533        22.800
 *  3  *  3          3.511        31.600
 *  4  *  1          3.211        28.900
 *  4  *  2          3.144        28.300
 *  4  *  3          3.700        33.300
-----------------------------------------------------------
 *  *  1  1          1.717        20.600
```

```
 *  *  1  2        1.792       21.500

 *  *  1  3        2.458       29.500

 *  *  2  1        1.975       23.700

 *  *  2  2        1.925       23.100

 *  *  2  3        2.708       32.500

 *  *  3  1        2.058       24.700

 *  *  3  2        1.942       23.300

 *  *  3  3        2.942       35.300

------------------------------------------------------------

 *  1  1  1        0.067        0.200

 *  1  1  2        0.133        0.400

 *  1  1  3        0.667        2.000

 *  1  2  1        0.467        1.400

 *  1  2  2        0.600        1.800

 *  1  2  3        0.667        2.000

 *  1  3  1        0.633        1.900

 *  1  3  2        0.867        2.600

 *  1  3  3        1.000        3.000

 *  2  1  1        1.733        5.200

 *  2  1  2        1.433        4.300

 *  2  1  3        2.133        6.400

 *  2  2  1        1.767        5.300

 *  2  2  2        1.200        3.600

 *  2  2  3        2.800        8.400

 *  2  3  1        1.533        4.600

 *  2  3  2        1.367        4.100

 *  2  3  3        3.533       10.600

 *  3  1  1        1.933        5.800

 *  3  1  2        2.167        6.500
```

```
  * 3  1  3          3.300        9.900
  * 3  2  1          2.533        7.600
  * 3  2  2          2.767        8.300
  * 3  2  3          3.533       10.600
  * 3  3  1          2.700        8.100
  * 3  3  2          2.667        8.000
  * 3  3  3          3.700       11.100
  * 4  1  1          3.133        9.400
  * 4  1  2          3.433       10.300
  * 4  1  3          3.733       11.200
  * 4  2  1          3.133        9.400
  * 4  2  2          3.133        9.400
  * 4  2  3          3.833       11.500
  * 4  3  1          3.367       10.100
  * 4  3  2          2.867        8.600
  * 4  3  3          3.533       10.600
 ----------------------------------------------------------

      A N A L Y S I S   O F   V A R I A N C E   T A B L E

 K                   Degrees of   Sum of      Mean       F
Value   Source       Freedom      Squares     Square     Value        Prob
------------------------------------------------------------------------------------------------

 2   Factor A            3        119.592      39.864   9160.2534    0.0000
 4   Factor B            2        1.965         0.982    225.7234    0.0000
 6   AB                  6        1.794         0.299     68.7021    0.0000
 8   Factor C            2        15.430         7.715    1772.8294  0.0000
10    AC                 6        4.566          0.761    174.8723   0.0000
12    BC                 4        0.361          0.090    20.7660    0.0000
14    ABC               12        3.131           0.261   59.9574     0.0000
-15    Error            72        0.313            0.004
```

```
-----------------------------------------------------------------------------------------------------
   Total        107      147.153
-----------------------------------------------------------------------------------------------------


  Coefficient of Variation: 3.04%

   s_ for means group 2:     0.0127       Number of Observations: 27
   y
   s_ for means group 4:     0.0110       Number of Observations: 36
   y
  s_ for means group 6:     0.0220       Number of Observations: 9
   y
   s_ for means group 8:     0.0110       Number of Observations: 36
   y
   s_ for means group 10:     0.0220      Number of Observations: 9
   y
   s_ for means group 12:     0.0190      Number of Observations: 12
   y
   s_ for means group 14:     0.0381      Number of Observations: 3
   y
```

Appendix 3e: Quality of bioethanol from NaOH pretreated sugarcane cake samples

Corresponding to Tables 4.8 and 4.9 in the main text

```
Function: FACTOR

Experiment Model Number 3:

Three Factor Completely Randomized Design

Data case no. 1 to 108.

Factorial ANOVA for the factors:

   Replication (Replication) with values from 1 to 3

   Factor A (Temp) with values from 1 to 4

   Factor B (Time) with values from 1 to 3

   Factor C (Conce) with values from 1 to 3

Variable 5: Quality

Grand Mean = 9.655   Grand Sum = 1042.749   Total Count = 108

          T A B L E   O F   M E A N S

 1  2  3  4          5          Total

-------------------------------------------------------------

 *  1  *  *        0.021         0.556

 *  2  *  *        5.126       138.393

 *  3  *  *       13.816       373.043

 *  4  *  *       19.658       530.757

-------------------------------------------------------------

 *  *  1  *        8.189       294.809

 *  *  2  *        9.907       356.661

 *  *  3  *       10.869       391.279

-------------------------------------------------------------

 *  1  1  *        0.005         0.041

 *  1  2  *        0.014         0.126

 *  1  3  *        0.043         0.389
```

```
 *  2  1  *         3.477        31.290

 *  2  2  *         5.215        46.931

 *  2  3  *         6.686        60.171

 *  3  1  *        11.900        107.097

 *  3  2  *        14.039        126.354

 *  3  3  *        15.510        139.592

 *  4  1  *        17.376        156.380

 *  4  2  *        20.361        183.250

 *  4  3  *        21.236        191.127

-----------------------------------------------------------

 *  *  *  1         2.643        95.159

 *  *  *  2         8.564       308.321

 *  *  *  3        17.757        639.269

-----------------------------------------------------------

 *  1  *  1         0.010         0.093

 *  1  *  2         0.013         0.116

 *  1  *  3         0.038         0.346

 *  2  *  1         2.942        26.474

 *  2  *  2         4.279        38.514

 *  2  *  3         8.156        73.405

 *  3  *  1         3.343        30.084

 *  3  *  2        13.371        120.337

 *  3  *  3        24.736        222.622

 *  4  *  1         4.279        38.507

 *  4  *  2        16.595        149.354

 *  4  *  3        38.100        342.896

-----------------------------------------------------------

 *  *  1  1         1.905        22.864

 *  *  1  2         7.318        87.811
```

```
 *  *  1  3        15.344       184.134

 *  *  2  1         2.811        33.734

 *  *  2  2         8.751       105.007

 *  *  2  3        18.160       217.920

 *  *  3  1         3.213        38.561

 *  *  3  2         9.625       115.503

 *  *  3  3        19.768       237.215

-----------------------------------------------------------

 *  1  1  1         0.000         0.000

 *  1  1  2         0.007         0.021

 *  1  1  3         0.007         0.020

 *  1  2  1         0.013         0.039

 *  1  2  2         0.014         0.042

 *  1  2  3         0.015         0.044

 *  1  3  1         0.018         0.054

 *  1  3  2         0.018         0.053

 *  1  3  3         0.094         0.282

 *  2  1  1         1.203         3.610

 *  2  1  2         3.210         9.630

 *  2  1  3         6.017        18.050

 *  2  2  1         3.610        10.830

 *  2  2  2         4.412        13.237

 *  2  2  3         7.621        22.864

 *  2  3  1         4.011        12.034

 *  2  3  2         5.216        15.647

 *  2  3  3        10.830         32.491

 *  3  1  1         2.808         8.423

 *  3  1  2        11.632         34.896

 *  3  1  3        21.259         63.778
```

```
     *  3   2   1           3.209           9.627

     *  3   2   2          12.836           38.508

     *  3   2   3          26.073           78.219

     *  3   3   1           4.011          12.034

     *  3   3   2          15.644           46.933

     *  3   3   3          26.875           80.625

     *  4   1   1           3.610          10.830

     *  4   1   2          14.421           43.264

     *  4   1   3          34.095          102.286

     *  4   2   1           4.412          13.237

     *  4   2   2          17.740           53.220

     *  4   2   3          38.931          116.793

     *  4   3   1           4.813          14.440

     *  4   3   2          17.623           52.869

     *  4   3   3          41.273          123.818
   -----------------------------------------------------------

        A N A L Y S I S   O F   V A R I A N C E   T A B L E

 K                 Degrees of   Sum of        Mean        F

Value   Source     Freedom      Squares       Square      Value        Prob

----------------------------------------------------------------------------------------------------------

 2     Factor A       3         6229.112     2076.371   16128085.0244   0.0000

 4      Factor B      2         132.691      66.346       515336.3122    0.0000

 6       AB           6         46.849        7.808        60648.9695     0.0000

 8      Factor C      2         4176.121     2088.061     16218886.1710   0.0000

 10     AC            6          3292.098     548.683    4261863.0778     0.0000

 12     BC            4         30.975        7.744       60148.7532      0.0000

 14     ABC            12        32.442      2.703        20999.1680      0.0000

-15    Error           72        0.009       0.000

----------------------------------------------------------------------------------------------------------
```

```
      Total           107    13940.297
---------------------------------------------------------------------------------------------------


 Coefficient of Variation: 0.12%

  s_ for means group 2:    0.0022      Number of Observations: 27
  y
  s_ for means group 4:    0.0019      Number of Observations: 36
  y
  s_ for means group 6:    0.0038      Number of Observations: 9
  y
  s_ for means group 8:    0.0019      Number of Observations: 36
  y
  s_ for means group 10:    0.0038      Number of Observations: 9
  y
  s_ for means group 12:    0.0033      Number of Observations: 12
  y
  s_ for means group 14:    0.0066      Number of Observations: 3
  y
```

Appendix 3f: Quality of bioethanol from POM pretreated sugarcane cake samples

Corresponding to Tables 4.10 and 4.11 in the main text

```
Function: FACTOR

Experiment Model Number 3:

Three Factor Completely Randomized Design

Data case no. 1 to 108.

Factorial ANOVA for the factors:

   Replication (Replication) with values from 1 to 3

   Factor A (Temp) with values from 1 to 4

   Factor B (Time) with values from 1 to 3

   Factor C (Conce) with values from 1 to 3

Variable 5: Volume

Grand Mean = 15.741   Grand Sum = 1700.068   Total Count = 108

          T A B L E   O F   M E A N S

 1  2  3  4            5             Total

-------------------------------------------------------------

 *  1  *  *         0.594         16.041

 *  2  *  *        16.179        436.836

 *  3  *  *        21.545        581.728

 *  4  *  *        24.647        665.464

-------------------------------------------------------------

 *  *  1  *        14.205        511.380

 *  *  2  *        15.607        561.836

 *  *  3  *        17.413        626.852

-------------------------------------------------------------

 *  1  1  *         0.072          0.649

 *  1  2  *         0.519          4.669

 *  1  3  *         1.191         10.722
```

```
 *  2  1  *        12.970       116.734

 *  2  2  *        16.313       146.813

 *  2  3  *        19.254       173.289

 *  3  1  *        20.512       184.610

 *  3  2  *        21.260       191.339

 *  3  3  *        22.864       205.779

 *  4  1  *        23.265       209.386

 *  4  2  *        24.335       219.015

 *  4  3  *        26.340       237.063

--------------------------------------------------------

 *  *  *  1         3.487      125.534

 *  *  *  2        13.222       475.976

 *  *  *  3        30.516      1098.558

--------------------------------------------------------

 *  1  *  1         0.376         3.384

 *  1  *  2         0.606         5.455

 *  1  *  3         0.800         7.201

 *  2  *  1         2.206        19.858

 *  2  *  2         9.895        89.052

 *  2  *  3        36.436       327.926

 *  3  *  1         4.012        36.105

 *  3  *  2        19.388       174.491

 *  3  *  3        41.237       371.133

 *  4  *  1         7.354        66.188

 *  4  *  2        22.998       206.978

 *  4  *  3        43.589       392.298

--------------------------------------------------------

 *  *  1  1         2.860        34.324

 *  *  1  2        11.042       132.499
```

```
 *  *  1  3         28.713        344.557

 *  *  2  1          3.245         38.939

 *  *  2  2         13.071        156.857

 *  *  2  3         30.503        366.040

 *  *  3  1          4.356         52.272

 *  *  3  2         15.552        186.620

 *  *  3  3         32.330        387.961

------------------------------------------------------------

 *  1  1  1          0.009          0.026

 *  1  1  2          0.042          0.127

 *  1  1  3          0.165          0.496

 *  1  2  1          0.143          0.428

 *  1  2  2          0.541          1.622

 *  1  2  3          0.873          2.619

 *  1  3  1          0.977          2.930

 *  1  3  2          1.235          3.705

 *  1  3  3          1.362          4.086

 *  2  1  1          1.404          4.212

 *  2  1  2          5.215         15.644

 *  2  1  3         32.293         96.879

 *  2  2  1          2.407          7.220

 *  2  2  2          9.226         27.678

 *  2  2  3         37.305        111.914

 *  2  3  1          2.809          8.426

 *  2  3  2         15.243         45.730

 *  2  3  3         39.711        119.133

 *  3  1  1          3.209          9.627

 *  3  1  2         17.249         51.747

 *  3  1  3         41.079        123.236
```

```
   *  3   2   1          3.611          10.832

   *  3   2   2         19.655           58.965

   *  3   2   3         40.514          121.541

   *  3   3   1          5.215          15.645

   *  3   3   2         21.260           63.779

   *  3   3   3         42.118          126.355

   *  4   1   1          6.819          20.458

   *  4   1   2         21.660           64.981

   *  4   1   3         41.315          123.946

   *  4   2   1          6.820          20.459

   *  4   2   2         22.864           68.591

   *  4   2   3         43.322          129.965

   *  4   3   1          8.424          25.271

   *  4   3   2         24.469           73.406

   *  4   3   3         46.129          138.386
  ---------------------------------------------------------

     A N A L Y S I S   O F   V A R I A N C E   T A B L E

 K                    Degrees of    Sum of        Mean          F

Value   Source       Freedom       Squares       Square      Value              Prob

----------------------------------------------------------------------------------------------------

  2     Factor A          3              9250.903     3083.634   88846.9841     0.0000

  4     Factor B           2             186.174       93.087        2682.0563     0.0000

  6     AB                   6              67.332       11.222         323.3343       0.0000

  8     Factor C           2              13492.533    6746.267   194376.3086   0.0000

 10      AC                  6             4557.569      759.595     21885.7698    0.0000

 12     BC                   4              29.250         7.313         210.6913        0.0000

 14    ABC                 12             52.482          4.374         126.0121        0.0000

-15    Error               72              2.499            0.035

-------------------------------------------------------------------------------------------------------
```

```
  Total        107    27638.743
---------------------------------------------------------------------------

 Coefficient of Variation: 1.18%

 s_ for means group 2:     0.0359      Number of Observations: 27

 y

 s_ for means group 4:     0.0310      Number of Observations: 36

 y

 s_ for means group 6:     0.0621      Number of Observations: 9

 y

 s_ for means group 8:     0.0310      Number of Observations: 36

 y

 s_ for means group 10:     0.0621      Number of Observations: 9

 y

 s_ for means group 12:     0.0538      Number of Observations: 12

 y

 s_ for means group 14:     0.1076      Number of Observations: 3

 y
```

Appendix 4: Fourier transform infrared spectra for Mn-Anderson POM salt (FT-IR) that was done using a Bruker Vector 22 infrared spectrometer using KBr pellet method.