

**OPTIMIZATION OF PRETREATED CASSAVA PEELS BIOETHANOL
PRODUCTION USING TAGUCHI DESIGN INTEGRATING KINETICS
OF YEAST (*Saccharomyces cerevisiae*) REACTION AND BIOETHANOL
CHARACTERIZATION**

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**A Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Master of Science in Chemistry of Murang'a University of
Technology**

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DECLARATION

Student Declaration

I hereby declare that this Thesis is my original work and to the best of my knowledge has not been presented for a degree award in this or any other university. I understand what plagiarism is and I am aware of the University's policy in this regard. I understand that any false claim in respect of this work shall result in disciplinary action in accordance with the University Anti-Plagiarism Policy.

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Supervisors' Approval

The undersigned certify that they have read and hereby recommended for acceptance of Murang'a University of Technology a thesis entitled **"Optimization of Pretreated Cassava Peels Bioethanol Production using Taguchi Design Integrating Kinetics of Yeast (*Saccharomyces cerevisiae*) reaction and Bioethanol Characterization."**

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DEDICATION

I dedicate this thesis to Mrs. Mary Wanjiru Nderi for her support and to the memory of my beloved mother, Millicent Atieno.

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ABSTRACT

Majority of African rural homes use harmful energy sources like wood and fossil fuels, which are hazardous to human health and not environmentally friendly. Bioethanol is a form of ethanol produced from fermentation process. Bioethanol is an alternative clean source of energy that can be obtained from biomass and agricultural wastes. Producing bioethanol from agricultural wastes cleans up the environment and prevents the cutting of trees for wood fuel, thus solving the problem of deforestation. Bioethanol also gives farmers an additional source of income from otherwise wasted agricultural materials, reduces indoor pollution and is more cost-effective due to its higher calorific value. Pretreatment of lignocellulose biomass involves chemical, biological, and physical treatments. While enzymes from microorganisms are studied, plant-derived enzymes and the use of citric acid have not been extensively investigated for the pretreatment of cassava peels. Taguchi method, despite its potential in optimizing fermentation settings like surface response and Box-Behnken design, the use of Taguchi method would be beneficial in this field. This work primarily focused on the pretreatment of cassava (*Manihot esculenta* Crantz) peels, Taguchi optimization of fermentation conditions, determining the K_m (measure of the affinity an enzyme has for its substrate) and V_{max} (maximum velocity of an enzymatically catalyzed reaction when the enzyme is saturated with its substrate) of *Saccharomyces cerevisiae*, and characterization of bioethanol. The feedstock was subjected to 0.5000 M sulphuric acid, pearl millet enzyme extract and 0.00026 M citric acid pre-treatments to increase the glucose content and reduce lignin content. Taguchi optimization design of four factors (temperature, pH, yeast to substrate ratio and time) at four levels was adopted to improve production efficiency. Lineweaver-Burk equation was used to obtain the K_m and V_{max} of *Saccharomyces cerevisiae*. The best pre-treatment method was sulphuric acid pre-treatment that produced a total sugar content of 14.50 % brix followed by citric acid 8.500 % brix then finally pearl millet enzyme extract pre-treatment at 0.800% brix. Taguchi's analysis of the optimized condition showed that the optimal conditions for fermentation were a pH of 4.5, temperature of 40 °C, yeast to substrate ratio of 4.500 g/50ml and fermentation time of 90 minutes. The V_{max} was 0.4300 Mol/L/Sec with K_m of 583.5 Mol/L. The bioethanol produced was characterized and the following parameters registered; density of 0.8763 g/cm³, specific gravity of 0.8779 g/cm³, ethanol concentration of 73.63 %v/v, ash content of 0.005, kinematic viscosity of 3.677 centistokes (cst), sulphur content of 0.0788 %, flash point of 17.00 °C, pH of 4.286, the calorific value of 21.89 MJ/kg and conductivity of 8.800 μ s/m. These characteristics of the bioethanol produced were found comparable to those of the ASTM standards for bioethanol. Cassava bioethanol emerges as a promising alternative source of fuel given to its exceptional qualities. The bioethanol can be used as disinfectants and fuel for cook stove

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ACRONYMS AND ABBREVIATIONS

IPCC	Intergovernmental panel on climate change
MTBE	Methyl tert-butyl ether
ETBE	Ethyl tertiary butyl ether
FW	Food waste
WMO	World Meteorological Organization
UNEP	United Nations Environment Program
UN	United Nations
INC	International Negotiating Committee for a Framework Convention on
UNCED	United Nations Conference on Climate Change Environment Development
EPRA	Energy and Petroleum Regulatory Authority
NERSA	National Energy Regulator of South Africa
REFIT	Renewable Energy Feed-in Tariff
PPA	Power Purchase Agreement
RFI	Request for Information
NBC	National Biofuels Committee
MoE	Ministry of Energy
LHW	Liquid Hot Water
OVAT	One variable at a time
MTBE	Methyl tert-butyl ether
IR	Infrared Radiation
C₆H₁₂O₆	Glucose
C₁₂H₂₂O₁₁	Maltose
H₂O	Water
CO₂	Carbon (IV) Oxide
CMC	Carboxy Methyl Cellulose
Cts	Centistokes

CHAPTER ONE

INTRODUCTION

1.1 Background information of the study

The reliance on fossil fuels as the dominant source of energy has resulted in many detrimental environmental consequences (Martins *et al.*, 2019). Emissions of hazardous particles and gases play a significant role in climate change (Perera, 2017). Pollutant gases cause a variety of serious health and developmental defects in children (Perera, 2017). Carbon dioxide (CO₂) traps sunlight energy by allowing light to enter the atmosphere and prevents it from escaping (Manieniyani *et al.*, 2009). According to Intergovernmental Panel on Climate Change (IPCC) report, the increase in anthropogenic greenhouse gas concentrations are responsible for the observed majority of the recorded escalation in globally averaged temperatures since the mid-20th century (Manieniyani *et al.*, 2009). Substitute sources of biofuel production are required to improve the current levels.

Biofuels are advocated as a low-carbon substitute for fossil fuels because they can help decrease greenhouse gas emissions and mitigate the associated climate change effects (Jeswani *et al.*, 2020). The preponderance used liquid biofuel in the market today is bioethanol (Bhuvaneswari & Sivakumar, 2019). To prevent gasoline vapors and compressed air in the combustion engine cylinders from prematurely igniting before reaching the appropriate degree of compression, anti-knocking agents such as tetra-ethyl lead and methyl tert-butyl ether (MTBE) are often used (De Vasconcelos, 2015). Ethanol has a high-octane rating of 150 and effectively replaces the harmful anti-knocking additives (De Vasconcelos, 2015). Producers use bioethanol to make ethyl tertiary butyl ether (ETBE) (Norkobilov *et al.*, 2017). Therefore, biofuels are alternative sources of clean and affordable energy (Tsai *et al.*, 2008).

Different types of biomass sources can be used to produce bioethanol (Zabed *et al.*, 2017). Many countries have set lofty goals for replacing petroleum-based fuels with bioethanol (Sekoai & Yoro, 2016). The US has proposed raising bioethanol output to 36 billion gallons (US EPA, 2014) while China has also supported biofuel by establishing goals of 10 million tons of bioethanol each year and 12 million metric tons per year for biodiesel (USAID, 2009). India intends to use 20% biofuels in its

transportation (USAID, 2009). Global nations viz Japan and Australia have developed initiatives to develop biofuels (Sekoai & Yoro, 2016). Programs on biofuel have not advanced in Africa due to several obstacles such as funding limitations, lack of technical expertise, lack of available land and government policies (Sekoai & Yoro, 2016). Today edible crops are first-generation bioethanol (Muktham *et al.*, 2016). Biofuel makes up most of the 25 billion gallons of bio-ethanol generated globally (Bertrandetal *et al.*, 2016). The United States and Brazil are two of the world's largest economies producing bioethanol in the world (Bertrandetal *et al.*, 2016). Bio-fuel fabrication threatens food security along with competition for land space (Mitchell, 2011).

The secondary generation bio-ethanol is from inedible waste lignocellulose biomass (Lavoie, 2011). In keeping with this lignocellulose biomass include; food waste, kitchen garbage and municipal solid waste (Dhiman & Mukherjee, 2020). Due to the vast number of diverse fruit plantations and vegetable growing areas worldwide, the disposal of agro-wastes has become a serious problem (Bhuvaneswari & Sivakumar, 2019). This cassava roots can be used to produce second-generation bioethanol (Sriroth *et al.*, 2012).

Algal biomass forms third-generation bioethanol, it offers a distinct growth yield in comparison to traditional biomass derived from lignocellulose (Brennan & Owende, 2010). Species with a high lipid content like chlorella, typically determine the production of biofuels from algae as their lipid content plays a crucial role (Liang *et al.*, 2009; Lee & Lavoie, 2013). Chlorella are targeted for producing bioethanol (Ngamsirisomsakul *et al.*, 2019). Cyanobacteria's synthetic biology creates fourth-generation biofuels (Berla *et al.*, 2013). Artificial photosynthesis and solar-powered water (water splitting oxidation) can both significantly increase the amount of fuel produced on a worldwide scale (Inganäs & Sundstrom, 2015). The amount of (CO₂) released during bioethanol combustion is the same as that plants extract from the atmosphere during growth (net zero carbon), so it does not increase the effects of the greenhouse (Abo *et al.*, 2019).

Before fermentation, lignocellulose must undergo pre-treatment (Asgher *et al.*, 2014). Various methods are available such as using physical, chemical and physicochemical methods and biological agents like *Aspergillus niger*, *Aspergillus oryzae*, *Humicola*

insolens and *Trichoderma reesei*, that deconstruct the lignocellulose structure (Gusakov, 2011; Wilkinson *et al.*, 2017). Literature extensively documents the utilization of biological agents such as fungi and bacteria for lignocellulose deconstruction (Wu *et al.*, 2022). Amylase enzymes extracted from pearl millet may offer unique properties that could enhance lignocellulose degradation processes. However, despite their potential benefits, the utilization of plant-based amylase enzymes, especially those from pearl millet, remains less investigated compared to microbial counterpart. The fermentation process is then achieved by using species of fungi such as *Neurospora* and bacteria like *Bacillus cereus* and *Zymomonas mobilis* (Egbosiuba, 2014).

Factors that affect bioethanol yield include; pH, fermentation time, yeast to substrate ratio and temperature (Okoth *et al.*, 2023). Based on the results, the greatest bioethanol yield was 45.50% at the same time the fermentation temperature was 35 °C, the yeast concentration was 10 %, the C₆H₁₂O₆ concentration was 100 g/L and the fermentation time was 6 days (Egbosiuba, 2014). Eighty-three percent of ethanol was obtained from cassava peel using *Zymomonas mobilis* (Sivamani & Ramesh, 2014). It is important to optimize fermentation conditions of cyanic cassava peels to reduce wastage of materials during the production process. When optimizing fermentation conditions, it takes a lot of time and effort to conduct multiple experiments using one variable at a time (OVAT) method to identify various independent variables and their effects (Vishwanatha *et al.*, 2009; Das *et al.*, 2014).

Taguchi orthogonal array design is a statistically based experimental design that summarizes the compilation and classification of the factors to be considered (Antony, 1998; Das *et al.*, 2014). The method calculates the number of variables, examines the variable at various parameters and finally examines the impact of the variable error (Antony, 1998; Das *et al.*, 2014). Taguchi's design of the experiment aims for cost-effective, robust products and processes (Antony, 1998; Das *et al.*, 2014). Thus, Taguchi design of experiment was used in this work to optimize the fermentation conditions. Michaeli-Menten K_m and V_{max} fermentation measure reaction rate (velocity) at various substrate concentrations (Counotte & Prins, 1979). The reaction velocity gets closer to V_{max} when the substrate concentration reaches saturating levels (Michaelis and Menten, 1913). Substrate concentration with half V_{max} reaction speed

is K_m (Michaelis and Menten, 1913). K_m measures an enzyme's affinity for its substrate; a lower value denotes a stronger affinity (Michaelis and Menten, 1913). Researchers have not yet established the best way to produce bioethanol from cassava in terms of pH, reaction time, and the yeast-to-substrate ratio. Michaeli-Menten K_m and V_{max} fermentation parameters was used to establish the interaction between the substrate and the yeast to determine whether the produced bioethanol can be used as a green fuel.

1.2 Statement of the problem

The global dependence on fossil fuel energy for an extended period has led many adverse effects (Azni *et al.*, 2023). The environment has suffered greatly from ongoing reliance on fossil fuels, primarily as a result of the steady rise in greenhouse gas concentrations. Carbon dioxide and other pollutants are released into the atmosphere when fossil fuels are burned, which has a major impact on climate change and global warming. This phenomenon manifests in various ways, including changes in precipitation patterns, rising global temperatures and the exacerbation of extreme weather events such as heat waves and flash floods. Furthermore, the production and use of fossil fuels frequently involves harmful activities including habitat loss, deforestation, air and water pollution, which exacerbates environmental degradation. The process of turning biomass into bioethanol is more sustainable. First-generation bioethanol may unintentionally cause deforestation and food insecurity therefore environmentally friendly methods that make use of waste materials is vital. For example, the waste products from cassava are among the many agricultural wastes that are produced in large quantities in rural areas. Rather than letting this garbage decompose and release methane, a strong greenhouse gas, into the atmosphere, it can be used to produce bioethanol, which would reduce environmental risks and serve as a source of renewable energy. Therefore, this research used second-generation sources such as cyanic cassava peels. Previous study has reported starch breakdown can be achieved by chemical, physicochemical, and enzymatic pre-treatment. The best pre-treatment method for the hydrolysis of cassava peel starch is necessary for enhancing the quality and the yield of cassava peel bioethanol. This work utilized acids and amylase enzymes from the pearl millet for the pre-treatment of cyanic cassava peels for the hydrolysis of starch into $C_6H_{12}O_6$ for bioethanol production. Researchers have

also reported different pH, time, yeast to substrate ratio and temperature of bioethanol production for *Saccharomyces cerevisiae*.

1.3 Objectives

This section stipulates the main and the specific objectives

1.3.1 General objective

To optimize bioethanol production from pretreated cassava peels using Taguchi design by integrating the kinetics of *Saccharomyces cerevisiae* and characterizing the resulting bioethanol

1.3.2 Specific objective

- i. To determine the best pre-treatment method between sulphuric acid, pearl millet amylase enzyme extract and citric acid for the hydrolysis of cassava peel starch into simple sugar
- ii. To optimize pH, time, yeast to substrate ratio and temperature of bioethanol production for *Saccharomyces cerevisiae* using Taguchi method
- iii. To determine the reaction kinetics parameters of K_m and V_{max} for *Saccharomyces cerevisiae*
- iv. To analyze the produced bioethanol for its density, specific gravity, ash content, kinematic viscosity, sulphur content, flash point, pH, calorific value and electrical conductivity

1.4 Null hypotheses

H₀₁. Sulphuric acid pre-treatment for the hydrolysis of cassava peels is not different from pearl millet amylase enzyme extract pre-treatment and citric acid pre-treatment

H₀₂ Bioethanol production from *Saccharomyces cerevisiae* is not affected by pH, time, yeast to substrate ratio and temperature

H₀₃. There is no significant difference in the reaction kinetics parameters (K_m and V_{max}) for *Saccharomyces cerevisiae* under the conditions tested

H₀₄. The bioethanol produced is not a suitable fuel for use based on its density, specific gravity, ash content, kinematic viscosity, sulphur content, flash point, pH, calorific value and conductivity

1.5 Significance of the study

This research provides information on alternative cleaner energy. By incorporating bioethanol into medical sector to make surgical spirits, the healthcare industry can reduce its reliance on petroleum-based ethanol while maintaining the necessary standards of cleanliness and sterilization. The bioethanol produced can also be used for domestic purpose in cook stoves.

1.6 Limitation of the study

The study focuses on using a single species of cassava for producing bioethanol. It employs only one yeast strain, *Saccharomyces cerevisiae*, as the fermentation agent. The research optimizes specific fermentation conditions including pH, time, yeast to substrate ratio and temperature.

1.7 Contribution of the thesis

The research identified the optimal conditions for bioethanol production from pretreated cassava peels using the Taguchi design method, which can significantly enhance the efficiency and yield of bioethanol in industrial applications.

The study provided a comprehensive kinetic analysis of the yeast (*Saccharomyces cerevisiae*) reaction during the bioethanol production process, offering valuable insights for improving fermentation efficiency.

The characterization of the produced bioethanol was conducted, ensuring that the bioethanol meets industry standards and is suitable for commercial use as a fuel for cook stoves.

The findings from this research can be applied to other agricultural waste materials, promoting sustainable and eco-friendly bioethanol production practices.

1.8 Organization of the thesis

The content of this research is divided into five chapters as highlighted below:

Chapter one is the introduction. The chapter discusses the background of the research, statement of the problem, objectives of the study, research hypotheses, significance of the study, scope of the study and limitations of the study and contribution of the thesis.

Chapter two represents literature review. The chapter discusses on the literature review on the Vienna Convention for the protection of the Ozone layer, renewable energy, pretreatment of cassava peels, fermentation, description of analytical instruments used in bioethanol research and derivation of Michaeli Menten's equation.

Chapter three describes the materials and methods. The chapter is divided into various sections that include geographical location where the cassava samples were obtained, detailed description of the pretreatment methods used (e.g., chemical and enzymatic pretreatments), factors considered for optimization (e.g., temperature, pH, yeast concentration), levels of factors and their combinations as per Taguchi design, experimental setup for fermentation, methods for collecting data on fermentation kinetics, statistical methods used for analyzing the data and methods for characterizing the produced bioethanol (e.g., specific gravity , calorific value).

Chapter four present the results of the research findings and discussions. This chapter focuses on data analysis, presentation, interpretation and discussion. The first section describes the amount of sugar obtained from different pretreatments that was used to pretreat the cassava peels powder, optimal fermentation conditions from Taguchi experimental design, ANOVA analysis, Michaeli Menten's fermentation kinetics and characterization of cassava peel bioethanol.

Chapter five covers the conclusion made by the study findings, the recommendations from the study and recommendation for future works.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

In this chapter, the literature on treaties that govern green energy, the current available renewable energy, sources of bioethanol, situation worldwide on bioethanol, drawbacks of existing renewable energy sources, history of cassava, classification of cassava, characterization of cassava, economic importance of cassava and its nutritional value, cassava peel lignocellulose biomass composition, pretreatment methods for lignocellulose biomass, fermentation, factors affecting bioethanol yield, methods of analysis and Machaeli- Menten kinetics are discussed.

2.2 Vienna treaty on climate change

Based on the Vienna Convention and Montreal Protocol protection of the ozone layer there has been an international agreement that States ought to think about creating a climate convention with legal force. The convection is used to address greenhouse gas emissions that are not protected by the ozone layer. United Nations Environment Program (UNEP) established IPCC to assess Policy implications for mitigation and adaptation based on scientific research (Chazournes, 1992).

UN established the International Negotiating Committee for Climate Convention. INC was to "negotiate a framework convention, containing appropriate commitments. INC was also tasked with other related legal instruments as might be agreed upon" for it to be prepared for signing at the UNCED conference in 1992 in Rio de Janeiro (Chazournes, 1992). The Convention's long-term goal and accompanying legal documents are to stabilize atmospheric amounts of greenhouse gases that would make it impossible for humans to change the climate system (Chazournes, 1992). Following the treaty various countries are thus trying to cut their greenhouse emission by using cleaner fuels. Thus, for Kenya to effectively contribute, sustainable source of bioethanol needs to be explored.

2.3 Current available renewable energy

The International Energy Agency predicts that by 2040, solar photovoltaic energy will account for 47 % of the technological mix used to generate electricity in African countries (Renewable energy in Kenya, 2022). According to 2016 World Resources Institute research, Kenyan solar energy provider M-KOPA solar has installed 225,000 solar energy products (Renewable energy in Kenya, 2022). Kenya has pledged to cut its (CO₂) release by 30 % by 2030 (Bwire, 2019). Every year, more Kenyans opt for solar energy over connecting their homes to the nation's electrical grid since Kenya Power's electricity is expensive (Renewable energy in Kenya, 2022). Kenya has solar power installations with a 4 to 8-megawatt capacity (Renewable Energy in Kenya, 2022). Only India has a more vibrant commercial photovoltaic system market than Kenya, which is located in the developing world (solarge2, 2022). Annual sales of solar modules range from 25,000 to 30,000 (Renewable Energy in Kenya, 2022). About 200,000 rural homes have solar home systems installed in them (Renewable Energy in Kenya, 2022). As a national initiative to boost the share of renewable energy sources in some districts that are far from the national grid, the government is presently installing solar photovoltaic systems to power schools and other organizations (Kiplagat *et al.*, 2011).

Kenya is fortunate to have a wealth of geothermal resources (Simiyu & Keller, 2000; Kiplagat *et al.*, 2011). Most of which are concentrated in the Rift Valley's volcanic hot spots (Simiyu & Keller, 2000; Kiplagat *et al.*, 2011). According to estimates, the untapped geothermal power potential is between 4000-7000 MW (Simiyu & Keller, 2000; Kiplagat *et al.*, 2011). The government of Kenya has focused heavily on encouraging geothermal development because it has been determined that geothermal energy is the most affordable Kenya's energy source (Kiplagat *et al.*, 2011). To increase the production of geothermal the Geothermal Development Company Ltd (GDC) will be in charge of geothermal resource exploration, drilling and evaluation until a clear and accurate resource potential statement, with sufficient assurance to encourage potential developers to invest in designated generation projects (Kiplagat *et al.*, 2011).

Ideally, when comparing development by nation, the report reveals that China is in the lead with about 80% of the new capacity installed globally in 2021 (Iha, 2022). China

has the largest installed hydropower capacity in the world (Iha, 2022). Hydroelectric power plants are accessible, reliable and adaptable (Kumar, 2020). On progress toward the UN's global Sustainable Development Goal of achieving universal access to electricity by 2030, Kenya has been one of Africa's success stories (African Business, 2022). Over the past ten years, the rate of electrification in the nation has increased in Africa (African Business, 2022). According to Kenya's Energy and Petroleum Regulatory Authority (EPRA) by 2020, greater than 75 % of the population had access to electricity. The government has stated that it intends to increase that number to 100% within this decade (African Business, 2022).

2.4 Cassava bioethanol

In low-income and middle-income countries, millions of homes get their energy mostly from burning solid fuels (Puzzolo, 2013). Ending the subsequent vicious cycle of poverty and illness can be accomplished in part by promoting clean cooking fuels as an alternative to unclean ineffective stoves (Connors, 2023). Ethanol has gained recognition as a fuel option that offers noteworthy health advantages recently (Puzzolo, 2013). However, only a few countries have started using ethanol for cooking up until this point (Puzzolo, 2013). Bioethanol is produced through the process of fermentation followed by distillation, converting organic materials such as crops or agricultural residues into a renewable and environmentally friendly fuel source (Akponah *et al.*, 2013).

Based on findings the highest amounts of ethanol produced by *Zymomonas mobilis* following 48 hours of fermentation were 15.0 and 16.5 (%v/wt) in hydrolysates of acid and enzymes (Akponah *et al.*, 2013). *Saccharomyces cerevisiae* produced ethanol of 2.9 and 2.84 (%v/wt) in acid and enzyme hydrolysate respectively. Acid hydrolysates with *Zymomonas mobilis* and *Saccharomyces cerevisiae* decreased pH during fermentation (5.5 to 4.41 and from 5.5 to 4.9 respectively) (Akponah *et al.*, 2013). When *Zymomonas mobilis* and *Saccharomyces cerevisiae* were used to ferment enzyme hydrolysates, pH steadily also reduced (5.5 to 4.46 and 5.5 to 5.1) subsequently. The time for fermentation was from 0h to 48h. Sugar concentration decreased in *Zymomonas mobilis* and *Saccharomyces cerevisiae* acid hydrolysates (63.94 to 30.09 and 63.94 to 9.40 subsequently (Akponah *et al.*, 2013).

Utilizing sulphuric acid solutions at concentrations of 2 %, 6 %, and 10 % during hydrolysis was used to produce bio-ethanol from cassava peels using a mixture of *Aspergillus niger* and *Saccharomyces cerevisiae* (Hauwa *et al.*, 2019). According to the data, a 10 % H₂SO₄ concentrated acid pre-treatment sample produced the most ethanol (37.35 g/ml after 4 days at a pH of 4.55, fermentation temperature of 28 °C and sugar content of 15.5 % (Hauwa *et al.*, 2019).

A research on enzymatic liquefaction of potato wastes which have carbohydrate content of ninety percent for ethanol generation, some parameters were shown to be important using the Plackett-Burman design (Izmirlioglu, 2016). Solid-state fermentation of (glucoamylase and *Zymomonas mobilis*) combination (5.15 %-25.75 % [v/v]), substrate concentration ranges from 10 to 70 g/L, while α -amylase concentration ranges from 5 % to 25 % (v/v) (Sivamani & Ramesh, 2014). Based on the mathematical model and experiments, the highest concentration of ethanol that could be produced under ideal conditions of 69.82 g/L of substrate, 24.74 % (v/v) of α -amylase, and 5.22 % simultaneous saccharification and fermentation mixture were 35.018 and 35.010 g/L (83 % yield) (Sivamani & Ramesh, 2014).

The generation of ethanol from cassava wastewater, using native variety of *Zymomonas mobilis* and *Saccharomyces cerevisiae* obtained from rafia palm sap produced the maximum ethanol after 48 hours of fermentations (Akponah *et al.*, 2013). In acid and enzyme, hydrolysates had 15.0 % v/wt and 16.5 % v/wt respectively. In the particular case of *Saccharomyces cerevisiae*, acid and enzyme hydrolysates yielded 2.9 and 2.84 (%v/wt) ethanol respectively (Akponah *et al.*, 2013). During fermentation, the pH of acid hydrolysates seeded with *Zymomonas mobilis* and *Saccharomyces cerevisiae* decreased from 5.5 to 4.41 and 5.5 to 4.9 respectively (Akponah *et al.*, 2013). As fermentation time rose from 0 to 48 hours, the pH of *Zymomonas mobilis* and *Saccharomyces cerevisiae* fermented enzyme hydrolysates consistently decreased from 5.5 to 4.46 and 5.5 to 5.1 respectively (Akponah *et al.*, 2013).

The optimal parameters for producing the most amount of bio-ethanol were 30 °C, 6 days' duration of the fermentation, 10 % yeast dose, 100% glucose concentration, pH 4.0, and 180-rpm agitation rate (Umeuzuegbu, 2022). The density was 780 kg/m³ with kinematic viscosity of 1.55 cst at 40 °C, a flash point of 13 °C, a refractive index of

1.362 and octane number of 102 (Umeuzuegbu, 2022). The sulphur content was 0.06 %, water content of 0.05 ppm, boiling point of 78.5 °C, lower heating value of 20.4 KJ/dm³ and a pH of 6.5 were the physiochemical properties of the produced bio-ethanol (Umeuzuegbu, 2022).

The best yield of 45.50 % of bioethanol was produced using the traditional method for optimizing the impact of temperature, pH, yeast concentration, C₆H₁₂O₆ concentration and fermentation time on bioethanol (Egbosiuba *et al.*, 2014). The optimum C₆H₁₂O₆ yield was 78 mg/ml obtained at 100 °C, 45-minute hydrolysis period with 0.40 mole acid concentration. Following the results, fermentation occurred at a pH of 5, temperature of 35 °C, fermentation time of 6 days, yeast concentration of 10 %, C₆H₁₂O₆ concentration of 100g/L. The described bioethanol's kinematic viscosity was found to be 1.21 10³ cst at 20 °C, 0.83 10³ cst at 40 °C, and 0.6 10³ cst at 60 °C. The outcome further demonstrated a decreasing viscosity trend as temperature was raised from 20 °C (Egbosiuba *et al.*, 2014).

The bioethanol made from cassava peel complies with the established standards for bioethanol and gasoline in terms of kinematic viscosity quality (Egbosiuba *et al.*, 2014). The generated bioethanol had specific gravities of 0.750 kg/L and 0.785 kg/L at 27 °C room temperature and 15 °C standard temperature respectively. The specific gravity of the bioethanol established in the study was between the 0.750 kg/L and 0.850 kg/L range recommended by ASTM standards (Egbosiuba *et al.*, 2014). The produced bioethanol's flash point, which is 14.2 °C, was within the range of the ASTM guidelines for combustible and flammable liquids (Egbosiuba *et al.*, 2014).

A newly identified yeast strain named 267 was used to produce ethanol effectively using thermotolerant yeast strains (Kaewkrajaya *et al.*, 2014). According to the findings, 33 strains were able to create ethanol at 45 °C in a cassava starch hydrolysate medium with a pH of 4.5 and ingredients of 180 g/l reducing sugar and 0.5 g/l (NH₄)₂SO₄ when the shaking speed was 120 rpm (Kaewkrajaya *et al.*, 2014). The various strains of *Pichia kudriavzevii* had the highest ethanol concentrations (26.2, 26.2, 23.6, and 22.5 g/l) respectively (Kaewkrajaya *et al.*, 2014).

The yeast strain that produced the highest ethanol produce was selected for ethanol production in the shaking flask at 45 °C. In a medium with, 0.5 g/l KH₂PO₄, 0.5 g/l

MgSO₄ 7 H₂O, 180 g/l reducing sugar, 0.5 g/l (NH₄)₂SO₄ and 1 g/l yeast extract. Ethanol synthesis peaked after 36 hours with a productivity of 1.03 (g/l)/h and a yield of 40 % of the theoretical yield, it generated 37 g/l ethanol. By batch fermentation at 45 °C, ethanol was produced with a yield of 46 % of the theoretical yield and a maximum concentration of 42.4 g/l after 48 hours (Kaewkrajaya *et al.*, 2014). From the best optimized condition determined by Taguchi method, the produced bioethanol can be characterized to establish its suitability for use as a green fuel.

2.5 Global perspective and policies on bioethanol

The process of producing bioethanol is new in Turkey (Bayrakci & Koçar, 2014). Some factories in Turkey produce first-generation bioethanol (Bayrakci & Koçar, 2014). Based on Thailand's research, the government of Thailand has pushed for the usage of biofuels in road transportation to lessen the importation of oil (Silalertruksa & Gheewala, 2011). Currently, biodiesel is from animal fat, leftover cooking oil and palm oil whereas bio-ethanol is from sugar cane, molasses and cassava (Silalertruksa & Gheewala, 2011). These liquid biofuels are used in automobiles (Silalertruksa & Gheewala, 2011). The Royal Thailand Government has set many goals for the production of bioethanol than that of biodiesel in the transportation sector to replace the use of gasoline (Silalertruksa & Gheewala, 2011).

Ghana has gained a lot of experience with bioenergy technologies in recent years, including better cook stoves, better charcoal production techniques, briquetting, cogeneration from sawmills and palm leftovers, as well as biogas from municipal/farm waste (Ahiataku-Togobo & Ofosu-Ahenkorah, 2009; Janssen & Rutz, 2011). The primary goals for Ghana's bioenergy development include expanding access to contemporary energy services (Janssen & Rutz, 2011). Through regulation and financial incentives, we can encourage the long-term regeneration of woody biomass resources (Janssen & Rutz, 2011). Supporting buildout of an indigenous bioenergy based alternative transportation fuel company (biofuels) to replace petroleum-based fuels, as well as passing legislation to stimulate the use of bioenergy resources (Janssen & Rutz, 2011).

To boost South Africa's renewable energy industry, in 2009 South African National Energy Regulator (NERSA) introduced South Africa Renewable Energy Feed-in

Tariff Program (REFIT) (Janssen & Rutz, 2011). Additionally, NERSA released a draft power purchase agreement (PPA), regulatory guidelines and basis for selecting renewable energy projects for the REFIT program (Janssen & Rutz, 2011). The formal launch of the government's procurement procedure for electricity production using renewable energy sources took place in September 2010 (Janssen & Rutz, 2011). In reply to a Request for Information (RFI), private project developers are encouraged to submit viability study for wind, solar, biomass, biogas, landfill gas and small hydropower projects (Janssen & Rutz, 2011). The South African Department of Energy announced in 2013 that it intended to make the mandatory blending of gasoline and diesel with biofuels (bioethanol and biodiesel) as the nation works to develop its biofuel sector and lessen its reliance on imported fossil fuels (Sekoai & Yoro, 2016). Furthermore, the state proposed 5-year pilot phase master plan to deploy 2% biofuel countrywide (Sekoai & Yoro, 2016). Five firms have been given licenses to manufacture bioethanol and biodiesel in South Africa (Sawahel, 2006; Sekoai & Yoro, 2016).

The Tanzanian government's vision for the growth of the bioenergy industry is to replace fossil fuels for transportation while promoting socioeconomic through rural electrification projects for development (Kiwele, 2009; Janssen & Rutz, 2011). Benefits anticipated because of bioenergy project implementation include increased energy security, decreased reliance on foreign currency and oil imports and new job and income-generating opportunities for farmers by providing alternative markets (Kiwele, 2009; Janssen & Rutz, 2011).

The Zambian government is to ensure environmentally sound biomass exploitation to achieve fuel supply security and stable price (Kalumiana, 2009; Janssen & Rutz, 2011). Opportunities for the development of biodiesel exist in Kenya (Takase *et al.*, 2021). Arid and semi-arid makes up about 80 % of the country's land, where some biodiesel feedstock can flourish without endangering the nation's food security (Takase *et al.*, 2021). This presented an opportunity to lessen dependency on high-priced fossil oil, provide much-needed jobs and stimulate the economy, especially for farmers who would be farming the feedstock (Kiplagat, 2011).

The goal is to expand energy access through sustainable biofuel production and reduce fossil fuel imports (Janssen & Rutz, 2011). In Kenya, the creation of a biodiesel

strategy came first in the process of developing policy, followed by the development of a bioethanol strategy with the ultimate goal of developing a national biofuel policy (Janssen & Rutz, 2011). The Kenyan Ministry of Energy (MoE) founded the National Biofuels Committee (NBC) in 2006 to coordinate the activities of many stakeholders in the biofuels sector (Janssen & Rutz, 2011). Therefore, there is a need to set up several bioethanol industries within counties, especially within the reserves because farming generates a large amount of biomass. The produced bioethanol will not only help to combat climate change but also create employment opportunities for the farmers.

2.6 Shortcomings of already available renewable energy

The accessibility along with technological limitations of renewable sources of energy are key determining factors when evaluating whether to use them to produce electricity (Kumar, 2020). Every resource has limitations, photovoltaic power generation is limited to daylight hours, except during foggy seasons (Kumar, 2020). To prevent damage to the wind turbine, the speed should not go beyond 25 m/s (Karadza, 2023). The production of electricity is not possible at 3m/s wind speed (Kumar, 2020). The low capacity factor of wind power, however, is the issue that needs to be resolved. The grid must be able to handle the instability because the wind does not always blow at a constant rate (Kiplagat *et al.*, 2011).

Even though geothermal energy is renewable, its energy is limited by the availability of resources to provide 24-hour power (Kumar, 2020). Hydropower resources may face difficulties as they grow in the future. This is due to the rapidly increasing demand for water resources for other uses as well as other irrigated agriculture. H₂O resources will be less accessible in the future, than they were in the past (Tang, 2021). This is because it depends on differences in climate and weather, which are unpredictable (Tang, 2021). There is ought to be precautions to protect river resources, such as maintaining a sufficient amount of forested area to ensure rainfall and implementing programs to control soil erosion in the catchment areas (Kumar, 2020).

2.7 History of cassava

There are numerous names for cassava (*Manihot esculenta crantz*) in Uganda (Akinpelu *et al.*, 2022). Around Lake Victoria, cassava is referred as muwogo, while in the districts of Lira, Gulu, and Moyo, it is known as mogo, gwam, and gbanda (Ameny, 1995). The 1950s saw the introduction of cassava as a staple meal in Uganda, where many people today depend on it to prevent starvation (Ameny, 1995). For at least 4,000 years, local Indians in Latin America have grown cassava (*Manihot esculenta crantz*) (Akinpelu *et al.*, 2022). Upon its discovery in the Americas, European merchants brought cassava to Africa as a potential food crop (Akinpelu *et al.*, 2022).

The Asian traders introduced cassava in Uganda between 1862 and 1875 and it quickly spread over practically the entire nation (Nakabonge *et al.*, 2017). Cassava resistance to dry spells and tolerance to a variety of agro-ecological conditions led to the widespread cultivation and distribution of cassava (Nakabonge *et al.*, 2017).

In Kenya sixty percent of the nation's entire production comes from cassava, which is regarded as a significant food crop in the Western region (Kalro, 2023). Cassava roots fall into one of two major categories for food consumption (Iragaba *et al.*, 2020). First, foods are prepared from freshly collected roots and secondly, flour manufactured from roots make variety of foods (Iragaba *et al.*, 2020). Cassava roots are low in protein but high in calories because they are mostly made of starch and soluble carbohydrates (Montagnac *et al.*, 2009). The other third of cassava roots can be used to make secondary products (Akinpelu *et al.*, 2022).

Fresh cassava roots contain approximately 30 % starch, making it one of the most fermentable components in light of ethanol synthesis (Bakky *et al.*, 2021). The most important bio-fuel is bio-ethanol, which represents more than 90 % of overall biofuel use (Bakky *et al.*, 2021). Bioethanol offers better properties than petrochemical-based gasoline when blended with 95 % gasoline because it can cut CO₂ emissions by around 90 % and SO₂ emissions by about 60-80 % (Sirajuddin *et al.*, 2019). Bioethanol can also increase the fuel's octane rating (Wibowo *et al.*, 2018). The C₆H₁₂O₆ obtained from the hydrolysis process undergoes fermentation to make bioethanol (Soperton *et al.*, 2019). The objective of hydrolysis is the creation of monosaccharides from

cellulose and hemicellulose, which undergo fermentation to create ethanol (Sirajuddin *et al.*, 2019).

2.8 Classification of cassava

Cassava is a woody plant that grows in the tropics as a perennial crop with branched stems and tuberous roots (Wassie, 2018). Cassava can be classified as a single species based on its morphology or the characteristics of its foliage, stem and tubers (Aloys & Ming, 2006; Li *et al.*, 2017). There are various cyanogenic glycosides in cassava (Li *et al.*, 2017). The two main categories of cassava cultivars are bitter cultivars, which have tuberous roots with high concentrations of cyanogenic glycosides and sweet cultivars, which have peel with lower quantities of glycosides (Li *et al.*, 2017). On a fresh weight basis, the hydrogen cyanide content of sweet cassava roots is less than 50 mg per kilogram, whereas the amount in the bitter variety can reach 400 mg per kilogram (Ifeabunike *et al.*, 2017). Peeling and thorough cooking can usually render sweet cassava roots safe for consumption (Food safety focus, 2018). The processing of bitter cassava roots must be more thorough (Ukonu & Olasunbo, 2022).

Peeling and grating the bitter cassava roots is one of the traditional preparation methods (Ukonu & Olasunbo, 2022). The grated roots are soaked in H₂O for a long time to allow leaching and fermentation to occur (Ukonu & Olasunbo, 2022). Finally, the roots are cooked to release the volatile hydrogen cyanide gas (Food safety focus, 2018). Farmers in Uganda favor many regional cassava varieties such as Mingoro, Gbasumenge, Abiria, Kwatamumpale, Bukalasa, Magana and Ofumbachai (Kawuki *et al.*, 2016; Nakabonge *et al.*, 2017; Faizo *et al.*, 2020). Shibe, Tajirika, Karembo, Siri, Nzalauka, and Karibuni are some of the cassava varieties grown in Kenya (Anyango, 2022). These varieties can yield up to 28 tonnes per acre and reach maturity in eight to twelve months (Anyango, 2022).

2.9 Characterization of cassava

Carbohydrates, mostly as starch in their comestible, subterranean roots (Kajiwara & Maeda, 1983). The H₂O content of the root's ranges from 59 to 79 % weight for weight (w/w) (Kajiwara & Maeda, 1983). On a dry solid basis, the starch in cassava roots makes up 77-94 % of the total weight, followed by protein, fat, fiber and ash content

(1.7-3.8 % w/w, 1.5-3.7 % w/w, 1.8-2.5 % w/w respectively) (Sriroth *et al.*, 2012). Some sugars, such as sucrose, $C_6H_{12}O_6$, and fructose are also present in the roots at 4-8 % w/w (Sriroth *et al.*, 2012). Other than starch, the roots also contain cellulosic fiber (Kajiwara & Maeda, 1983). Hemicellulose and pectin materials are polysaccharides, as shown by the fact that the hydrolyzed cell wall materials contain monosaccharides such as rhamnose, fucose, arabinose, xylose, mannose, galactose, and $C_6H_{12}O_6$ (Kajiwara & Maeda, 1983).

2.10 Economic importance of cassava and its nutritional value

2.10.1 The economic and nutritional worth of cassava roots

Cassava roots have become a crucial component considering the manufacture of bioethanol and other bio-based chemicals (El-Sharkawy, 2004; Li *et al.*, 2017). In several African countries' humid and sub-humid tropics, cassava is becoming a popular staple food (Bayata, 2019). Cassava roots contain nutrients such as protein and vitamins (Bayata, 2019). Cassava protein has a small concentration of several essential amino acids (10.0–20.0 g/kg) (Diallo, 2013; Li *et al.*, 2017). Cassava is rich in niacin, thiamin, riboflavin, calcium and vitamin C (Diallo, 2013; Li *et al.*, 2017). The roots of cassava are also high in calories and contain varying amounts of other nutrients (Li *et al.*, 2017). However, the cultivar, location, age of the plant, environmental factors and processing techniques all have an impact on the nutritional makeup of cassava (Tewe, 2004; Li *et al.*, 2017). Before use for nutrition, careful cooking and overnight soaking can detoxify the anti-nutrients (Prathibha, 1995; Bayata, 2019). Cassava is bio-fortified to improve its protein, mineral and vitamin content while lowering its anti-nutrient and carcinogenic levels (Bayata, 2019). By adding, other crops with high protein contents to cassava's powder increases protein level (Prathibha, 1995; Bayata, 2019).

In Tanzania, 84% of all production is used to make food for people (Bennett *et al.*, 2019). The remainder is used for export, starch production and animal feed (Kapinga *et al.*, 2005). There are several uses for cassava (Edhirej *et al.*, 2015). It is employed in the creation of a variety of foods, bio-fibers, bio-composites and biopolymers (Edhirej *et al.*, 2015). Cassava is used to manufacture a wide range of products, despite being widely employed as a food industry raw material (Akinpelu *et al.*, 2011). The newly peeled tubers are eaten like vegetables after being boiled or roasted (Akinpelu

et al., 2011). In Nigeria, this is referred to as "Fufu" and the tubers are regularly used for soups and stews after being boiled and crushed into a paste. Additionally, it can be eaten after frying or after being ground into a paste, which is known as "our" in West Africa, as sundried chips (Akinpelu *et al.*, 2011). In addition to being used to make adhesives, cassava flour is used to make bread, cookies, candy, pasta and couscous-like goods (Nginya, 2018).

2.10.2 Economic importance and nutritional value of leaves

The leaves of cassava can produce high levels of crude protein based on the cultivar and environmental factors (Li *et al.*, 2017). Cassava has low contents of manganese, zinc, iron and sodium (Awoyinka, 1995; Li *et al.*, 2017). In sub-Saharan African nations, cassava leaves are valued as a vegetable (Achidi *et al.*, 2005; Li *et al.*, 2017). Cassava leaves could be a significant source of healthy food for a large majority of people after effective and affordable detoxification (Li *et al.*, 2017). Cassava leaves for instance, have been prepared as green vegetables, included in a sauce and served with starchy foods cassava fufu (Latif & Müller, 2015; Li *et al.*, 2017).

2.10.3 Economic importance and nutritional value of the stem

Although cassava biomass stems can make up half of the root mass, their significance in the production of starch and energy has far gone unnoticed (Howeler, 2012; Zhu *et al.*, 2013). The stems have a long past of being unusable, even for feed, except for 10–20 % that are used for propagation (Zhu *et al.*, 2013). This is due to their particularly woody nature (Wei *et al.*, 2018). The stems are utilized as cooking fuel in some countries (Howeler, 2012; Zhu *et al.*, 2013). Therefore, it is extremely important and of the utmost importance to conduct research on making the best use of currently available effluent biomass in energy production, for example, crop residues (Perlack *et al.*, 2005; Gao *et al.*, 2011; Zhu *et al.*, 2013).

However, cyanic cassava does not have many applications (Cardoso *et al.* 2005; Bayitse *et al.* 2015). The cassava plant produces linamarin and a trace of lotaustralin (Azmi, 2019). The two cyanogenic glucosides that protect the plant against predators (Nyirenda, 2020). The plant's entire surface is covered in cyanogen with significant concentrations in the cortex (skin layer) of the roots and leaves (Cardoso *et al.* 2005; Bayitse *et al.* 2015). Livestock cannot eat the peels therefore the peels are discarded.

This creates a pollution issue for the environment (Adedeji, 2019). Cassava peels are ideal for bioethanol production since it does not pose any food insecurity.

2.11 Cassava peel lignocellulose biomass composition

Lignocellulosic Biomass (LB) is a profuse and sustainable plant source comprising largely of an aromatic polymer (lignin) and polysaccharides (cellulose and hemicelluloses) (Broda *et al.*, 2022). LB offers a significant promise as a fossil-resource-free alternative for producing second-generation biofuels, BioSource chemicals and minerals without jeopardizing worldwide food sovereignty (Zoghلامي & Paës, 2019). Cellulose and hemicellulose being the most abundant, accounting for about 70% of the total biomass (Ufodike *et al.*, 2020; Vasić *et al.*, 2021). Figure 2.1 shows the cassava lignocellulose composition. Because of the covalent with also hydrogen bonds that bind cellulose and hemicellulose to component lignin, the lignin's structure is strengthened and more resistant to treatments (Ufodike *et al.*, 2020; Vasić *et al.*, 2021). Cassava peels are highly rich in starch (Aruwajoye *et al.*, 2017).

To make lignocellulose biomass more accessible to enzyme and microbial breakdown, pre-treatment is necessary to alter its physical and chemical structure (Aruwajoye *et al.*, 2017).

Plant cell walls include cellulose, a naturally occurring nanofiber structure that holds the cells together (Aro, 2015). It must first be broken down into sugar for bioethanol production (Aro, 2015). Expensive enzymes are required for the hydrolysis of sugar during the biochemical processing of cellulosic biomass. It is necessary to break down these densely packed nanofibers into soluble sugars while utilizing fewer enzymes (Aro, 2015). Utilizing specialized microorganisms that can transform carbohydrates into ethanol without the use of pricey enzymes can also significantly improve the economics of cellulosic biofuels (Aro, 2015).

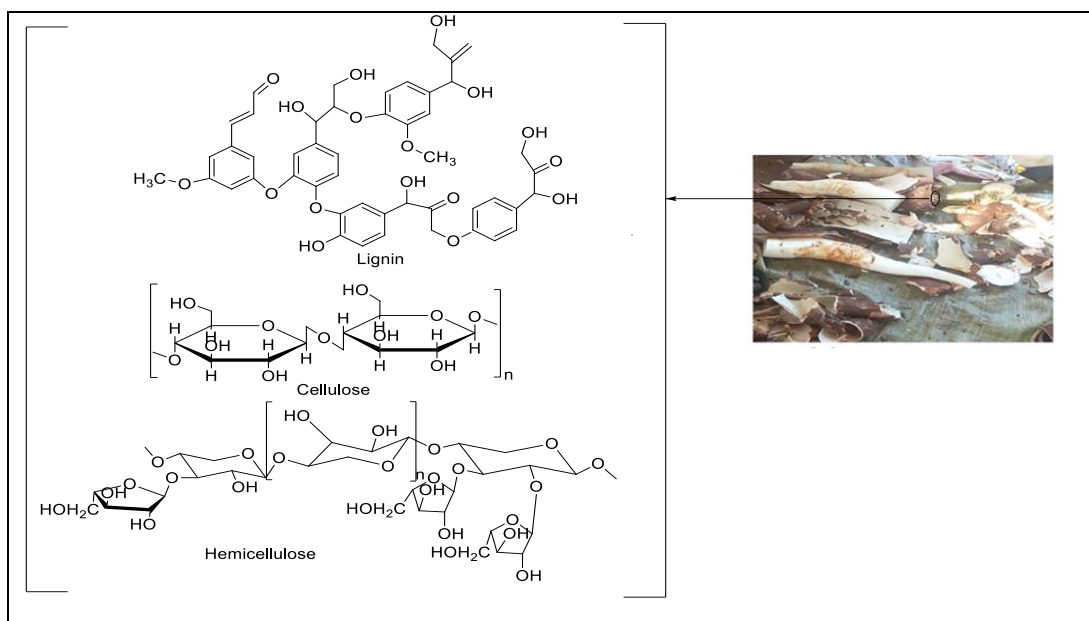


Figure 2.1 Cassava lignocellulose composition

2.12 Pre-treatment methods for lignocellulose biomass

Based on the numerous forces or resources used in the pre-treatment, the procedures are categorized as physical, chemical, biological, physio-chemical, or a combination of these (Gallego-García *et al.*, 2023). A type of hydrothermal pre-treatment named liquid hot water pre-treatment (LHW) does not need a catalyst, chemical addition, rapid decompression, or any other further procedures (Awoyale & Lokhat, 2021). LHW pre-treatment uses temperatures and pressures that are between 170 °C and 230 °C and greater than 5 Mpa respectively (Awoyale & Lokhat, 2021). By removing the hemicellulose from lignocellulose materials, the LHW pre-treatment exposes the cellulose (Awoyale & Lokhat, 2021). This makes the cellulose available for fermentation (Awoyale & Lokhat, 2021). After pre-treatment, the slurry is sieved to separate a liquid fraction with hemicellulose sugars from some solid portion rich in cellulose (Awoyale & Lokhat, 2021).

2.12.1 Physical techniques milling

The size of the pores and the surface area of lignocellulose biomass can be increased, whereas cellulose crystallinity and degree of polymerization can be decreased (Aftab *et al.*, 2019). Milling is employed to increase the accessibility of lignocelluloses to cellulases (Wu *et al.*, 2022). Enzymes called cellulases help break down cellulose (Chatterjee *et al.*, 2015). However, for the catalysis to occur and for the optimum

outcomes; the substrate availability must be improved (Taherzadeh 2008; Aftab *et al.*, 2019). There are several different types of milling processes (Taherzadeh 2008; Aftab *et al.*, 2019). Ball milling, colloid milling, hammer milling and two-roll milling are used for wet and dry materials, ensuring efficient grinding (Taherzadeh 2008; Aftab *et al.*, 2019).

2.12.2 Chemical pre-treatment

Both acid and alkali pretreatments are vital steps in the process of biomass conversion (Loow *et al.*, 2016). One of the most efficient ways to solubilize the hemicelluloses and improve the cellulose's accessibility is with acids (Jia *et al.*, 2019). Hemicellulose hydrolysis and lignin condensation and precipitation are the two primary processes that occur during acid treatment (Jia *et al.*, 2019). Partial hemicelluloses may hydrolyze during the acid pre-treatment, which increases the availability of cellulose to enzymes (Amiri & Karimi, 2018). Recently, methods for the hydrolysis of cellulosic materials have been used that use very little acid with high temperature, which has increased $C_6H_{12}O_6$ yields (Jia *et al.*, 2019). The cassava peels' polysaccharides are broken down by sulphuric acid and citric acid into constituent sugars, or monosaccharides, which can subsequently be fermented.

On the other hand, pre-treatment with alkaline involves treating biomass at standard temperature and pressure with bases like ammonium hydroxide, potassium hydroxide, calcium hydroxide and sodium hydroxide (Harmsen *et al.*, 2010). However due to the alkaline environment, the solubilization of hemicellulose is limited, resulting in less degradation sugar (Javanmard *et al.*, 2024). The technique's primary benefit is successful lignin removal from biomass (Harmsen *et al.*, 2010). This approach increases the accessibility of the enzyme that breaks down hemicellulose by removing $RCOCH_3$ and uronic acid groups from the hemicelluloses (Harmsen *et al.*, 2010). Alkali pre-treatment procedures use lower temperatures and pressures than other pre-treatment technologies (Wu *et al.*, 2011).

At ambient temperatures, alkali pre-treatment is possible, but the pre-treatment process takes hours or days (Aftab *et al.*, 2019). Base pre-treatment is less likely to degrade starch than acid processes (Kumar *et al.*, 2009). Lignocellulose materials swell when exposed to diluted NaOH, which increases internal surface area, reduces polymerization, reduces crystallinity, breaks down the structural bonds between lignin

and carbohydrates and lignin itself (Kumar *et al.*, 2009; Behera *et al.*, 2014). According to reports, the digestibility of hardwood treated with NaOH rose from 14 % to 55 % by reducing lignin content from 24–55 % to 20 % (Kumar *et al.*, 2009; Behera *et al.*, 2014).

2.12.3 Physicochemical treatment

Steam-explosion breaks down the composition of the lignocellulose material using both chemical and physical methods (Ballesteros *et al.*, 2002; Brodeur *et al.*, 2011). After briefly exposing the material to high pressures and temperatures, the hydrothermal pre-treatment process rapidly depressurizes the composition, disrupting the fibril anatomy (Pérez-Limiñana *et al.*, 2022). When the fibrils are broken, the cellulose is more easily accessible to hydrolysis by the enzymes (Ballesteros *et al.*, 2002; Brodeur *et al.*, 2011). Figure 2.2 shows the structure of cellulose

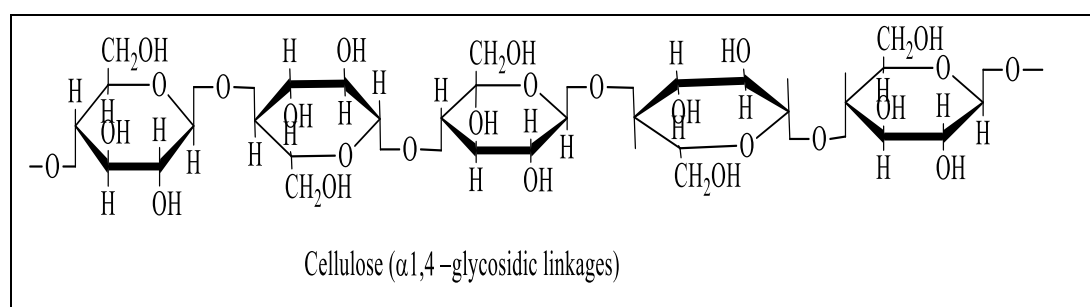


Figure 2.2 Structure of cellulose

2.12.4 Biological pre-treatment

Despite substantial lignin breakdown, biological treatment employs different types of rot fungi (Millati *et al.*, 2011). In biological pre-treatment procedures, brown, white and soft-rot fungi hydrolyze lignin and hemicellulose (Kumar *et al.*, 2009). White and soft rots break down both cellulose and lignin, but brown rots break down only cellulose (Kumar *et al.*, 2009). The most efficient biological preparation for lignocellulosic material is a white-rot fungus (Kumar *et al.*, 2009). Endoglucanase is an enzyme that breaks down the β 1, 4-glucosidic linkages that hold cellulose chains together, resulting in shorter cello-dextrins (Zabed *et al.*, 2016; Basso *et al.*, 2015).

Exoglucanase extracts $C_6H_{12}O_6$ or cellobiose from cellulose and cello-dextrin chains and β -glucosidases hydrolyze cellobiose into $C_6H_{12}O_6$ (Devendran *et al.*, 2016). Exoglucanases and endoglucanases function differently (Zabed *et al.*, 2016; Basso *et*

al., 2015). Exoglucanases, as opposed to endoglucanases, are enzymes that break down lengthy chains at the extremities of intramolecular cleavages to liberate $C_6H_{12}O_6$ and other sugars from the cellulose chain (Zabed *et al.*, 2016; Basso *et al.*, 2015). Liquefaction and saccharification are two steps in the two-stage process of enzyme-mediated starch hydrolysis (Sriroth *et al.*, 2012).

Typically, liquefying enzymes can begin to function at temperatures above 85 °C, which allows them to aid in reducing the viscosity of starch paste while it is cooked (Sriroth *et al.*, 2012). The dextrins, or by-products of the liquefaction process, are hydrolyzed to $C_6H_{12}O_6$ by breaking 1, 4, and 1, 6 glycosidic linkages using glucoamylase enzyme (Sriroth *et al.*, 2012). The utilization of pearl millet enzymes presents a promising approach for the deconstruction of the complex structure of cassava peels subsequently converting it into simple sugars that is fermentable. Figure 2.3 shows the pre-treatment methods for lignocellulose biomass

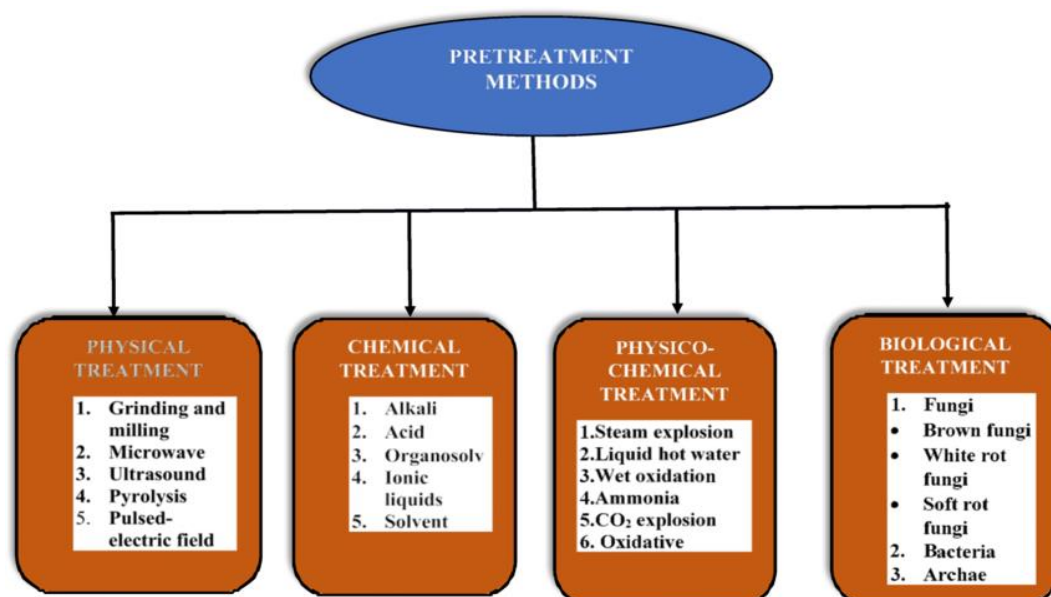


Figure 2.3 Pre-treatment methods for lignocellulose biomass

Source (Das *et al.*, 2021)

Yeast belong to the phylum Ascomycota and very few to Basidiomycota (Wang *et al.*, 2010). Yeasts can be found on plant surfaces and in soils all over the world (Britannica, 2023). Ascomycete yeasts come in hundreds of varieties, many of which are significant economically in the making of bread and many other applications (Britannica, 2023). Only a few strains of *Saccharomyces cerevisiae* are frequently

used (Britannica, 2023). Yeasts require less nutrition than other microbes like lactic acid bacteria (Maicas, 2020).

However, the availability of essential elements like $C_6H_{12}O_6$, amino acids, vitamins, minerals and oxygen (O_2) support their growth (Maicas, 2020). Yeasts have a highly divergent morphology the most common shapes are round, ellipsoidal and oval (Maicas, 2020). Yeasts are unique in that they can ferment a variety of carbohydrates, the majority of which are present in processed grains and ripe fruit (Maicas, 2020). These sugars include ($C_6H_{12}O_6$), maltose ($C_{12}H_{22}O_{11}$) and maltotriose (Maicas, 2020). Yeasts may thrive in pH settings as low as 3.5 (Maicas, 2020). For the sake of technological convenience, yeast is categorized into *Saccharomyces* and non-*saccharomyces* (Maicas, 2020).

One of the most noteworthy characteristics of *Saccharomyces cerevisiae* quickly converts carbohydrates to ethanol and CO_2 under both anaerobic and aerobic environments (Dashko *et al.*, 2014). Acetaldehyde (C_2H_4O) is converted into ethanol by exclusively fermentative development in the absence of O_2 as the ultimate electron acceptor (Deken, 1966; Hagman & Piškur, 2015). *Saccharomyces cerevisiae* can respire aerobically with O_2 serving as the last electron acceptor, although it will continue to ferment alcohol until all of the $C_6H_{12}O_6$ in the medium has been consumed and this is referred as the Crabtree effect (Deken, 1966; Hagman & Piškur, 2015). The rise of ethanol production by microorganisms depends on the use of the best microbial variety (Akponah *et al.*, 2013). The following qualities make microorganisms appropriate for fermentation; tolerance to high substrate concentrations, high ethanol yield and sugar absorption, tolerance to high ethanol level, tolerance to little or no oxygen and tolerance to low pH (Akponah *et al.*, 2013).

2.13 Fermentation

The fermentation process of simple sugars by microorganisms is frequently employed instead of chemical synthesis to create ethanol from renewable sugar-containing biomass (Sriroth *et al.*, 2012). Starch and cellulose feedstock, on the other hand, are polymers of $C_6H_{12}O_6$ and cannot be used by yeast directly (Sriroth *et al.*, 2012). Before yeast fermentation, they must be converted or depolymerized to $C_6H_{12}O_6$ (Sriroth *et al.*, 2012). Starch can be depolymerized or hydrolyzed using an acid, an enzyme, or a

combination of the two, which is much easier and less expensive than doing the same for cellulosic materials (Sriroth *et al.*, 2012). Starch is composed solely of $C_6H_{12}O_6$ monomers connected through glycosidic linkages (Sriroth *et al.*, 2012). Amylopectin and amylose are two types of glucan that are also present in the starch (Sriroth *et al.*, 2012). $C_6H_{12}O_6$ polymer branches with mostly α -1, 4 glycosidic linkage in the linear section and a few α -1, 6 in the branch structure (Joseph *et al.*, 2024).

The majority of starches have an amylose content of 20–30 %, with amylopectin making up the remainder (Cornejo-Ramírez *et al.*, 2018). Some starches contain extremely high amylose levels of up to 50 % to 70 %, such as high amylose maize starches (Obadi *et al.*, 2023). Glucose polymers become more soluble and extremely vulnerable to enzyme attacks when the granular structure of starch is broken down during cooking with too much H_2O (Sriroth *et al.*, 2012). The starch slurry also intensifies at the same time (Sriroth *et al.*, 2012). Gelatinization is the process, and gelatinization temperatures are the ranges of temperature where the characteristics of starch are modified (Sriroth *et al.*, 2012). The gelatinization temperatures of various starches vary, which suggests that they cook more or less easily (Sriroth *et al.*, 2012). Compared to cereal starches, cassava starch requires less heat to cook the recommended pasting temperatures for cassava is 60 to 65 (Sriroth *et al.*, 2012). Figure 2.4 shows the structure of amylose and amylopectin.

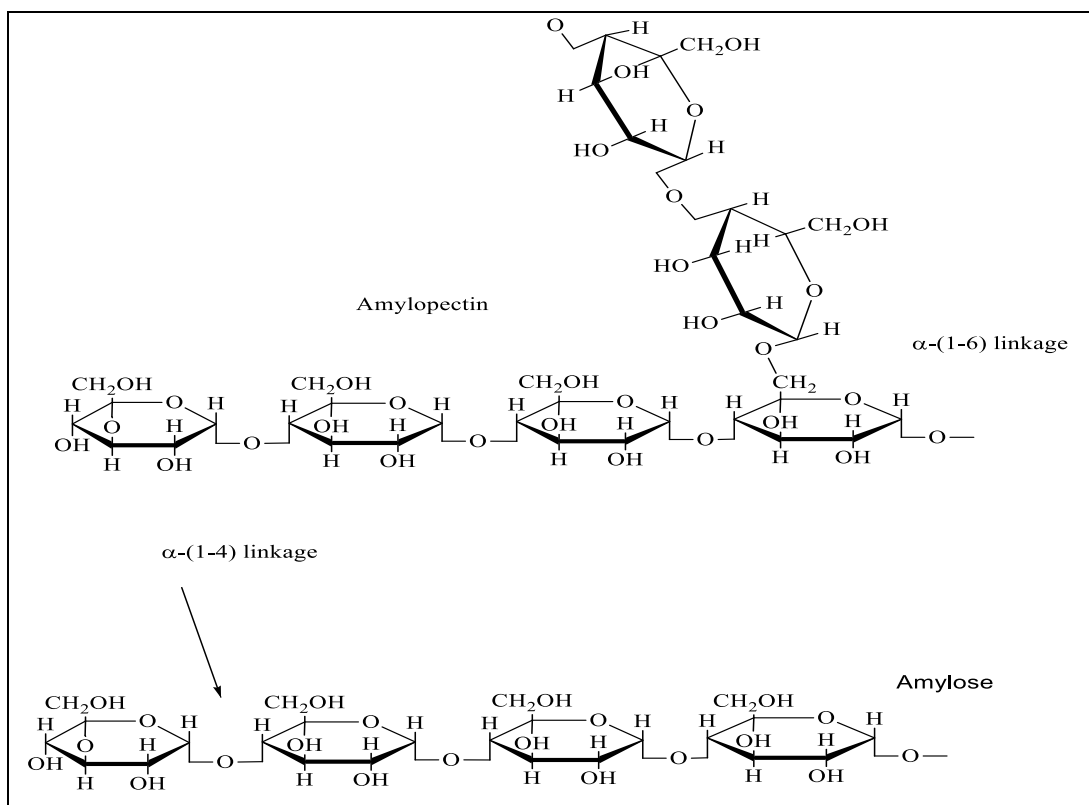


Figure 2.4 The structure of amylose and amylopectin

Although fermentation is a natural process, microorganisms are still needed for the conversion of $C_6H_{12}O_6$ into ethanol or lactic acid or many other products (Vasić *et al.*, 2021). Numerous industrial yeasts, including, *Saccharomyces cerevisiae* are employed for these purposes (Vasić *et al.*, 2021). *Saccharomyces cerevisiae* is the primary fermentative strain employed in industries producing sugar-based biofuels (Vasić *et al.*, 2021). Pre-treatment transforms the cellulosic material or slurry into fermentable sugars, making it suitable for either enzymatic or acidic hydrolysis. *Saccharomyces cerevisiae* is used in fermentation to convert $C_6H_{12}O_6$ into ethanol under anaerobic conditions and at a specific temperature (Vasić *et al.*, 2021). Figure 2.5 show yeast fermentation of glucose into ethanol.

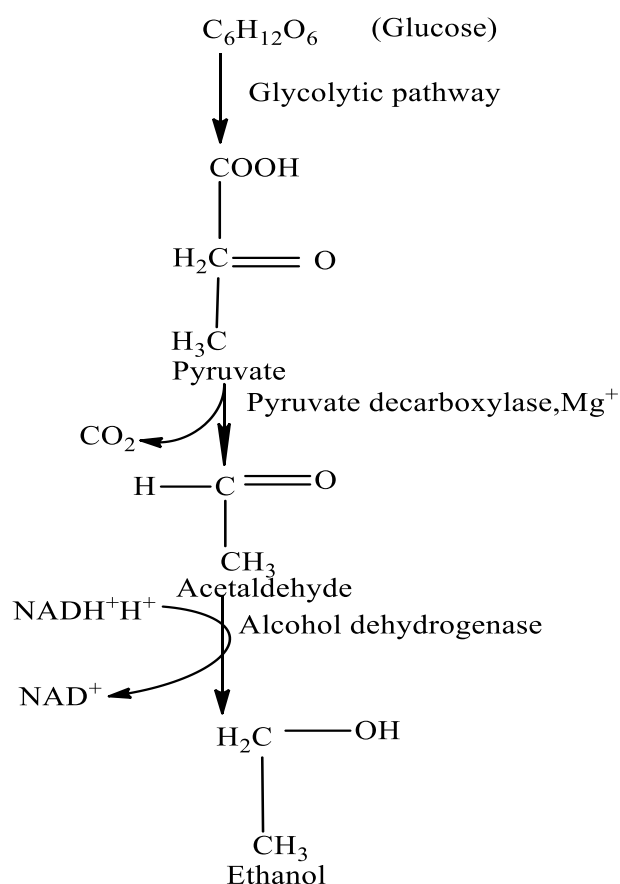
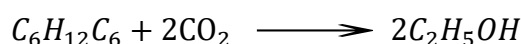


Figure 2.5 Yeast fermentation of glucose into ethanol

Source (Biradar *et al.*, 2016; Osei, 2021)

The yeast cells directly metabolise glucose through the glycolysis pathway in order to produce energy for biosynthesis (Talebnia, 2008; Osei, 2021). For every mole of glucose consumed, the fermentation process' total reactions provide two moles of ethanol and carbon dioxide (Talebnia, 2008; Osei, 2021). Such yeast fermentation produces CO_2 as a byproduct (Vasić *et al.*, 2021). The fermentation procedure involves high production of bioethanol (Vasić *et al.*, 2021).

The summary of the fermentation process is shown using equation 2.1



2.1

2.14 Factors affecting bioethanol yield

The majority of yeasts are capable of glycolysis of a variety of hexose carbohydrates into ethanol (Tse *et al.*, 2021). However, due to its durability and tolerances,

Saccharomyces cerevisiae is a prevalent yeast strain for alcoholic fermentation being a facultative anaerobe that can survive in situations that are both aerobic and anaerobic while being supplied with $C_6H_{12}O_6$ (Tse *et al.*, 2021).

Over other yeasts, *Saccharomyces cerevisiae* offers several benefits (Tse *et al.*, 2021). *Saccharomyces cerevisiae* is also tolerant to high ethanol concentrations (Tse *et al.*, 2021). *Saccharomyces cerevisiae* will create acetaldehyde in anaerobic environments, which is then further converted to ethanol (Tse *et al.*, 2021). Temperature, sugar content, pH level, fermentation time, agitation rate, and inoculum size all have an impact on the synthesis of bioethanol (Zabed *et al.*, 2014). Yeast cells experience a range of stressors during inoculation and fermentation that may have an impact on bioethanol output (Tse *et al.*, 2021).

These challenges can be biological (cellular aging, microbial competition), chemical (ethanol toxicity and metabolites, pH), or physical (temperature shock, osmotic pressure) (Tse *et al.*, 2021). Stress can cause an increase in mutations, microbial contamination, changed yeast flocculation, increased glycerol production and decreased ethanol production, among other things (Tse *et al.*, 2021). Unfavorable chemical formations such as aromatic molecules in fermented beverages (Tse *et al.*, 2021). The pH of the broth has an impact on the generation of ethanol because it affects byproduct formation, fermentation pace, yeast growth and bacterial contamination (Zabed, *et al.*, 2014).

The amount of H^+ in the fermentation broth influences nutrient penetration into the cells (Zabed, *et al.*, 2014). Furthermore, a pH between 2.75 and 4.25 is an important factor in the survival and development of yeasts (Fleet & Heard, 1993). *Saccharomyces cerevisiae* performs best when the fermentation process produces ethanol at a pH between 4.0 and 5.0 (Staniszewski *et al.*, 2007). When the pH is less than 4.0 the incubation duration must be extended (Staniszewski *et al.*, 2007). When the pH exceeds 5.0 the amount of ethanol decreases considerably (Staniszewski *et al.*, 2007).

The absorption of nutrients from the fermentation broth into the cells as well as the removal of ethanol from the cell to the fermentation broth are both regulated by the shaking rate (Zabed *et al.*, 2014). The amount of ethanol produced increases as the

agitation rate increases (Zabed *et al.*, 2014). Agitation boosts sugar intake and lessens ethanol ability to suppress cell growth (Zabed *et al.*, 2014). 150–200 rpm is the typical agitation rate for yeast cell fermentation (Zabed *et al.*, 2014). Excessive agitation is not ideal for efficient ethanol generation (Zabed *et al.*, 2014). One popular crucial process in industrial ethanol fermentation is mechanical stirring, which has several advantages including lower temperatures, prevention of "fermentation tank deposits", shorter fermentation times, increased ethanol yield, increased fermentation efficiency, height uniformity of the output, improved centrifuge performance and more representative product (De Vasconcelos, 2015). When properly carried out, mechanical stirring is the best method for hygienizing the fermentation environment, whether batch feeding is being used or continuous processes using free cells (De Vasconcelos, 2015). Agitation encourages uniformity in the fermentation environment, maintains yeast suspension and permits effective contact between the microbe and its substrate (De Vasconcelos, 2015).

Another factor is the temperature which has an immediate impact on the microorganisms' rate of growth (Charoenchai *et al.*, 1998). Fermenting organisms are stressed by temperatures above that are not favorable for cell growth (Cot *et al.*, 2007). The ideal temperature range for fermentation is 20 °C to 35 °C. *Saccharomyces cerevisiae* immobilized cells have a little higher optimal temperature than free cells (Liu & Shen, 2008; Topaloğlu *et al.*, 2023). The tertiary structure of several enzymes controls microbial activity and the fermentation process is temperature sensitive, which can render them inactive (Phisalaphong *et al.*, 2006).

The duration of fermentation affects the growth and reproduction of microorganisms (Zabed *et al.*, 2014). Shorter fermentation times result in ineffective fermentation (Zabed *et al.*, 2014). The high concentration of ethanol in the fermented broth causes extended fermentation times to harm microbial development, particularly when done in batch mode (Zabed *et al.*, 2014). Longer fermentation times with lesser ethanol yields can be used to complete fermentation at lower temperatures (Zabed *et al.*, 2014).

The rate of fermentation increases when sugar concentration increases up to a particular point (Fatima *et al.*, 2024). Too much sugar keeps the fermentation rate constant, this is due to the concentration of sugar usage being greater than what the microbial cells can effectively absorb (Zabed *et al.*, 2014).

The highest rate of ethanol generation is often attained when employing sugars with a concentration of 150 g/L (Zabed *et al.*, 2014). A crucial element in the creation of ethanol has also been thought to be the initial sugar content (Zabed *et al.*, 2014). By using a greater initial sugar concentration, high ethanol productivity and yield can be achieved in batch fermentation (Zabed *et al.*, 2014). However, a longer fermentation period and a greater recovery cost are required (Zabed *et al.*, 2014). The maximum ethanol production (4.76 g/L/h) and concentration (128.5 g/L) were reported by (Yan *et al.*, 2015), most likely as a result of favorable conditions for the yeast to create bioethanol (Yan *et al.*, 2015). Because water hyacinth has a low sugar concentration, which restricts the substrate for bioethanol synthesis, it produced the least amount of ethanol (9.5 g/L) and the least amount of ethanol per unit time (0.31 g/L/h) because of their diminished viability, stressed yeast cells are poor competitors for substrate and micronutrients (Hawkes *et al.*, 2019).

The losses also result from the creation of bacterial byproducts like organic acids, proteinaceous materials, cyclic peptides, and hydroxylated fatty acids (Brexó & Sant'Ana, 2017). A key contributor to the loss of bioethanol production output is the battle between yeasts and microbial contaminants for space in the fermentation environment (Brexó & Sant'Ana, 2017). Significant economic losses may result when the yield of bioethanol production is decreased by as much as 30% (Brexó & Sant'Ana, 2017).

2.15 Methods of analysis

2.15.1 Pensky martens flash point apparatus

Flashpoint is a key feature that is used to determine the fire hazard of combustible liquids (Le *et al.*, 2015). Flash point is defined as the lowest temperature at which a liquid produces enough vapor to ignite in air at atmospheric pressure when an ignition source is applied under specified test conditions (Le *et al.*, 2015). The closed cup and the open cup are the two fundamental techniques for figuring out a volatile liquid's flash point (Kelechava, 2023). The Pensky-Martens test method is a closed-cup test, which means it seeks to mimic a liquid spill in a closed setting as stated in ASTM D93-20 (Kelechava, 2023). Following Pensky-Martens test protocols, the range of biodiesel's flash points is 60 °C to 190 °C, and that of petroleum products is 40 °C to

370 °C can both be determined using manual or automated closed-cup apparatuses (Kelechava, 2023).

2.15.2 Digital density meter

Digital density meters use ISO 12185 (Draffin, 2008). The digital method entails passing a sample via a magnetically stimulated glass "U" tube. The tube 'resonates' like a violin string when it vibrates naturally at a particular frequency (Draffin, 2008). When a liquid is inserted into the tube, the resonance frequency changes. Fuel oil including H₂O may affect both test methods; as a result, if the sample contains a significant amount of H₂O, it is removed before the density test is carried out (Draffin, 2008). Figure 2.6 shows a digital density meter.



Figure 2.6 A photo of a digital density meter

The density of the fuel is divided by the density of H₂O when both numbers are measured at 60 degrees Fahrenheit, and this calculation is used to get the specific gravity of fuel oil (Draffin, 2008). The outcome is shown as SG at 60/60°F and lacks units (Draffin, 2008).

2.15.3 Capillary flow viscometers

The resistance of a fluid to progressive deformation brought on by tensile or shear stress is measured by its viscosity (Boda *et al.*, 2015). When it comes to liquids, it relates to the figurative notion of thickness. A characteristic that happens when adjacent particles in a fluid moving at various speeds collide (Boda *et al.*, 2015). The particles that make up the fluid often travel more swiftly when it is driven down a tube due to the fact that the fluid increases in speed near the axis of the tube (Boda *et al.*, 2015). The fluid moves more slowly near its wall because of the friction between the particle layers, therefore some stress (like pressure variation between the tube's two ends) is necessary to keep the fluid moving (Boda *et al.*, 2015). A fluid in a glass, when inverted, is subjected to gravitational forces, some fluids will flow easily out of the glass, some with difficulty and some not at all (Lewis, 1996). Viscosity is also a measure of the rate of flow (Lewis, 1996). Normal configurations for capillary flow viscometers resemble U-tubes (Lewis, 1996). Figure 2.7 displays a few common capillary flow viscometer configurations. Figure 2.7 (a) shows the Ostwald viscometer, also known as the U-tube viscometer, which is the most basic design. Using a capillary tube, the test fluid is precisely pumped up the opposite limb and through mark A until it reaches mark B in the viscometer. Under the effect of the produced pressure head, the fluid then passes through the capillary tube (Lewis, 1996). The time it takes for the fluid to pass through the capillary tube and fall from mark B to C is noted (Lewis, 1996). To calculate the fluid's kinematic viscosity, the time is multiplied by a constant for the instrument (Lewis, 1996).

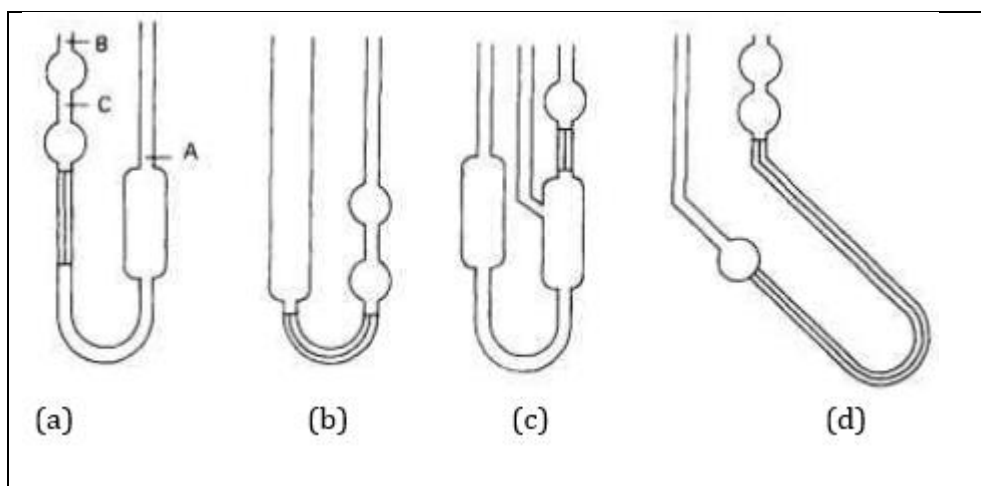


Figure 2.7 Types of capillary flow viscometer: (a) an Ostwald viscometer; (b) a reverse-flow viscometer; (c) a suspended-level viscometer; (d) a Cannon-Fenske Viscometer

Source (Lewis 1996)

2.15.4 Bomb calorimeter

The most crucial way to characterize the energy content of fuels as well as the stability of materials to measure the enthalpy shift that happens when a material burns in a reactive atmosphere, typically O_2 under pressure during complete combustion of organic molecules to CO_2 (g) and H_2O (l) (Haines, 2002). The energy released per kilogram of fuel burned is known as the calorific value (CV) of a fuel (Gujarati, 2023). A calorimeter consists of a thick-walled cylindrical jar with two electrodes supported by a lid (Gujarati, 2023). The electrodes are in contact with the fuse and a known weight of the fuel sample (Gujarati, 2023). The oxygen inlet valve is located on the lid and is used to supply high-pressure O_2 gas (between 25 and 30 atm) (Gujarati, 2023). The copper calorimeter with a known weight of H_2O holds the entire lid with the fuel sample (Gujarati, 2023). To ensure that the H_2O is heated evenly, a mechanical stirrer is provided (Gujarati, 2023).

Bomb calorimeter thermal dynamics uses lumped heat transfer analysis in a stirred water bath with a static air gap and insulated wall an isoperibol, or an adiabatic jacket (Abdul Jameel *et al.*, 2022). In an adiabatic flame calorimeter, the fuel is totally burned in an oxygen-rich atmosphere at a pressure of one atmosphere, the heat is then transported into a known mass of liquid, often H_2O , where it is then absorbed and the

temperature rise (T) is measured (Haines, 2002). Enthalpy change (H) per mole of fuel is given by equation 2.2 below, assuming that heat losses are kept to a minimum (Haines, 2002). Where ΔH_c is the enthalpy of combustion in kJ, m is the mass of water in kg, C_p is the specific heat capacity of water in kJ/kg °C, and ΔT is the temperature change of water in °C (Abdul Jameel *et al.*, 2022). Figure 2.8 shows a cross section of a bomb calorimeter

$$\Delta H_c = m \times C_p \times \Delta T$$

2.2

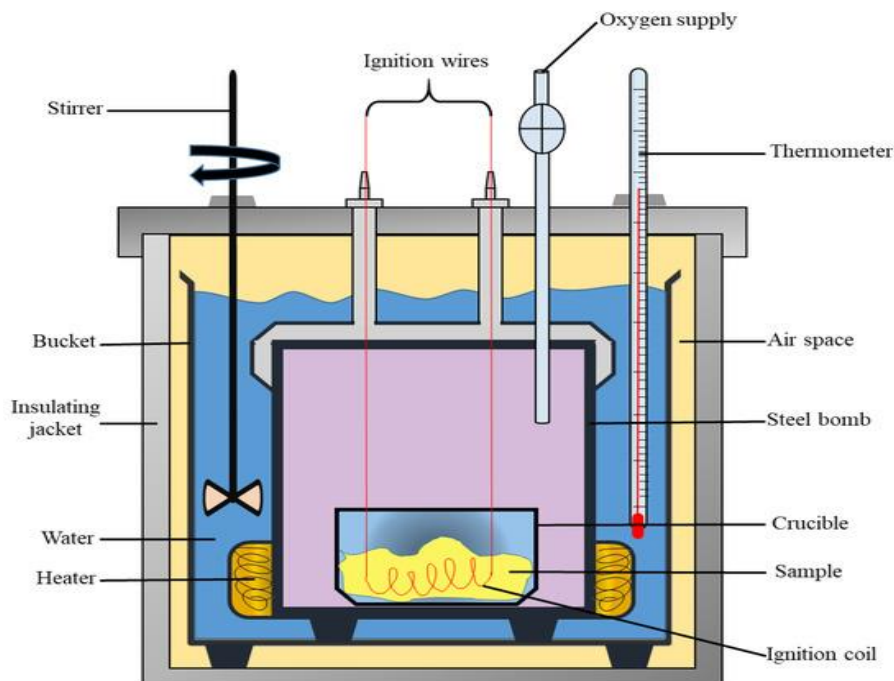


Figure 2.8 Cross section of a bomb calorimeter

Source (Abdul Jameel *et al.*, 2022)

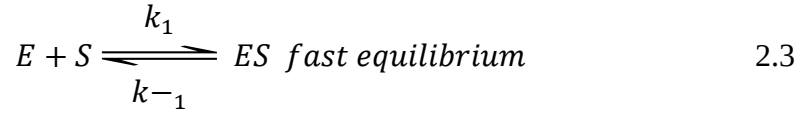
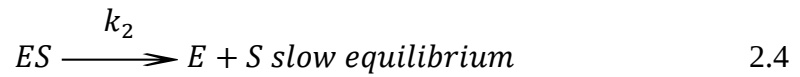
2.16 Taguchi experimental design

Taguchi experimental design is a large-scale method for studying industrial process experiments (Davis & John, 2018). The method uses a statistical approach to mathematical models and experimental trials for prediction (Davis & John, 2018). Taguchi method produces high-quality, low-cost DOE products (Davis & John, 2018).

2.17 Machaeli Menten $V_{\max}$ and K_m parameters

Enzyme-catalyzed reactions proceed via the reaction process, according to Michaelis-Menten shown in Equations 2.3 and 2.4

Equilibrium reactions



Rate Law:

$$v = \frac{k_2[E][S]}{K_m + [S]} \quad 2.5$$

Where $[S]$ is substrate concentration, v is rate, k_2 is enzyme rate constant, K_m is Michaelis constant and $[E]$ is enzyme concentration and $v_{\max}$ is the maximum velocity of an enzymatically catalyzed reaction when the enzyme is saturated with its substrate

By application of steady-state approximation, Michaeli-Menten equation reduces to Equation 2.6

$$v = \frac{v_{\max}[S]}{K_m + [S]} \quad 2.6$$

Equation 2.6 serves as the foundation for the current methods for calculating $v_{\max}$ and K_m . Due to the hyperbolic plot of v against $[S]$, finding both constants that fit the equation is challenging (Patiha *et al.*, 2021). The linear form Equation 2.7 is used to calculate $V_{\max}$ and K_m (Ude & Oluke 2021).

$$\frac{1}{v} = \frac{1}{v_{\max}} + \frac{k_m}{v_{\max}} \cdot \frac{1}{[S]} \quad 2.7$$

Linear plot of $\frac{1}{v}$ against $\frac{1}{[S]}$ gives $v_{max} = \frac{1}{intercept}$ and $K_m = \frac{v_{max}}{slop}$

$$v = v_{max} - \frac{V}{[S]} K_m \quad 2.8$$

The linear plot of v_o against $\frac{v}{[S]}$ gives v_{max} as the intercept and $k_m = -slope$ (Patiha *et al.*, 2021).

2.18 Summary

The chapter examined the state of renewable energy, with a focus on bioethanol production. It discussed international conventions on green energy and assessed current renewable sources and their challenges. The chapter also examined the global situation of bioethanol, history of cassava, classification, economic and nutritional importance of cassava. The chapter further explained the composition of cassava peel lignocellulose biomass, pretreatment methods and fermentation procedure, as well as the factors that affect the fermentation process, analytical methodologies and Michaelis-Menten kinetics equation.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

Understanding the enzymatic conversion of biomass to bioethanol is essential for researchers, policymakers and industry professionals seeking to harness the potential of this renewable energy source. In the following sections, we will explore the study area, cassava substrate preparation, fundamental pre-treatment methods (0.5M sulphuric acid, pearl millet extract, and 0.00026M citric acid) for the conversion of the cassava substrate into fermentable sugars, optimization of fermentation conditions, distillation and analysis of the bioethanol.

3.2 Reagents

Yeast (*Saccharomyces cerevisiae*) and citric acid were obtained commercially from Mathai's supermarket, Murang'a. Pearl millet obtained from a local market in Murang'a, Buffer tablets pH 4 and 7, NaOH, and H₂SO₄ of analytical grades were obtained from Kobia Kenya Ltd, Sigma Aldrich supplier in Nairobi.

3.3 Sample collection and preparation

The cassava used in this study was obtained from a farmer in Kodiaga and its geographical coordinates is 0°05'55.7" North and 34°26'14.8" East situated in North Gem, Siaya County, Kenya. Figure 3.1 shows a photo of cassava plantation.



Figure 3.1 A photo of cassava plantation

The cyanic cassava was washed with water to remove soil pollutants, fertilizers, and other foreign substances associated with biomass waste and then peeled off. The peels were sun-dried for one week to remove the moisture and finally milled to obtain cassava peel powder. Figure 3.2 shows a photo on preparation of the cassava peels



Figure 3.2 Preparation of the cassava peels

3.4 Preparation of amylase enzyme source from pearl millet

Enzymes are increasingly utilized in industrial settings to hydrolyze starch rather than more traditional techniques such as acid starch hydrolysis. Therefore, low-cost starch hydrolyzing enzymes are in high demand, Beta-amylase in cereal grains, releases reducing sugar by hydrolyzing starch (Kolawole *et al.*, 2018).

3.4.1 Pearl millet grains malting

Pearl millet grain malting was carried out as described by Kharkrang an Ambasht, (2012). Three hundred grams of the pearl millet was measured using a weighing balance. The grains were rinsed and soaked in distilled water for six hours. The soaked grains were sieved and spread out in a tray and allowed to germinate in an incubator set at 25 °C. The grains were sprinkled with a small amount of distilled water each day up to the fifth day (Macnicol *et al.*, 1993; Kolawole *et al.*, 2018). Figure 3.3 shows pearl millet grain preparation and malting process.



Figure 3.3 Pearl millet grain preparation and malting

3.4.2 Amylase enzyme extraction from malted pearl millet grains

Kharkrang and Ambasht's (2012) method was used with a few modifications to extract enzymes from the germinated grains. The samples were rubbed between the palms to remove any vegetative elements before being suspended in distilled water and decanted. The 300 g sample was weighed and homogenized in an electric blender with 800 mL of distilled water. The sample was centrifuged for 25 minutes at 35000 rpm. The obtained supernatant was used as the enzyme extract solution (Kolawole *et al.*, 2018).

3.5 Enzymatic pre-treatment

Enzymatic pre-treatment was done using the method reported by Yussouff *et al*, 2018 with some modifications. 950 ml of distilled water was combined with 100 g of cassava peel powder. 100g of cassava was used instead of a larger amount because plant-based beta amylase enzymes are not very effective at breaking down starch molecules into sugar. Using a smaller quantity of cassava helps ensure that the Beta-amylase enzymes adequately process the starch. 50 ml of pearl millet amylase enzyme extract was then added to the cassava peels sample. The content was then placed in a water bath maintained at a 40 °C for two hours as shown in figure 3.4. Sterilization was done using an autoclave for 15 minutes at 121 °C. The sample was allowed to cool, then later sieved and filtered through number 1 Whatman filter paper by vacuum filtration. After cassava was hydrolyzed, the quantity of total sugar in the hydrolysate

was established using refractive index. The filtrate was stored in the refrigerator set at 4 °C to be used for bioethanol production.



Figure 3.4 Heating of cassava peels containing pearl millet amylase enzyme extract in a water bath

3.6 Preparation of sulphuric acid 0.5 M concentration

To make 0.5 M sulphuric acid, 500 ml of distilled water was measured with a measuring cylinder and poured into flat bottomed flask. Sulphuric acid (98 % w/w) of 27.2 ml was added to the distilled water. The mixture was gently stirred after being diluted with distilled water up to the 1000 ml mark (Okoro et al., 2022).

3.7 Sulphuric acid pre-treatment

According to Yussouff *et al.*, (2018) methods, acid hydrolysis was carried out by adding 200 g of cassava peels powder to 1000 ml of 0.5 M sulphuric acid in a 1000 ml beaker. The samples were heated for 2 hours in a water bath at 98 °C, sterilized in an autoclave for 15 minutes at 121° C, allowed to cool, and then filtered through No. 1 whatman filter paper using vacuum filtration method. Figure 3.5 shows photos stages involving cassava peels sulphuric acid pre-treatment and the final filtrate. The sample was stored in the refrigerator at 4 °C and later used for bioethanol production.

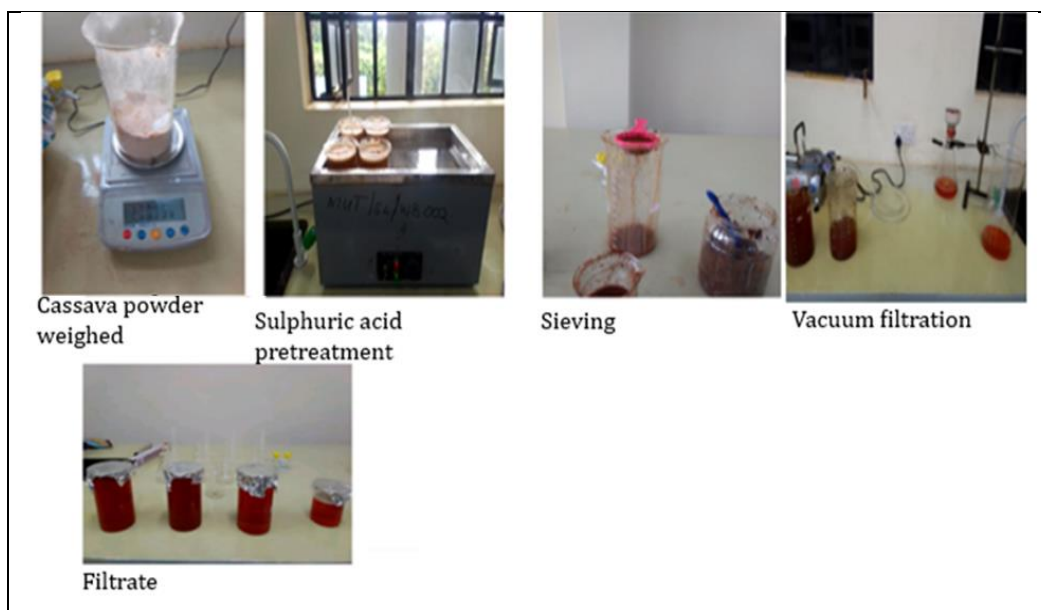


Figure 3.5 Photos showing stages of cassava peels sulphuric acid pre-treatment and the final filtrate

3.8 Citric acid pre-treatment

Fifty grams of citric acid was weighed and dissolved in 1000 ml distilled water. Using Yussouff *et al.*, (2018) methods, acid hydrolysis was carried out by adding 100 g of cassava peels powder to 1000 ml of 0.00026 M citric acid solution in a 1000 ml beaker. 100g of cassava was used because citric acid is a weak acid and therefore does not effectively break the glycosidic bond between the glucose molecules in the starch. The samples were heated for 2 hours in a water bath at 98 °C, sterilized in an autoclave for 15 minutes at 121 °C, allowed to cool and then filtered through No. 1 Whatman filter paper using vacuum filtration method, figure 3.6 shows the obtained filtrate. The sample were stored in the refrigerator and later used for bioethanol production.



Figure 3.6 A photo of the filtrate from citric acid pretreated cassava peels

3.9 Determination of the percentage sugar content

After cassava was hydrolyzed, the quantity of total sugar in the hydrolysate was established using refractive index. To do this, a drop of cassava hydrolysate was applied to the glass slide a graduated hand-held-refractometer model REF107 shown in figure 3.7 and the brix reading was expressed as a percentage. Following AOAC (2000), a hand-held-refractometer model REF107 was used to calculate the brix (%) (Hauwa *et al.*, 2019).



Figure 3.7 A photo of a hand-held- refractometer

3.10 pH test

The pH meter model -930 was calibrated using a buffer before being used to obtain the pH of the filtrates. The readings were taken as indicated by Ademiluyi *et al.*, (2013).

3.11 Optimization of fermentation condition process set-up for the pearl millet amylase enzyme extract, sulphuric acid and citric acid pretreated cassava peels

Before use, the pH was adjusted to 4.0, 4.5, 5.0 and 5.5 for different sample treatments. The pH of the sulphuric acid filtrate sample was adjusted using 1.0 M NaOH (Okoro *et al.*, 2022). The amylase enzyme filtrate was adjusted to pH levels of 4.0, 4.5, 5.0, and 5.5 using sulphuric acid and NaOH, while the pH of the citric acid filtrate was adjusted to the same levels using citric acid and NaOH. The reduced sugar was monitored using a hand-held refractometer model REF107 for each filtrate (Olayemi *et al.*, 2019). Using (Hauwa *et al.*, 2019) method with some modifications, 50 ml of the filtrate samples was poured into a 100 ml conical flask and *Saccharomyces cerevisiae* yeast was added to each flask containing the filtrate samples. In addition, a control group was included in which no yeast was added to 50 ml of sterile water in a separate conical flask, containing cassava peels that had not been pretreated. Balloons were tightly tied to the opening of the conical flask to collect the carbon dioxide produced during fermentation. The flasks were shaken and incubated at 30 °C, 35 °C, 40 °C, and 45 °C temperatures. To ensure a uniform distribution of yeast, the flasks were periodically shaken.

3.12 Optimization of fermentation parameters of pearl millet amylase enzyme extract, sulphuric acid and citric acid pretreated cassava peels by Taguchi method

Optimizing the fermentation process is crucial to maximize the yield and quality of the resulting products. The optimization of fermentation parameters for cassava peels pretreated with pearl millet amylase enzyme extract, sulphuric acid, and citric acid, using the Taguchi method was done with four factors at four levels as shown in Table 3.1.

Table 3.1 Factors and levels of fermentation parameters in Taguchi experimental design for pearl millet amylase enzyme extract, sulphuric acid and citric pretreated cassava peels

Factors	Levels			
	Low		High	
Temperature °C	30 .00	35.00	40.00	45.00
pH	4.00	4.50	5.00	5.50
Time (Min)	30.00	60.00	90.00	120.00
Yeast to amount (g/50ml)	0.03	0.05	0.07	0.09

Various combinations were optimized with some modifications (Ngui *et al.*, 2022). The independent variables selected were temperature °C, pH, time (min), and yeast to substrate ratio (g/l) and the dependent variables was ethanol concentration (g/l). The run was performed by Minitab 17.1 software at four levels and four factors, giving sixteen combinations as shown in Table 3.2 below

Table 3.2 Matrix layout of L₁₆ Taguchi orthogonal array design

Run/experiment number	Temperature (°C)	pH	Catalyst (g/ml)	Time (Min)
1	30	4.0	0.03	30
2	30	4.5	0.05	60
3	30	5.0	0.07	90
4	30	5.5	0.09	120
5	35	4.0	0.05	90
6	35	4.5	0.03	120
7	35	5.0	0.09	30
8	35	5.5	0.07	60
9	40	4.0	0.07	120
10	40	4.5	0.09	90
11	40	5.0	0.03	60
12	40	5.5	0.05	30
13	45	4.0	0.09	60
14	45	4.5	0.07	30
15	45	5.0	0.05	120
16	45	5.5	0.03	90

The response values in terms of ethanol and ratios of Taguchi experimental design in 16 runs were analyzed to determine the main effects of the factor. Figure 3.8 shows a set up for the optimization of pretreated cassava peels. The analysis of variance technique was then applied to determine which factors were statistically significant. The controlling factors were identified, with the magnitude of the effects qualified and the statistically significant effects determined. The optimal conditions were determined by combining the levels of factors that had the highest main effect value. The analysis of variance (ANOVA) for the responses of ethanol production was carried out according to the factors' contribution by the Taguchi method. The factors in the experimental design considered to be statistically significant at 95% confidence limit were used to determine the ratio (F) and the *p*-value ($p < 0.05$).



Figure 3.8 Optimization of pretreated cassava peels.

3.13 Scale up production of bioethanol using the optimized conditions

The scale-up production of bioethanol was conducted based on the established optimal conditions, which included a pH of 4.5, 4.5g of yeast, and a fermentation time of 90 minutes at 40 °C fermentation temperature. To initiate the scaled-up production, the volume of the substrate was increased to 500 ml, and the amount of yeast added to the substrate media was adjusted to 45g. Additionally, the fermentation time was slightly extended to 18 hours to ensure optimal ethanol production. Any fermentation time beyond this duration resulted in a slight decrease in ethanol volume and a cloudy appearance in the distillate, prompting reliance on the 18-hour fermentation period. For the scaled production, 1200 ml of sulphuric pretreated cassava filtrate was poured into a 2000 ml beaker. The sample was then placed on a magnetic stirrer, with the pH meter immersed in the sample, and the filtrate's pH was adjusted to 4.5 using 825 ml of sodium hydroxide base. Subsequently, 500 ml of the adjusted filtrate was poured into four 500 ml Erlenmeyer flasks with 45 g of yeast added to each flask. These flasks were then placed in a water bath set at 40 °C for six hours. The samples were transferred to an incubator set at 40 °C to continue fermenting for the next 12 hours, totaling the fermentation time to 18 hours. Following this, the samples were immediately distilled off for further processing.

3.14 Distillation

The fermented liquid was poured into a round-bottomed flask, placed on a heating mantle connected to a distillation column and then connected to a tap of running water. The distillate was collected in a vial placed to the opposite end of the distillation column at 78 °C. Ethanol boils at 78 °C and this temperature the solvent escaped. This was done for each fermented broth using the method indicated by Oyeleke *et al.*, (2012). The distillation process is as shown in figure 3.9.



Figure 3.9 A photo of distillation of ethanol

3.15 Estimation of bioethanol yield

The yield of bioethanol can be stated in a variety of ways, such as mL/g of substrate, g/g of substrate, mL/L of solution and g/L of solution. In order to improve comparability, the bioethanol yield unit was changed to the same unit. The unit was changed in this study into L bioethanol/ton for cassava peel substrate based on the following assumptions in the equation below (Dinata & Kartawiria, 2021).

$$\frac{g \text{ bioethanol}}{1 \text{ litre final solution}} = \frac{g \text{ substrate}}{1 \text{ litre initial solution}} \quad 3.9$$

$$\text{Bioethanol yield} = \frac{g \text{ bioethanol} \div 0.7893 \frac{g}{ml}}{g \text{ substrate}} \quad 3.10$$

$$= \frac{ml \text{ bioethanol} \div 1000 \frac{ml}{L}}{g \text{ substrate} \div 1000,000 \frac{g}{ton}} \quad 3.11$$

$$= \frac{L \text{ bioethanol}}{ton \text{ substrate}} \quad 3.12$$

3.16 Determination of density, specific gravity and ethanol concentration

The density meter model DMATM 4100 Instrument was calibrated using water as calibration standards. Using a syringe, the sample was slowly injected into the glass oscillator until it was completely filled and care was taken to ensure that no air bubbles was present. The readings were taken once constant temperature of 20.0 ± 0.3 °C was reached.

3.17 Determination of ash content

The A.O.A.C (1998) method was used to establish the amount of ash in the biomass samples 1g of each sample was placed in a pre-weighed crucible, which was then placed into a desiccator to dry after being burned at 550 °C for 30 minutes in a muffle furnace for ashing process. Ash content was calculated using Equation 3.5.

$$\text{Ash content} = \frac{W_2 - W_0}{W_1 - W_0} \times 100 \quad 3.13$$

Where: W_1 = weight of the container + biomass sample before burning

W_0 = Weight of the container

W_2 = weight of the container + biomass sample after burning

3.18 Determination of sulphur content

Using Ismail *et al.*, (2023) methods with some modifications 20 ml of 1:1 of 1.0M hydrochloric acid was added to 20 ml of bioethanol in a beaker, and the mixture was stirred. This step converted the sulfur in the bioethanol to sulphuric acid. The beaker was then placed on a sand bath and heated for 30 minutes, evaporating the excess

water and ethanol and concentrating the sulphuric acid. The solution was filtered using a filter paper, and the filtrate was collected in another beaker, while the residue on the filter paper was discarded. 20 ml of barium chloride solution was added to the filtrate, and the mixture was stirred well, precipitating the sulphuric acid as barium sulfate. The mixture was allowed to stand until the precipitate settled at the bottom of the beaker. If no precipitate formed, it indicated that the sulfur content was very low or negligible. The mixture was filtered again using a filter paper, and the precipitate was collected on the filter paper. The precipitate was washed with distilled water to remove any impurities. The filter paper with the precipitate was transferred to a pre-weighed crucible or beaker. Additionally, an empty filter paper was weighed to use as a blank. The filter paper with the precipitate and the blank were ashed until they turned white, removing the organic matter and leaving only the barium sulfate. The ashed samples and the blank were weighed, and the weight of the blank was subtracted from the weight of the sample to determine the weight of the barium sulfate. The weight of sulfur in the sample was calculated using Equation 3.14

$$\text{Weight of sulphur} = \text{weight of barium sulphate} \times \frac{32.06}{233.39} \quad 3.14$$

Where,

32.06 is the atomic weight of sulfur in g/mol

233.39 is the molecular weight of barium sulfate in g/mol

The percentage of sulfur in the sample was calculated using Equation 3.15

$$\text{Percentage of sulphur} = \frac{\text{weight of sulphur}}{\text{weight of bioethanol sample}} \times 100\% \quad 3.15$$

3.19 Determination of flash point

The flash point of bioethanol was determined using the Pensky Martens (ASTMD93) method. Figure 3.10 shows a photo of the Pensky-Martens closed cup. The test sample was placed in a metal cup in such a way that it just touched the prescribed mark on the

inside of the cup. The cover was then be positioned on the cup. The heating was set to provide a 7 °F per minute temperature rise, and the sample was constantly agitated. As the temperature of the flash approaches, the injector burner lights up and then injected in the sample at approximately 12-second intervals until a distinct flash is visible within the container and the injector burner is turned off. A thermometer was used to determine the close flash point at this moment (Suleiman *et al.*, 2016).



Figure 3.10 A photo of the Pensky-Martens closed cup

3.20 Determination of viscosity

The Ostwald viscometer was thoroughly rinsed with water. Subsequently, the apparatus was dried in the oven. It was then partially filled with the test sample (bioethanol) from the lower end of the tube. The liquid was introduced into the viscometer and drawn up to the upper bulb of the tube. The level was maintained stationary by holding it with a finger. The time taken by the sample to descend from the upper marked level to the lower marked level was carefully observed. This process was repeated twice and readings were recorded each time (Khadka, 2021).

3.21 Determination of calorific value

Calorimeter model BCY-1A shown in figure 3.11 was used to measure the calorific value of the fuel. A crucible was inserted into the bomb after adding of 1g of the fuel. The bomb was filled with oxygen and placed inside the jacket before the fuse was connected with a nichrome wire. The jacket was filled with 2000 ml of water and the calorimeter was put together. A thermometer was used to check the temperature once every minute until it reached a steady value (Kanna *et al.*, 2017)



Figure 3.11 Photo of a bomb calorimeter

3.22 Electrical conductivity

The instrument utilized was a digital electrical conductivity meter model 950. 30 ml of bioethanol was transferred into a 100 ml beaker. The electrode was submerged up to the point of censorship. After a stable reading was acquired, the meter was turned on to the necessary calibrated units, and readings was done in triplicate (Ademiluyi and Mepba, 2013).

3.23 Summary

In summary, understanding the enzymatic conversion of cassava peels biomass to bioethanol is crucial for advancing renewable energy solutions. This chapter provided a comprehensive exploration of the processes involved, starting with the preparation

of cassava peels substrates and the application of various fundamental pretreatment methods (sulphuric acid, pearl millet amylase enzyme extract and citric acid) to convert cassava into fermentable sugars. It also emphasized the importance of optimizing fermentation conditions to maximize bioethanol yield. Furthermore, the chapter covered the distillation process and the analysis of bioethanol

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

The implications of acid pretreatment and pearl millet amylase enzyme extract pretreatment on the bioethanol generation from cassava roots are explored in detail in this chapter. The chapter reveals the ideal conditions found using the Taguchi methodology, discusses yeast fermentation kinetics and characterizes the bioethanol produced from cassava peels.

4.2 Comparison of different types of pre-treatment: sulphuric acid, pearl millet amylase enzyme extract and citric acid pre-treatment

In this study, bioethanol was produced through fermentation using cassava peels (*Manihot esculenta crantz*). The high starch concentration of the substrate required hydrolysis to break down the starch and convert it to glucose for use in the fermentation. The process involves breaking the glycosidic linkages between glucose units in the starch molecule. A hand-held refractometer (model REF107) was used to measure the sugar content of the hydrolysate. Table 4.1 indicates that compared to pearl millet amylase enzyme extract and citric acid pretreatment, 0.5 M sulphuric acid pretreatment was more appropriate to be applied in particular for the production of bioethanol. In this study, the percentage brix yield of sugar in the sulphuric acid was higher (14.5 %) than that of citric acid pretreated cassava peels (8.5 %) followed by the pearl millet amylase enzyme extract (0.8 %).

Sulfuric acid, being a strong acid, provides a high concentration of protons (H^+) in solution. This high proton availability ensures that more glycosidic bonds in starch are protonated simultaneously, accelerating the hydrolysis process. Citric acid, provides fewer protons in solution. This lower proton availability results in slower protonation of glycosidic bonds, making the hydrolysis process less efficient. The amount of sugar obtained was 11.189 % when using 1 % sulphuric acid to pretreat cassava peel (Susilawati *et al.*, 2018). This shows that higher concentration of sulphuric acid (0.5 M) is more successful in extracting sugar from cassava peels than lower concentration (1 %). Compared to the plant-based enzyme, the crude enzymes from *Aspergillus*

niger appear to be far more effective at producing reducing sugars. This was demonstrated by the fact that the yield of reducing sugars of $72.38 \pm 0.06 \text{ mg g}^{-1}$ reported by Mohammed *et al.*, (2013) is more than that of the plant-based amylase enzyme from pearl millet 0.8 % brix in this work.

Mineral acids like sulphuric acid and organic acids like citric acid have different capacities for hydrolysis. Citric acid is less effective in breaking down the complex carbohydrates found in cassava peels. Sulphuric acid is a powerful mineral acid and can supply a lot of protons for the reaction thus properly catalyze the hydrolysis of the cassava peel starch into reducing sugar. The brix yield of 14.5 % from sulphuric acid pretreated cassava peels as presented in Table 4.1 falls within the range of 14-22 % reported for bioethanol production (Limtong, 2007; Suleiman *et al.*, 2016).

Table 4.1 Sugar yield from pearl millet amylase enzyme extract, sulphuric acid and citric acid pretreated cassava peels

Sample	Weight of cassava sample (g)	Pre-treatment method	Concentration of glucose (Brix %)
1	100 g	Pearl millet amylase enzyme extract	0.8
2	200 g	0.50000 M sulphuric acid	14.5
3	100g	0.00026 M Citric acid	8.5

4.3 Optimization of fermentation process parameters involving pearl millet amylase enzyme extract

Production of lignocellulose ethanol depends significantly on the fermentation process variables, including temperature, pH, yeast-to-substrate ratio, and time. Taguchi's experimental design was used to examine the impact of four parameters on the fermentation process over an entire set of 16 runs shown in Table 3.1 and Table 3.2. The response values are in g/L for the four factors (Temperature, pH, time, and yeast to amount of substrate) that were selected for the optimization of ethanol production

by fermentation process as shown in Table 4.2. The first five columns of Table 4.2 indicate the sixteen experimental runs generated by Taguchi design.

The efficiency of ethanol production ranges from 1.5723 g/L to 8.2055 g/L. The bioethanol production from the optimization of water hyacinth production ranged efficiency ranged from 0.138 %, v v⁻¹ to 0.215 %, v v⁻¹ (Das *et al.*, 2016). A Minimum ethanol of 1.572 (g/L) was noted in experiment /run 1, that had a combination with a temperature of 30 °C, pH of 4.0, yeast to substrate ratio of 0.03 g/ml and fermentation time of 30 minutes producing a minimal signal to noise ratio of 3.9307 this resulted to lower ethanol response of 1.5723 (g/L). According to Das *et al.*, (2016), the lowest ethanol obtained was 0.138% (v/v) with a signal-to-noise (S/N) ratio of -17.20.

Table 3.2 Response values with experimental bioethanol yield, S/N ratio and predicted bioethanol yield of L₁₆ Taguchi orthogonal array design for pearl millet amylase enzyme extract pretreated cassava peels

Experiment Number	Temperature (°C)	pH	Catalyst (g/ml)	Time (min)	Average bioethanol (g/L)	Standard deviation	Signal to noise ratio	Predicted bioethanol (g/L)
1	30	4.0	0.03	30	1.5723	0.0316	3.9307	1.3310
2	30	4.5	0.05	60	4.6400	0.6351	13.3304	4.8355
3	30	5.0	0.07	90	3.5266	0.4848	10.9471	3.7332
4	30	5.5	0.09	120	5.8635	0.0763	15.3631	5.7026
5	35	4.0	0.05	90	6.6968	0.1104	16.5173	6.5359
6	35	4.5	0.03	120	4.6527	0.0143	13.3541	4.8593
7	35	5.0	0.09	30	2.9817	0.0826	9.4893	3.1772
8	35	5.5	0.07	60	5.0154	0.0519	14.0061	4.7741
9	40	4.0	0.07	120	4.8852	0.2141	13.7776	5.0807
10	40	4.5	0.09	90	8.2055	0.3603	18.2821	7.9642
11	40	5.0	0.03	60	1.9642	0.5123	5.8637	1.8033
12	40	5.5	0.05	30	1.9185	0.0683	5.6592	2.1251
13	45	4.0	0.09	60	7.6194	0.3311	17.6384	7.8260
14	45	4.5	0.07	30	4.6626	0.1860	13.3726	4.5017
15	45	5.0	0.05	120	4.7155	0.8772	13.4706	4.4742
16	45	5.5	0.03	90	5.2546	0.5151	14.4108	5.4501

The highest yield was obtained from experiment/ run 10 with the combination of the following factors; a temperature of 40 °C, pH of 4.5, yeast to substrate ratio of 0.09 and time of 90 minutes gave the best signal to noise ratio of 18.282 with higher ethanol output of 8.2055 g/L. The highest ethanol titre of 0.215 (% v v⁻¹) was observed when maximum S/N ratio was -13.35 reported by Das *et al.*, (2016). The linear regression equation for the model developed correlated with the predicted response of all the process (Dhawane *et al.*, 2016).

The S/N ratio helps in determination of the deviation of the response from the desired value (Okoth *et al.*, 2023). The main effects plot for the means data and S/N ratios are as shown in Figure 4.1.

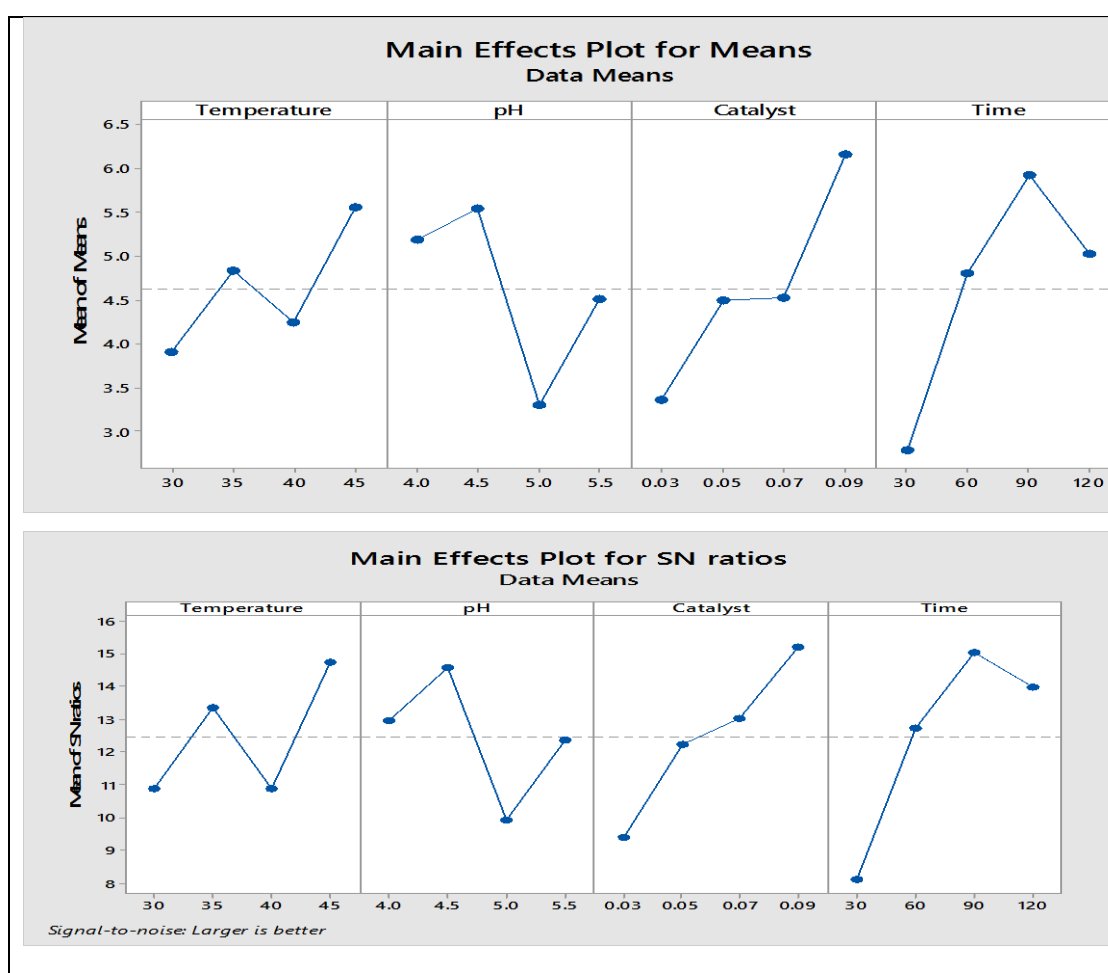


Figure 4.1 The main effect plot for data mean, and S/N ratio for the bioethanol yield for pearl millet amylase enzyme extract pretreated cassava peels

This study's findings however contradict the findings of Salihu *et al.* (2023) who reported that ethanol production decreased significantly at 40 °C as it can be seen at this particular temperature resulted to highest ethanol yield. This demonstrated unequivocally that during fermentation, each parameter pH or temperature has an impact on fermentation (Ogbonda & Kiin-Kabari, 2013).

The generation of ethanol declined or remained stagnant as the fermentation process goes for longer duration (Osemwengie *et al.*, 2020). Time also is a significant factor affecting the process of fermentation as prolonged fermentation can cause metabolic byproducts, including ethanol itself, to build up. The activity of the enzymes and microbes involved in the fermentation process can be inhibited presence of inhibitors in the fermentation media, which lowers their efficiency and slows down the synthesis of ethanol. According to Osemwengie *et al.*, (2020) possible causes for this include the synthesis of inhibitory substances including acetic acid, furfural, and phenolic compounds. The optimized fermentation process parameters for the production of bioethanol from pearl millet amylase enzyme extract pretreated cassava peels, as determined by Taguchi's design of experiment as shown in Figure 4.1 among the different conditions tested in the experiment time had the most pronounced effect on the fermentation process of the pearl millet amylase enzyme extract pretreated cassava peels. The ranking of parameters from the S/N ratios in Table 4.3 also shows that time had the strongest influence and it was ranked at 1, yeast to substrate ratio at 2, pH at 3 and finally the reaction temperature ranked at 4. This finding are similar to the one reported by Jambo *et al.*, (2019) that time had significant effect on fermentation and was ranked at 1.

The effects of pH ranked 2 and temperature ranked third (Jambo *et al.*, 2019). Temperature was also found to have the greatest impact on the generation of bioethanol reported by Okoth *et al.*, (2023). The generation of ethanol declined or remained stagnant as the fermentation process goes for longer duration (Osemwengie *et al.*, 2020). Prolonged fermentation can cause metabolic byproducts, including ethanol itself, to build up. The activity of the enzymes and microbes involved in the fermentation process can be inhibited by high ethanol concentrations, which lowers their efficiency and slows down the synthesis of ethanol.

According to Osemwengie *et al.*, (2020) possible causes for this include the synthesis of inhibitory substances including acetic acid, furfural and phenolic compounds.

Table 4.3 Response table for Signal to Noise showing the delta ranks

Level	Temperature	pH	catalyst	Time
1	3.901	5.193	3.361	2.784
2	4.837	5.540	4.493	4.810
3	4.243	3.297	4.522	5.921
4	5.563	4.513	6.168	5.029
Delta	1.662	2.243	2.807	3.137
Rank	4	3	2	1

These findings are similar to those attained by Zakaria *et al.*, (2020) who obtained fermentation temperature of 45 °C and pH 4.5. Another study established that a temperature of 40 °C and pH of 5.78 were found to be the ideal process parameters for achieving the highest bioethanol production reported (Chohan *et al.*, (2020). Additionally, another study discovered that the optimal pH and temperature for *Saccharomyces cerevisiae* to produce ethanol were 35 °C and 4.5, respectively (Salihu *et al.*, 2023). Figure 4.2 shows a plot of actual or experimental yields against anticipated yields used to assess the accuracy of the Taguchi model in this work (Kurt *et al.*, 2008).

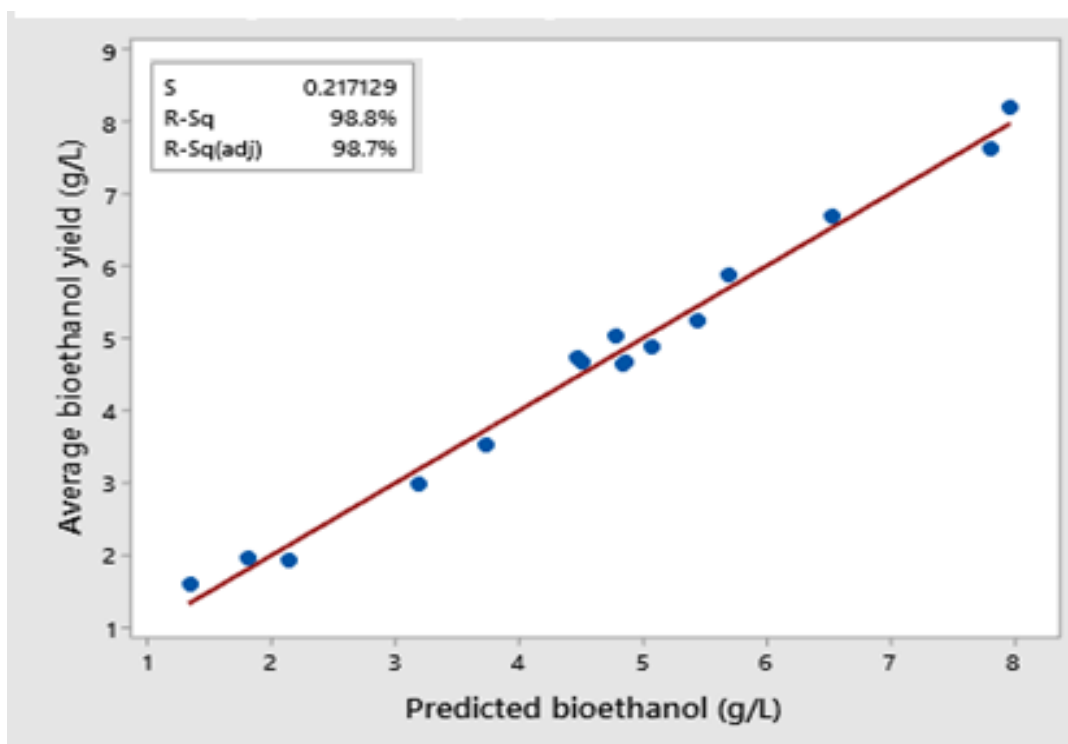


Figure 4.2 A plot of the experimental bioethanol verses predicted bioethanol yield

The independent variable (temperature, pH, time and yeast to substrate ratio in the model have a strong influence on the dependent variable for the bioethanol yield for pearl millet amylase enzyme extract pretreated cassava peels.

The actual R^2 (0.0988) and the adjusted R^2 (0.987) with high R^2 values for the enzyme pretreated cassava peels and the points' proximity shows that the model does an effective job of fitting the experimental data. According to (Qi *et al.*, 2009) straight regression line with high R values indicate that the model does an effective job of fitting the experimental data. Osemwengie *et al.*, (2020) also obtained 0.989 as the coefficient of determination suggesting that the Taguchi effectively captures the relationship between variables as observed in previous studies. The standard error (S) is 0.217129, which measures the average distance that the observed values fall from the regression line. A lower value indicates a better fit.

4.4 Analysis of variance for pearl millet amylase enzyme extract pretreated cassava peels

Taguchi technique was used to determine the components' contributions while doing an analysis of variance (ANOVA) for the responses related to ethanol production, as indicated in Table 4.4. It is possible to deduce that all the parameters taken into account in the experimental design are statistically significant for the pearl millet amylase enzyme extract pretreated cassava peels at the 95% confidence level based on the estimated ratios (F). The contribution of the chosen components to the generation of bioethanol is shown in Table 4.4 for the pearl millet amylase enzyme extract pretreated cassava peels based on the P -value ($P < 0.05$). Independent variables with a 5% significance level ($p < 0.05$) were deemed significant for their impact on bioethanol response. According to the F -Value, the order of influence is time > yeast to substrate ratio > pH > temperature. It is evident that every component had an impact on the production of bioethanol. However, time, with a p -value of 0.009 as shown in Table 4.4 was the most significant factor and had the most beneficial effect on the production of ethanol. Jambo *et al.*, (2019) also reported that fermentation time significantly affects the bioethanol fermentation of glucose by *Saccharomyces cerevisiae* with p value of 0.0035. Fermentation time is one of the most important parameters in the production of bioethanol as it determines the economical part of the whole production process as well as the viability of the fermentative microorganisms employed (Graves *et al.*, 2007).

Shorter fermentation time causes inefficient fermentation due to inadequate growth of microorganisms. On the other hand, longer fermentation time gives toxic effect on microbial growth especially in batch mode due to the high concentration of ethanol in the fermented broth (Azhar *et al.*, 2017). The result suggests that time had the most important influence on bioethanol yield, contrary to Okoth *et al.*, (2023) findings that temperature had the most significant effect, with a p -value of 0.02.

Table 4.4 Analysis of variance for S/N ratios

Source	D F	Adj ss	Adj MS	F- value	p- value
Temperature	3	6.378 50	2.126 2	9.660	0.047
pH	3	11.74 54	3.915 1	17.80	0.020
Yeast to substrate ratio	3	16.01 90	5.339 7	24.27	0.013
Time	3	21.06 58	7.021 9	31.92	0.009
Error	3	0.660 00	0.220 0		
Total	15	55.86 87			

DF: degrees of freedom, SS: sum of squares, and MS: mean of squares

4.5 Optimization of fermentation process parameters involving sulphuric acid pretreated cassava peels

Table 4.5 displays the ethanol production efficiency, which ranges from 3.5606 g/L to 24.5288 g/L. Experiment 1 (run 1) reported a minimum ethanol of 3.5606 g/L with a combination of 30 °C, pH of 4.0, yeast to substrate ratio of 0.03 g/ml, and duration of 30 minutes, resulting in a low signal to noise ratio of 11.03046. The best output from experiment/run 10 was attained when the following variables were combined: a temperature of 40 °C, pH of 4.5, yeast to substrate ratio of 0.09 g/ml and time of 90 minutes. The conditions gave the best signal to noise ratio of 27.79353 with a high ethanol yield of 24.5288 g/L.

Table 4.5 Response values with experimental bioethanol yield, S/N ratio and predicted bioethanol yield of L₁₆ Taguchi orthogonal array design for sulphuric acid pretreated cassava peels

Experiment Number	Temperature °C	pH	Catalyst (g/ml)	Time (min)	Average bioethanol yield (g/L)	Standard deviation	Signal to Noise ratio	Predicted bioethanol (g/L)
1	30	4.0	0.03	30	3.5606	0.1943	11.0305	3.2531
2	30	4.5	0.05	60	15.4372	1.3157	23.7714	15.0272
3	30	5.0	0.07	90	13.6091	1.3250	22.6766	14.4917
4	30	5.5	0.09	120	18.6161	0.3774	25.3978	18.4509
5	35	4.0	0.05	90	18.7131	0.9789	25.4429	18.5479
6	35	4.5	0.03	120	16.1489	0.5149	24.1629	17.0315
7	35	5.0	0.09	30	13.0186	2.7413	22.2913	12.6086
8	35	5.5	0.07	60	17.3331	0.1779	24.7775	17.0256
9	40	4.0	0.07	120	17.8153	0.3912	25.0159	17.4053
10	40	4.5	0.09	90	24.5288	1.3121	27.7935	24.2213
11	40	5.0	0.03	60	9.7524	0.6420	19.7822	9.5872
12	40	5.5	0.05	30	8.3716	1.3067	18.4562	9.2542
13	45	4.0	0.09	60	20.1930	0.1011	26.1040	21.0756
14	45	4.5	0.07	30	15.4322	0.0287	23.7686	15.2670
15	45	5.0	0.05	120	17.6606	0.6694	24.9401	17.3531
16	45	5.5	0.03	90	17.2220	3.2154	24.7217	16.8120

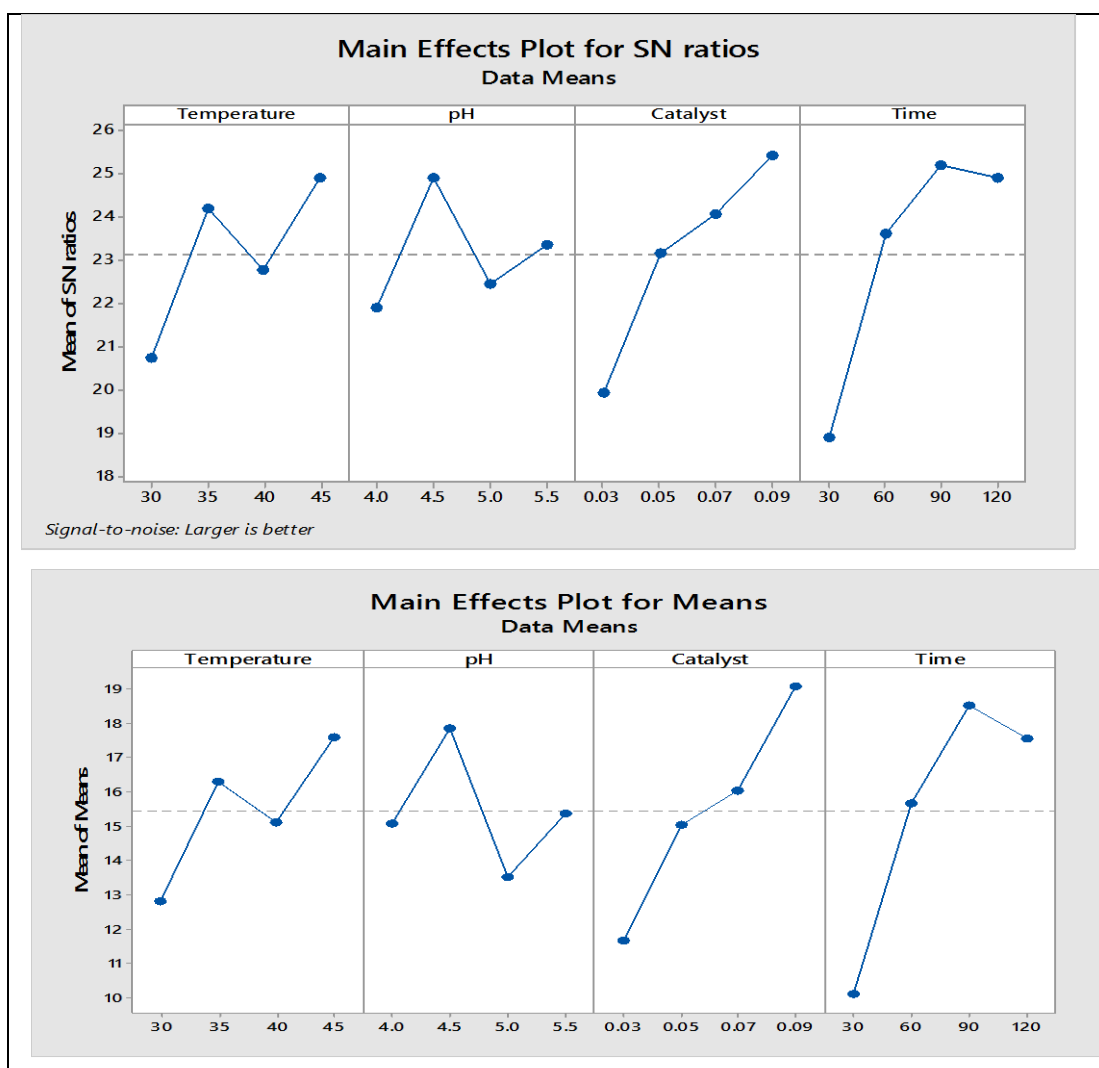


Figure 4.3 The main effect plot for data mean, and S/N ratio for the bioethanol yield for sulphuric acid pretreated cassava peel

Key insights are revealed by figure 4.3 estimated by Taguchi's experimental design for the synthesis of bioethanol from sulphuric acid pretreated cassava peels. The figure indicated that the optimum fermentation conditions was a temperature of 40 °C, pH of 4.5, yeast to substrate ratio of 0.09 g/ml and a duration of 90 minutes were the ideal condition for the generation of bioethanol. Figure 4.3 also shows that time had the most effect on the fermentation process for sulphuric acid pretreated cassava peels. The ranking of parameters from the S/N ratios in Table 4.6 also shows that time had the strongest influence and was ranked at 1, yeast to substrate ratio at 2, fermentation temperature at 3 and finally the pH at 4.

Environmental factors like pH and temperature interact with time. These elements may have an impact on metabolic activity and microbial growth rates, which in turn may

have an impact on ethanol yield. The duration of the fermentation process affects the production of byproducts in addition to ethanol. Prolonged fermentation times may increase the amount of secondary metabolites produced or reduce the amount of ethanol produced or the quality of the product (Azhar *et al.*, 2017). Therefore, controlling the ratio of ethanol production to undesired byproduct generation and this can be achieved by proper selection of fermentation time.

Table 4.6 Response table for signal to noise ratios showing the delta ranks

Level	Temperature (°C)	pH	Catalyst (g/L)	Time (min)
1	12.81	15.070	11.67	10.10
2	16.30	17.890	15.05	15.68
3	15.12	13.510	16.05	18.52
4	17.63	15.039	19.09	17.56
Delta	4.82	4.3800	7.42	8.42
Rank	3	4	2	1

Bioethanol production depends on the ideal temperature of the yeasts (Azhar *et al.*, 2017). The results in this work is similar to the optimum fermentation time of 24 hours with a pH of 4.5 reported by Mardina *et al.*, (2021). Most of the fermenting medium used for bioethanol production has pH in the range of 4.5–5.5 with various sugar concentration (Azhar *et al.*, 2017).

Furthermore, the study revealed that the most favorable conditions for *Saccharomyces cerevisiae* to produce ethanol were a temperature of 35 °C and a pH of 4.5, resulting in the highest ethanol yield, as documented by Salihu *et al.*, (2023). Longer fermentation times can lead to the accumulation of toxic metabolites. For example, in alcoholic fermentation, the accumulation of ethanol can become toxic to yeast cells, causing them to stress and produce unwanted increased glycerol production and decreased ethanol production (Tse *et al.*, 2021). According to Shanavas *et al.* (2011), utilizing an enzyme loading of 1.0 mg/L and a reaction period of 180 min, the maximum yield of ethanol from cassava starch was 2.0 %.

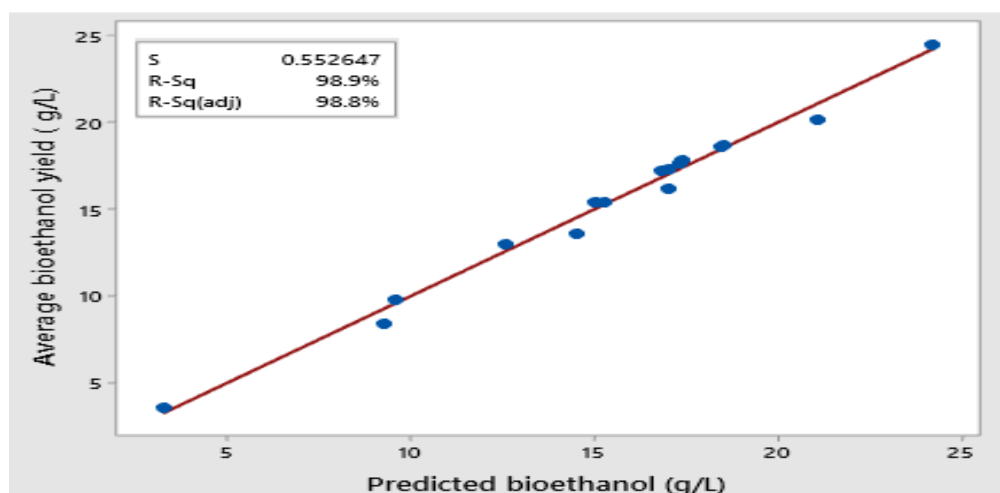


Figure 4.4 A plot of experimental bioethanol yield verses predicted bioethanol yield

The exceptionally high result of actual R^2 (0.989) and the adjusted R^2 (0.988) with high R^2 values from sulphuric acid pretreated cassava peels indicate that the Taguchi model captures the underlying relationships between the independent variables (pH, yeast to substrate ratio, temperature and time) and the dependent variable (bioethanol yield).

Osemwengie *et al*, (2020) obtained 0.989 as the coefficient of determination. The model's accuracy and dependability are further demonstrated when data points nearly match the regression line, which shows that the model's predictions closely match the actual observed values. Standard error of the regression was 0.552647 which a smaller value suggesting a better fit of the data to the Taguchi model.

4.6 Analysis of variance for sulphuric acid pretreated cassava peels

Table 4.7 displays the results of the analysis of variance (ANOVA) for the responses pertaining to ethanol production. Based on the calculated ratios (F), it is reasonable to infer that the experimental design's consideration of parameters is statistically significant for the sulphuric acid pretreated cassava peels at the 95% confidence level. Based on the P -value ($P < 0.05$) it is clear that each aspect affected the amount of bioethanol produced.

However, time stood out as the most important factor and had the greatest positive impact on ethanol production among the elements, with a p -value of 0.006 as shown

in Table 4.7 and a rank of 1 as shown in Table 4.6. Time was ranked first as a factor affecting fermentation at $p=0.0035$, inoculum concentration at $p=0.0056$, pH at $p=0.0109$ and fermentation temperature at $p=0.0219$ (Jambo *et al.*, 2019). However, the analysis of variance for bioethanol produced from sulphuric acid pretreated cassava peels contradicts the results of Das *et al.*, 2014 who reported that pH with a rank of 1 had the most influential factor on bioethanol production. The result also contradicts Okoth *et al.*, (2023) finding that temperature with a p -value of 0.02 had the most significant effect on bioethanol yield and therefore suggest that time has the most influential impact on bioethanol yield.

Table 4.7 Analysis of variance for the response of ethanol production

Source	D F	Adj SS	Adj MS	F-value	p - value
Temperature	3	50.2 79	16.7 60	11.76	0.036
pH	3	39.3 93	13.1 31	9.21	0.050
Yeast to substrate ratio	3	112. 176	37.3 92	26.23	0.012
Time	3	170. 348	56.7 83	39.84	0.006
Error	3	4.27 6	1.42 5		
Total	1 5	376. 471			

4.7 Optimization of fermentation process parameters involving citric acid

The ethanol production efficiency is shown in Table 4.8 and varies from 3.6916 g/L to 4.921 g/. However, Das *et al.*, (2014) reported that ethanol production ranged from 0.120 % (v/v) to 0.251 % (v/v), corresponding to the combined effect of the six factors within their specific ranges. The experiment's findings show that when these factors were at their ideal levels, ethanol production increased considerably. With

combination of 30 °C, pH 4.0, yeast to substrate ratio of 0.03 (g/ml), and 30 minutes of time, Experiment 1 (run 1) reported a minimum ethanol of 3.6916 (g/L).

This resulted to a minimal signal to noise ratio of 11.3443. An ethanol concentration of 0.120 (% v v⁻¹) was observed with a combination of recombinant GH5 cellulase volume (0.25 mL), recombinant GH43 hemicellulase volume (0.25 mL), *Saccharomyces cerevisiae* inoculum volume (0.25 mL), *Candida shehatae* inoculum volume (0.25 mL), pH (3), and temperature (26 C) reported by Das *et al.*, (2014).

Combining the following variables produced the best results from experiment/run 4: At 40 °C, pH of 5.0, yeast to substratum ratio of 0.09, and 120 minutes' duration and the highest ethanol output of 4.921 (g/L) was obtained with the best signal to noise ratio of 13.8411. With a combination of recombinant GH5 cellulase volume (1.0 mL), recombinant GH43 hemicellulase volume (0.5 mL), *Saccharomyces cerevisiae* inoculum volume (1.50 mL), *Candida shehatae* inoculum volume (0.25 mL), pH (5), and temperature (30 °C), the best response and maximum ratio (-11.99) were observed with a maximum ethanol titre of 0.251 (% v v⁻¹) ethanol reported by Das *et al.*, (2014). This implies that the superior signal-to-noise ratios are typically indicative of higher performance or quality in Taguchi experimental design. Thus, it can be said that the ethanol output that had a higher signal-to-noise ratio produced the most bioethanol, while the one that had a lower ratio produced less ethanol.

Table 4.8 Response values with experimental bioethanol yield, S/N ratio and predicted bioethanol yield of L₁₆ Taguchi orthogonal array design for citric acid pretreated cassava peels

Experiment	Temperature °C	pH	Catalyst (g/ml)	Time (min)	Average bioethanol (g/L)	Standard deviation	S/N ratio	Predicted bioethanol yield (g/L)
1	30	4.0	0.03	30	3.6916	0.0677	11.3443	3.7583
2	30	4.5	0.05	60	4.2380	0.0355	12.5432	4.3606
3	30	5.0	0.07	90	4.5791	0.0509	13.2156	4.3395
4	30	5.5	0.09	120	4.9210	0.0593	13.8411	4.9713
5	35	4.0	0.05	90	3.5592	0.0594	11.0270	3.6095
6	35	4.5	0.03	120	4.7113	0.1322	13.4628	4.4717
7	35	5.0	0.09	30	4.1539	0.0921	12.3691	4.2765
8	35	5.5	0.07	60	3.5251	0.0719	10.9434	3.5918
9	40	4.0	0.07	120	3.6575	0.1482	11.2637	3.7801
10	40	4.5	0.09	90	4.5905	0.2664	13.2372	4.6572
11	40	5.0	0.03	60	4.2811	0.2886	12.6311	4.3314
12	40	5.5	0.05	30	4.2535	0.0403	12.5749	4.0139
13	45	4.0	0.09	60	4.1013	0.1365	12.2584	3.8617
14	45	4.5	0.07	30	3.7243	0.2803	11.4209	3.7746
15	45	5.0	0.05	120	4.6129	0.1613	13.2795	4.6796
16	45	5.5	0.03	90	4.1471	0.1515	12.3549	4.2697

Important details on the production of bioethanol from cassava peels pretreated with citric acid pretreated cassava peels are shown in Figure 4.5, which was also produced from Taguchi design of experiment.

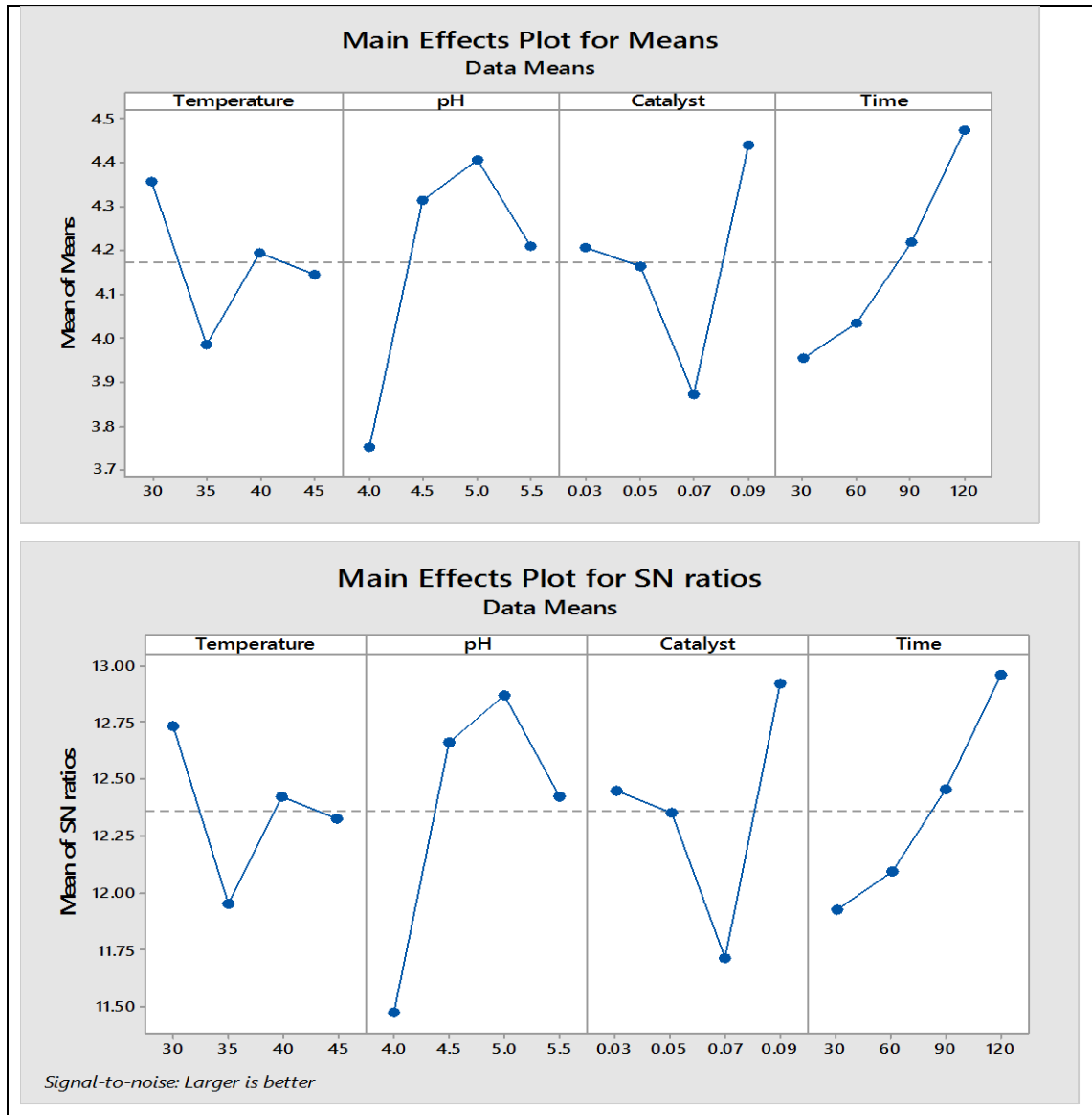


Figure 4.5 A plot of experimental bioethanol verses predicted bioethanol yield

The figure 4.5 suggests that for the purpose of maximizing the generation of bioethanol, the ideal fermentation conditions are temperature of 40 °C, pH 5.0, yeast to substrate ratio of 0.09 g/ml, and 120 minutes.

The image also shows how pH had a major impact on the fermentation process for cassava peels that have been prepared with citric acid. This conclusion is supported

by the examination of S/N ratios in Table 4.9, where pH (at 5.0) shows the largest influence on bioethanol yield, followed by yeast to substrate ratio (at 0.09 g/mL), time (at 120 minutes) and temperature (at 40 °C). In the statistical Taguchi optimization of fermentation process parameters for increased bioethanol production, pH, ranked first, was the most significant factor and demonstrates the highest positive impact on fermentation, as reported by Das *et al.*, (2014).

4.9 Response table for signal to noise ratio showing the delta ranks

Level	Temperature (°C)	pH	Catalyst (g/L)	Time (min)
1	4.357	3.752	4.208	3.956
2	3.987	4.316	4.166	4.036
3	4.196	4.407	3.872	4.219
4	4.146	4.212	4.442	4.476
Delta	0.370	0.654	0.570	0.520
Rank	4	1	2	3

The optimal condition for the citric pretreated cassava peels were a temperature of 40 °C, pH of 5.0, yeast-to-substrate ratio of 0.09, and 120 minutes were the ideal fermentation conditions for excellent results. This result was similar to the findings obtained by (Kularathne *et al.*, 2020). Which revealed that the highest bioethanol yield was attained under conditions of pH 5, a fermentation temperature of 35 °C, and a yeast concentration of 10 %.

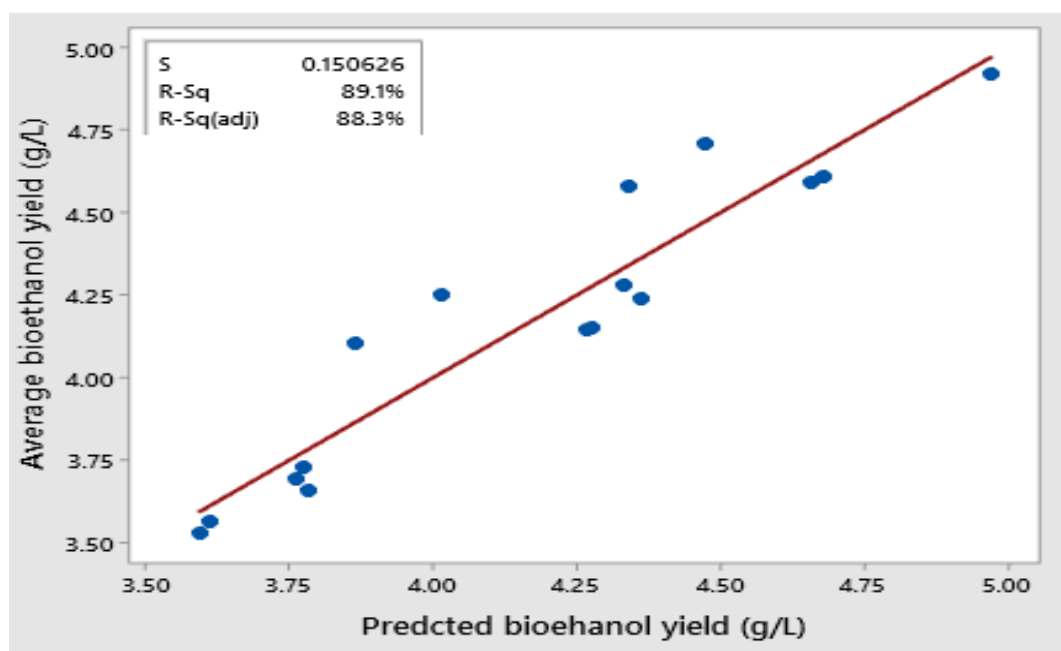


Figure 4.6 A plot of experimental bioethanol yield verses predicted bioethanol yield

The citric pretreated cassava peels exhibit the following R-squared values, actual R^2 (0.891) and the adjusted R^2 (0.883) indicating that the model still captures in the data very well.

4.8 Analysis of variance for citric acid pretreated cassava peels

The ANOVA analysis conducted on the responses related to ethanol production, as detailed in Table 4.10, indicates that the parameters ((Temperature, pH, Yeast to substrate ratio, Time) considered in the experimental design does not exhibit statistical significance for the citric acid pretreated cassava peels at $P < 0.05$, as all p -values are greater than 0.05. This is contrary different with the reported values in literature where some parameters such as pH with a p -value 0.001 had the highest rating (1), making it the most relevant and having the greatest positive impact on the production of ethanol reported by Das *et al.*, (2014).

Many researchers have documented that the pH of the fermentation medium influences the transfer of chemical products and enzymes across the cell membrane, hence impacting numerous enzymatic reactions (Liang *et al.*, 2009). The results of ANOVA showed that the reaction temperature was the most significant at 0.020,

followed by the yeast-to-substrate ratio at 0.086, pH at 0.216, and finally time at 0.564 (Okoth *et al.*,2023).

Table 4.10 Analysis of variance for the response of ethanol production

Source	D F	Adj SS	Adj MS	F- Value	p-Value
Temperature	3	0.27 87	0.09 291	0.88	0.541
pH	3	1.01 40	0.33 798	3.19	0.183
Yeast to substrate ratio	3	0.65 74	0.21 912	2.07	0.283
Time	3	0.63 82	0.21 273	2.01	0.291
Error	3	0.31 76	0.10 588		
Total	1 5	2.90 59			

From the three optimized methods it can be concluded that the use sulphuric acid optimized fermentation conditions were the most relevant to be applied in this study since it gave the highest yield of bioethanol giving the best output.

4.9 Michaeli's K_m and V_{max} parameter from the best bioethanol response

Enzymes are biological catalysts that speed up chemical reactions by lowering the activation energy required to convert substrates into products. The study concentrated on the kinetics of enzymatic fermentation of acid-hydrolyzed cassava peels in order to produce ethanol. Graphing the reciprocal of rate ($1/v$) against the reciprocal of substrate concentration ($1/[S]$) as shown in figure 4.7 produced a straight line with an intercept of $1/V_{max}$ and a slope of K_m / V_{max} , indicating that the Michealis-Menten model is suitable for the experimental data. The kinetics of *Saccharomyces cerevisiae* V_{max} was 0.4300 Mol/L/sec with a K_m value of 583.49 Mol/L. The K_m value for this reaction was large compared to the one obtained by Ude & Oluke, (2021) of 125.5

g/dm³. This suggests that the enzyme binds less tightly to the substrate, and therefore requires higher substrate concentrations for efficient catalysis.

The high K_m value was attributed to enzyme inhibition resulting from interactions between the enzymes produced by the yeast and the various derivatives generated during hydrolysis. Peels that have been hydrolyzed can contain small molecular weight molecules, like furan derivatives, phenolic compounds and amine-based compounds like vanillin, which impede fermentation reported (Endô *et al.*, 2008)

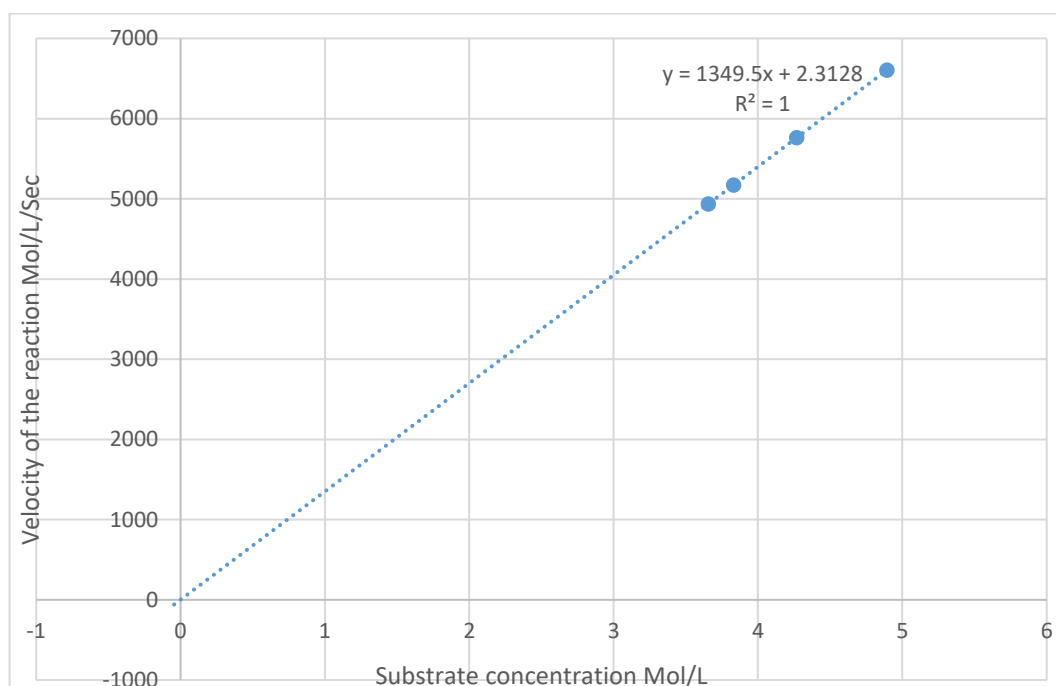


Figure 4.7 Lineweaver-Burk kinetic plot for enzymatic fermentation of sulphuric acid pretreated cassava peels

4.10 Characterization of bioethanol

In this study, instead of the typical expression of bioethanol yield as grams of bioethanol per gram of substrate, the yield was measured as liters of bioethanol per fresh cassava, to facilitate comparison with previous research findings. In this study, the yield of bioethanol was established using equation 3.11 and 3.12. The yield of bioethanol was 200 L/ton was close to 160 L/ton reported by Dinata & Kartawiria, (2021).

Density is a crucial characteristic of fuel that plays a significant role in determining its energy content and practical utility. Ethanol combustion efficiency is crucial for optimizing the quantity of energy converted from the fuel to heat. Incomplete combustion reduces cooking efficiency and results to energy loss. The key to burning ethanol efficiently is to achieve full combustion, which reduces the amount of unwanted byproducts like carbon monoxide produced and instead converts the fuel to CO₂ and H₂O. The density of the produced bioethanol was 0.8763 g/cm³ with a specific gravity of 0.8779 g/cm³ as shown in Table 4.11, which was lower than 0.9970 g/cm³ and 0.955 g/cm³ specific gravities reported by Omoruyi *et al.*, (2022) and Ogunsuyi *et al.*, (2016) respectively.

Table 4.11 Properties of bioethanol produced from sulphuric acid pretreated cassava peels

S/N	Property of the produced bioethanol	Units	Experimental Results	ASTM Standards for Bioethanol
1	Density @ 20 °C	g/cm ³	0.8763	0.75-85
2	Specific gravity	g/cm ³	0.8779	0.87
3	Concentration of EtOH	% v/v	73.6300	-
4	Ash		0.0050	-
5	Kinematic Viscosity @ 20 °C	Cts/sec	3.6765	5.00 max
7	Sulphur content	% wt	0.0788	0.05
8	Flash point (closed cup)	°C	17.00	18.60
9	pH		4.2860	-
10	Calorific value	MJ/kg	21.8900	26.89-29.70
11	Electrical conductivity	µS/m	8.8100	500.00 max

The concentration of ethanol refers to the amount or proportion of ethanol present in a solution. The concentration of ethanol in the produced bioethanol was 73.63 %v/v, which was higher than 45.50 % reported by Egbosiuba et al., (2014).

Ash buildup can decrease airflow and impair efficient combustion. Ash and other byproducts may be produced when ethanol is not completely burned. A portion of these byproducts might contain contaminants that worsen indoor air quality, which has an impact on the environment and public health. The ash content of the produced

bioethanol was 0.005, which can be considered very negligible. The ash content was low just as reported by Amalia *et al.*, (2021).

Viscosity is a measure of a fluid's resistance to progressive deformation brought on by shear or tensile stress. It corresponds to the widely accepted definition of liquid thickness. When adjacent particles in a fluid collide while moving at different speeds, viscosity is produced (Boda *et al.*, 2015). The atomization of the fuel during combustion is impacted by the fuel's viscosity. Increased viscosity can lead to inefficient combustion and poor atomization, which would reduce the burning efficiency and increase emissions. Low kinematic viscosity of ethanol produces less emission during combustion, especially when it comes to particle matter. Reduced production of smoke and other pollutants as well as more thorough combustion can result from the fuel's fine atomization. The produced bioethanol had a kinematic viscosity of 3.6765 cst which was lower but within the ASTM standards than the kinematic viscosity of 1.21×10^3 cst reported by Egbosiuba *et al.*, (2014).

The amount of sulphur in bioethanol can affect how well it burns. Incomplete combustion of sulphur can produce sulphur dioxide (SO₂). The sulphur level of bioethanol is typically a cause for worry. A high sulphur level may cause partial combustion by interfering with the burning process. In addition to producing less energy, incomplete combustion releases hazardous byproducts such carbon monoxide (CO) and particulate particles. The amount of sulphur in the produced bioethanol was 0.0788012 % which was close to the one reported by Umezuegbu, (2022) of 0.06 %.

The flashpoint parameter is the lowest temperature at which, under test conditions, the introduction of an ignition source allows the vapour of a test part to ignite and the flame to spread across the liquid's surface (Blomqvist *et al.*, 2015). Flame and combustible-grade liquids are defined as those with flash points below 37.8°C (ASTM, 2011). Due to its volatile nature, bioethanol vapor and oxygen may combine to create a combustible mixture. Because of this, bioethanol evaporation into the air occurs more quickly at higher concentrations, leading to increased combustibility. Result obtained that the flash point of the produced bioethanol is 17 °C which falls closely within the range of ASTM standards for combustible and flammable liquids but lower than the flash point of 46 °C reported by Ogunsuyi *et al.*, (2016). Higher burning efficiency is preferred to minimize fuel use, guarantee lower emissions, and

enhance fuel's efficiency. The low flash point of 17 °C of ethanol implies that ethanol can quickly combine to create a combustible mixture, which can ignite spontaneously.

Another parameter that was tested was the pH of the bioethanol.

The acidity of a substance is determined by the concentration of hydrogen ions (H^+) in the solution. In terms of acidity, ethanol is rather a weak acid. When contaminants are present in the ethanol, they may behave as bases or acids, changing the solution's overall acidity. Pollutants or impurities can significantly impact the suitability of fuel for various applications. Extremely low pH levels can corrode fuel storage tanks and transportation infrastructure, potentially rendering them unfit for use in certain environment. The pH of the produced bioethanol was 4.286, which was higher and thus less acidic than a pH of 2.85 (Nuwamanya *et al.*, 2011).

The calorific value of fuel bioethanol reveals the amount of energy one can obtain by burning a specific mass of bioethanol. A higher calorific value implies that the fuel can release more energy when combusted, making it more efficient for use as a source of heat. The calorific value of fuel bioethanol was 21.89 MJ/kg, which was lower than the 29.78 MJ/kg calorific value of ethanol reported by Nwifo *et al.*, (2013). However, the calorific value can be increase by adding the number of Carboxy Methyl Cellulose (CMC) (Hermawan and Sudarmanta 2018). The presence of carbon and hydrogen content elements in CMC has the potential to enhance the fuel's calorific value. This is due to the combustion process, where carbon interacts with oxygen, resulting in the generation of heat (Hermawan and Sudarmanta 2018).

Fuel ethanol's electrical conductivity is a crucial factor to consider when assessing the quality of the fuel. Electrochemical or galvanic corrosion is more likely to occur in high-conductive fluids due to the correlation between conductivity and the presence of corrosive ions like chlorine (Luecke and McCormick 2014). Higher purity bioethanol typically has lower electrical conductivity.

Bioethanol boasts a commendably low electrical conductivity, attributed to its pure composition resulting from fermentation, where the absence of ionic components prevents an increase in its conductivity. The bioethanol had an electrical conductivity of $8.81\mu s/m$. The electrical conductivity was lower than the electrical conductivity of

329 $\mu\text{s/m}$, 330 $\mu\text{s/m}$, 230 $\mu\text{s/m}$, 300 $\mu\text{s/m}$, 150 $\mu\text{s/m}$ obtained from the following varieties of cassava TMS 92B/00068, TMS 91/02324, TMS 92B/0061, TMS 98/0505, TMS 98/058 respectively reported by Ademiluyi and Mepba (2013). The low electrical conductivity can be attributed to the absence of corrosive ions in the produced bioethanol, thus making it ideal for use in cook stoves.

4.11 Summary

This chapter investigated the effects of acid (sulphuric and citric acid) and pearl millet amylase enzyme extract pretreatment on bioethanol production from cassava peels, offering a thorough study of their impacts. The chapter discussed the results from the Taguchi process to identify the ideal pretreatment settings thus increasing bioethanol yield. Examined yeast fermentation kinetics to determine the efficiency of the fermentation process. The characterization of bioethanol made from cassava peels is also reviewed, providing information about its quality and prospective applications. Overall, the findings highlight the significance of pretreatment strategies and process optimization in optimizing bioethanol production, providing information for researchers and industry experts intending to advance renewable energy technology.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The study findings suggest that there is statistical significant variation in the hydrolysis efficiency of cassava peels when sulphuric acid was used for the pre-treatment of cassava peels. This was due to the ability of sulphuric acid to convert the complex carbohydrates found in cassava peels into simpler sugars. Sulphuric acid was found to have the highest sugar content of 14.5% brix. The hydrolysis process is facilitated more effectively by the strong acidic nature of sulphuric acid rather than by the pearl millet amylase enzyme extract or citric acid. The result emphasizes the importance of choosing the right pretreatment technique for cassava peels when using them in industrial settings to produce bioethanol. Because acid pretreatment, particularly sulphuric acid pretreatment, is more effective at producing sugars from cassava peels than pearl millet amylase enzyme extract which produced sugar content of 0.8 % brix and citric acid pretreatment of 8.5 % brix.

Taguchi experimental design can indeed be used to optimize fermentation conditions. The optimum fermentation conditions for pearl millet amylase enzyme extract pretreated cassava peels and sulphuric acid pretreated cassava peels were a temperature of 40 °C, a pH level of 4.5, a yeast to substrate ratio of 0.09 g/ml and a fermentation time of 90 minutes.

Fermentation conditions for citric acid-pretreated cassava peels were a temperature of 40 °C, a pH of 5.0, and a yeast to substrate ratio of 0.09 and fermentation time of 120 minutes.

Saccharomyces cerevisiae's enzymatic kinetics are impacted by the particular experimental conditions used, as shown by the examination of K_m of 583.49 Mol/L and V_{max} of 0.4300 Mol/L/Sec which shows notable differences from previously reported value of a K_m value of 81.63 mol/l with a V_{max} of 0.71 Mol/L/sec. When evaluating enzyme kinetics, these variations emphasize how crucial it is to take into account experimental conditions like pH level, time, yeast to substrate ratio and temperature. It is clear from the observed variation in the K_m and V_{max} of values that

enzymatic processes are dynamic and that *Saccharomyces cerevisiae* is sensitive to change in pH, temperature, yeast to substrate ration and time of fermentation.

The thorough examination of bioethanol's characteristics indicates that it is, in fact, a useful fuel. Its advantageous qualities not only meet ASTM legal requirements but also provide advantages for the environment. Bioethanol can therefore be suggested as a practical alternative fuel source for a range of applications, supporting sustainable energy practices and lowering reliance on fossil fuels, based on the empirical evidence provided in this work that the produced bioethanol from the cassava peels had a density of 0.8763g/cm³, specific gravity of 0.8779g/cm³, ethanol concentration of 73.63 %v/v, ash content of 0.0050, kinematic viscosity of 3.6765 cst, sulphur content of 0.0788%, flash point of 17.00 °C, pH of 4.286, calorific value of 21.89 MJ/kg and electrical conductivity of 8.81µs/m.

5.2 Recommendations

5.2.1 Recommendation for policy and practice

The recommendation is that acid pretreatment is the best option for the pretreatment of cassava peels since it resulted in the highest sugar content of 14.5 % brix.

The optimal fermentation conditions to include a pH of 4.5, a temperature of 40 °C, a duration of 90 minutes and a yeast concentration of 0.09, as these conditions were found to produce the highest bioethanol yields.

Saccharomyces cerevisiae is sensitive to change in fermentation conditions. Therefore, once the optimum conditions have been established, it should be maintained to avoid a large K_m value and to ensure fast reaction rate (V_{max}).

Cassava peel bioethanol can be used as a substitute fuel source fuel due to its exceptional qualities. The bioethanol produced closely aligns with the recommended ASTM standards for bioethanol, making it a viable and sustainable option for fuel production.

5.2.2 Future works

Research to explore alternative solvents for extracting amylase from malted pearl millet grains, which is crucial for breaking down starch into simpler sugars for

fermentation. This could improve the utilization of malted grains in pre-treatment of cassava peels, contributing to more efficient and sustainable processing methods in the bioethanol industry.

Studying citric acid concentrations on cassava peels could increase fermentable sugar production. By manipulating citric acid concentrations, scientists can observe its impact on fermentation, breaking down complex carbohydrates into simpler sugars. This could optimize biochemical processes, transforming cassava peels into valuable bioethanol resource.

Optimizing hydrolysis conditions and exploring alternative processes can enhance sugar yield from pearl millet amylase enzyme extract and citric cassava pretreated peels. Pre-treatment breaks down lignocellulose biomass, releasing fermentable sugars, and enhancing enzyme accessibility to cellulose and hemicellulose components.

Investigating why citric pretreated cassava peels does not yield enough ethanol despite higher fermentable sugar content is crucial for optimizing bioethanol production from cassava waste. Factors beyond sugar content, such as citric acid interactions with cellulose, hemicellulose and lignin, and inhibitors introduced during citric pre-treatment, could provide insights.

Minerals can be added as cofactors in ethanol synthesis. Optimizing their concentration and availability in fermentation media can boost yeast metabolic activity and reduce inhibitor stress. Adsorbents like activated charcoal or resin can effectively remove inhibitors, creating an ideal environment for ethanol synthesis.

The study to explore the fermentation dynamics of citric acid and pearl millet amylase enzyme extract pretreated cassava peels. The Michaelis-Menten constant and maximum reaction velocity can explain the difference in ethanol yield, providing insights for optimizing cassava ethanol production.

Bioethanol, while meeting biofuel requirements, there is needs to improve its calorific value. Carboxymethyl cellulose (CMC) can increase ethanol's calorific value by adding more flammable material and increasing the carbon-to-hydrogen ratio. CMC

also improves fuel atomization and combustion efficiency, allowing for more thorough mixing of fuel with air.

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APPENDICES

Appendix I: MUT Research Authorization letter

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Ms. Odongo Irene Akinyi (AS401/5746/2021)
School of Pure, Applied and Health Sciences
Murang'a University of Technology,
P.O. Box 75-10,200
Murang'a.

Dear Ms. Odongo,

RE: APPROVAL OF RESEARCH PROPOSAL AND SUPERVISORS.

I am pleased to inform you that the Directorate of Postgraduate Studies on 25th March, 2024 considered and approved your Masters research proposal entitled "*Optimization of Bioethanol Production from Cassava Peels using Saccharomyces cerevisiae and Neurospora intermedia as Yeast Source*" and appointed the following as supervisors:

1. Dr. Ely Tetty Osewe – Murang'a University of Technology
2. Prof. Sylvia Opiyo - Murang'a University of Technology

You may now proceed with your data collection subject to obtaining research permit from NACOSTI, if required. You should also begin consulting your supervisors and submit through them quarterly progress reports to the Director Postgraduate Studies through your CoD and School Dean. Progress Reports forms can be accessed in the Student Portal.

It is the policy and regulations of the University that you observe deadlines. The guidelines on Postgraduate supervision can be accessed in the post graduate Handbook.

Your responsibilities as a student will include, among others;

- i. Maintain regular consultation with your supervisor(s), at least once a month
- ii. Submit quarterly reports on time, through your supervisors, CoD, Dean and to the Director of Postgraduate Studies;
- iii. Ensure quality work all through;



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- iv. Present your research findings at 2-3 seminars/conferences prior to thesis examination.
- v. Publish one article from your research findings in a refereed journal prior to thesis examination.

For any further clarification please contact the undersigned.

Yours Sincerely,


Prof. Geoffrey Muchiri, PhD
Director, Postgraduate Studies
GM/PIM



Copy to:

1. Registrar, Academics, Research and Students Affairs
2. Dean, School of Pure, Applied and Health Sciences



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Appendix II: Publication

The paper listed below has been published from this thesis

Odongo, I. A., Osewe, E. T., & Opiyo, S. A. (2024). Production And Characterization Of Bioethanol From Cassava Peels. *IOSR Journal of Applied Chemistry (IOSR-JAC)*, 17, (5), 7-13