PRODUCTION OF BIOETHANOL FROM OIL PALM TRUNK USING SACCHARIFICATION AND COFERMENTATION METHOD

BY

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CERTIFICATION

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DEDICATION

This project is dedicated to **Almighty God**, whose grace, wisdom, and strength made this work possible.

It is also lovingly dedicated to my parents and siblings, whose encouragement and sacrifices have been my greatest motivation throughout this academic journey.

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I give all glory and praise to **God Almighty** for His guidance, protection, and unfailing love throughout the period of this research work.

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NOMENCLATURE

- OPT – Oil Palm Trunk
- GHG – Greenhouse Gas
- GMOs – Genetically Modified Organisms
- VB – Vascular Bundles
- SSCF - Simultaneous Saccharification and Co-Fermentation
- SEM - Scanning Electron Microscopy
- PSSF - Pre-hydrolysis Simultaneous Saccharification and Fermentation
- SSF - Simultaneous Saccharification and Fermentation
- ZFN – Zinc Finger Nuclease
- TALEN – Transcription-like Effective Nuclease
- CRISPR/Cas9 – Clustered Regularly Interspaced Palindromic Sequences
- RAE – Renewable Energy Association
- NIFFER – National Institute for Freshwater Fisheries Research
- SA – Succinic Acid
- HTL – Hydrothermal Liquifaction
- AD – Anaerobic Digestion
- CSF – Combined Severity Factor
- AFEX – Ammonia Fibre Explosion
- SAA – Soaking in Aqueous Ammonia
- WAO – Wet Air Oxidation
- APAWAO – Alkaline Peroxide-assisted Wet Air Oxidation

- MTBE - Methyl Tertiary Butyl Ether
- NCYC – National Collection of Yeast Cultures
- RSM – Response Surface Methodology
- ILs – Ionic Liquids
- HMF – Hydroxymethylfurfural
- WDM – Wet Disk Milling
- HPLC – High Performance Liquid Chromatography

ABSTRACT

The increasing global demand for renewable and sustainable energy sources has intensified research into bioethanol production from non-food lignocellulosic biomass. This study investigates the production of bioethanol from oil palm trunk (OPT), an abundant agricultural residue generated during replanting cycles in oil palm plantations in Nigeria, to optimize pretreatment and fermentation conditions to maximize fermentable sugar yield and ethanol production efficiency.

Oil palm trunk samples were collected from Idogbo, Benin City, Nigeria, and processed through size reduction, drying, and sieving to a 500 µm particle size. Chemical composition analysis confirmed that OPT contains 29 to 45% cellulose, 12 to 29% hemicellulose, and 18 to 23% lignin, validating its suitability as a second-generation bioethanol feedstock. Pretreatment was carried out using dilute sodium hydroxide (NaOH) at a concentration of 20% to disrupt the lignocellulosic matrix and enhance cellulose accessibility for enzymatic hydrolysis.

Response Surface Methodology (RSM) based on a Box-Behnken design was employed to optimize three key pretreatment variables, namely acid concentration (1 to 6%), reaction time (10 to 120 minutes), and temperature (30 to 120°C), with fermentable sugar yield as the response variable. A total of 17 experimental runs were conducted, and the results were fitted to a quadratic model. Analysis of variance (ANOVA) confirmed the statistical significance of the model, with an F-value of 115.99 and a p-value of less than 0.0001. The model demonstrated excellent predictive accuracy, with a coefficient of determination (R^2) of 0.9933, an Adjusted R^2 of 0.9848, and an Adequate Precision ratio of 28.27, confirming a strong signal-to-noise ratio and reliable navigability of the design space. Acid concentration (A), reaction time (B), temperature (C), their interaction terms (AB and BC), and quadratic terms (A^2, B^2, and C^2) were all identified as statistically significant factors influencing sugar yield (p less than 0.05).

The optimum pretreatment condition was established at an acid concentration of 3.5%, a temperature of 120°C, and a reaction time of 120 minutes, yielding a maximum fermentable sugar concentration of 553.54 mg/g. Three-dimensional response surface plots demonstrated that sugar yield increased progressively with moderate acid concentration and rising temperature, but declined at extreme values due to thermal and acid-induced sugar degradation and the formation of inhibitory compounds, including furfural and hydroxymethylfurfural (HMF).

Enzymatic hydrolysis of the pretreated OPT biomass was performed using commercial cellulase enzymes, followed by fermentation with *Saccharomyces cerevisiae*. Fermentation performance was monitored over four days using the 3,5-dinitrosalicylic acid (DNS) colorimetric method at 610 nm. Sugar concentration decreased progressively from 3.8 mg/g on day one to 0.405 mg/g by day four, confirming active microbial metabolism and efficient conversion of released fermentable sugars into ethanol.

The findings of this study demonstrate that oil palm trunk is a technically viable and sustainable lignocellulosic feedstock for second-generation bioethanol production. The optimized pretreatment conditions effectively balanced lignin disruption and cellulose preservation, maximizing sugar recovery while minimizing inhibitor formation. The results support the potential of OPT waste valorization as a pathway toward renewable energy generation, reduced agricultural waste burden, and enhanced energy security in palm oil-producing regions of Nigeria. Future work should focus on co-culture fermentation systems capable of utilizing both hexose and pentose sugars, detailed techno-economic analysis, and life-cycle assessment to establish the commercial and environmental viability of large-scale OPT-based bioethanol production.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

The global transition from a fossil fuel-based economy toward a sustainable bioeconomy has intensified research into biorefinery technologies capable of converting renewable biomass into biofuels (Gajula et al., 2021). Within the biorefinery framework, diverse feedstocks, including sugar crops, starch, lignocellulose, aquatic biomass, oil-containing crops, and organic residues, are processed into a range of bioenergy products. Among these, bioethanol has emerged as a particularly promising renewable fuel, produced through the microbial fermentation of fermentable sugars derived from biomass. To avoid competition with food supply chains, waste materials and non-food crops have been proposed as viable alternative feedstocks for sustainable bioethanol production (Wardani et al., 2021).

The growing global demand for energy, coupled with the progressive depletion of fossil fuel reserves, has driven increased scientific interest in renewable energy alternatives. In this context, lignocellulosic biomass encompassing agricultural residues, forest by-products, and crop waste represents an abundant and low-cost feedstock for bioethanol production, with the potential to significantly reduce dependence on crude oil (Balat, 2011). Its widespread availability and comparatively low-cost relative to fossil fuels render it an economically attractive option for large-scale biofuel production.

Oil palm (*Elaeis guineensis*) is predominantly cultivated in tropical regions, particularly across Southeast Asian countries such as Indonesia, Malaysia, and Thailand. Palm oil, the primary derivative of oil palm, serves both as an edible commodity and as an industrial energy source, with the global palm oil market valued at over USD 50 billion annually (Research, 2014; Nutongkaew et al., 2019). Nigeria ranks fifth globally in palm oil production, contributing approximately 1.4 million metric tons in 2023, with projections of 1.5 million metric tons for the 2024/2025 season representing roughly 2% of global output (Statista; USDA Foreign Agricultural Service). Despite this steady growth, a significant supply-demand gap persists, as domestic consumption exceeds 3 million tons annually. The rapid expansion of the palm oil industry has generated substantial volumes of palm biomass waste, most notably oil palm trunk (OPT). OPT is a particularly attractive bioethanol feedstock, containing approximately 70% fermentable sugars and 30% (w/w) cellulosic residue. Oil palm trunks are typically felled after approximately 25 years of productive cultivation, generating considerable quantities of lignocellulosic biomass that are largely underutilized (Yamada et al., 2010). These characteristics position OPT as a promising second-generation bioethanol feedstock, offering dual benefits of agricultural waste valorization and renewable energy generation.

The conversion of lignocellulosic biomass into bioethanol involves several sequential processing stages: pretreatment (delignification), enzymatic saccharification to release fermentable hexose and pentose sugars, fermentation, and distillation for ethanol recovery (Kucharska et al., 2018). Pretreatment is a critical preparatory step, as the removal or disruption of lignin significantly enhances cellulose accessibility to hydrolytic enzymes and fermenting microorganisms (Alvira et al., 2010). Established pretreatment approaches include physical methods such as milling and grinding, chemical methods involving acids, alkalis, or organic solvents, and physicochemical methods combining both effects (Haldar & Purkait, 2021; Rodríguez, 2021). In the present study, three pretreatment methods, alkali, alkali-microwave, and alkaline peroxide, each combined with autoclave treatment, were evaluated for their effectiveness in delignifying OPT biomass. To maximize the utilization of both hexose and pentose sugars liberated during saccharification, a Simultaneous Saccharification and Co-Fermentation (SSCF) system was employed, using *Saccharomyces cerevisiae* NCYC 479 and *Pichia stipitis* NCYC 1541. The SSCF configuration offers several notable process advantages: the co-presence of yeast and enzyme complexes minimizes inhibitory glucose and cellobiose accumulation, thereby improving saccharification efficiency and ethanol yield; the use of a single bioreactor reduces capital investment; and rapid sugar consumption coupled with ethanol accumulation reduces the risk of microbial contamination (Ferreira et al., n.d.; Koppram et al., 2013; You et al., 2017). Key parameters governing the SSCF process include cell inoculation ratio, fermentation temperature, and fermentation time (Chauhan et al., 2021). *S. cerevisiae*, recognized for its high ethanol tolerance and robust industrial performance, was paired with *P. stipitis*, which is noted for its efficient xylose fermentation capability. The cell ratio between the two organisms directly influences the relative assimilation rates of glucose and xylose, making it a critical determinant of overall fermentation efficiency. To systematically optimize these parameters, Response Surface Methodology (RSM) coupled with Central Composite Design (CCD) was applied, enabling the identification of factor combinations that maximize ethanol yield (Chohan et al., 2020; Tesfaw et al., 2021).

Beyond process optimization, this study incorporates an assessment of energy consumption and greenhouse gas (GHG) emissions across the production process to develop a more efficient and environmentally responsible bioethanol production pathway from OPT waste.

1.2 PROBLEM STATEMENT

The production and conversion of raw biomass into biofuels offers considerable socioeconomic benefits, including the creation of local employment, regional economic development, and increased income for farming and forestry communities (Jamaludin, 2010). A common concern surrounding biomass-based fuel production is its potential competition with food supply chains; however, the present study circumvents this issue by utilizing waste residues from the palm oil industry rather than food or feed crops. The use of oil palm trunk (OPT) waste, a material regularly discharged from plantation sites at the end of the oil palm economic lifespan, ensures a consistent biomass supply without implications for food security (Yutaka et al., 2009).

Despite its potential, the conversion of OPT into bioethanol presents several technical and economic challenges that motivate this research. Oil palm trunks are typically felled after an economic lifespan of approximately 20–25 years, generating substantial quantities of biomass waste that currently remain underutilized and present environmental disposal challenges (Hambali et al., 2017). Although OPT contains considerable concentrations of fermentable sugars, its complex lignocellulosic structure, particularly its high lignin content, significantly reduces enzymatic digestibility and fermentation efficiency, thereby limiting bioethanol yield. The pretreatment processes required to disrupt the lignin matrix and enhance sugar accessibility are often associated with high capital expenditure and variable process efficiency. Additional operational challenges, including the recovery and recycling of fermenting microorganisms and enzymes, further compromise the economic viability of OPT-based bioethanol production. Moreover, the optimization of fermentation parameters to maximize ethanol yield from both the sap and cellulosic fractions of OPT remains an active area of investigation.

From an environmental standpoint, bioethanol utilization is associated with a net reduction in carbon dioxide emissions and improved valorization of agricultural waste streams. Nevertheless, production costs remain a significant barrier to commercial-scale implementation (Jamaludin, 2010). In particular, batch processing, the conventional mode of bioethanol production, is considerably more cost-intensive than continuous processing systems, in which the product is withdrawn progressively without repeated process interruption. Addressing these combined technical, economic, and environmental challenges is therefore essential for establishing OPT waste as a sustainable and cost-effective feedstock for renewable bioethanol production, with broader implications for energy security in palm oil-producing regions.

1.3 AIM AND OBJECTIVES

This study aims to investigate the optimization of bioethanol production from oil palm trunk (OPT) through acid pretreatment, enzymatic saccharification, and microbial co-fermentation, to achieve high ethanol yield and sustainable biofuel production.

The following objectives were set to achieve the aim of this study:

1. To reduce the size of the oil palm trunk (OPT)
2. To characterize the chemical composition of oil palm trunk (OPT) biomass, including cellulose, hemicellulose, lignin, and total fermentable sugar content, to assess its suitability as a feedstock for bioethanol production.
3. To carry out acid pretreatment of OPT biomass and evaluate its effect on the structural breakdown of lignocellulosic components and the release of fermentable sugars.
4. To perform enzymatic saccharification of the pretreated OPT biomass using cellulase enzymes and to determine the optimal enzyme loading, hydrolysis time, and temperature for maximum sugar yield.

5. To screen and select the most effective microbial strains, including *Saccharomyces cerevisiae* and other relevant co-fermenting organisms, for the fermentation of both hexose and pentose sugars derived from OPT hydrolysate.
6. To optimize co-fermentation process parameters, including initial pH, temperature, agitation rate, and fermentation time, using Response Surface Methodology (RSM) to maximize bioethanol yield.
7. To quantify bioethanol concentration and sugar conversion efficiency at the end of fermentation using High-Performance Liquid Chromatography (HPLC) and other standard analytical techniques.
8. To evaluate the techno-economic feasibility and environmental sustainability of the proposed bioethanol production process from OPT at a commercial scale.

1.4 SCOPE OF STUDY

This study focuses on the production of bioethanol from oil palm trunk (OPT) as the primary lignocellulosic biomass feedstock, employing saccharification and co-fermentation as the core processing strategies. The study is delimited to laboratory-scale investigations and encompasses the following key areas:

I. **Biomass Collection and Pretreatment:** The study covers the collection and processing of oil palm trunk biomass, including size reduction and acid pretreatment of OPT fiber. Pretreatment is carried out to disrupt the lignocellulosic matrix, thereby enhancing the accessibility and availability of fermentable sugars for enzymatic hydrolysis and subsequent fermentation.

II. **Chemical Composition Analysis:** The chemical characterization of pretreated OPT fiber is conducted to determine its total sugar content, including glucose, xylose, and other relevant constituents, as well as their cellulose, hemicellulose, and lignin fractions. This analysis is essential to establish the feedstock's suitability and potential for bioethanol production.

III. **Enzymatic Saccharification:** Enzymatic hydrolysis of the pretreated OPT biomass is performed using appropriate cellulase and hemicellulase enzyme preparations. This step focuses on the conversion of complex polysaccharides into simple, fermentable monomeric sugars, and includes the evaluation of enzyme loading, hydrolysis duration, and reaction temperature as key process variables.

IV. **Fermentation Design and Optimization:** The study encompasses the design and optimization of co-fermentation conditions using selected yeast strains, particularly *Saccharomyces cerevisiae,* where applicable, co-fermenting microorganisms capable of utilizing pentose sugars. Key fermentation parameters, including initial pH, temperature, and fermentation time, are systematically investigated to maximize bioethanol yield from OPT hydrolysate.

V. **Statistical Process Optimization:** Response Surface Methodology (RSM) is applied as the primary statistical optimization tool to model the interaction effects of fermentation parameters and to identify the optimal conditions for improved bioethanol production efficiency. Other relevant statistical tools may be employed where appropriate.

VI. **Analytical Measurement and Characterization:** Ethanol concentration and residual sugar content are quantified post-fermentation using High-Performance Liquid Chromatography (HPLC) and other standard analytical techniques. Sugar conversion rates and ethanol yield are computed to evaluate the overall efficiency of the saccharification and co-fermentation process.

VII. **Sustainability and Economic Assessment:** An assessment of the environmental sustainability and economic viability of utilizing oil palm trunk waste as a renewable feedstock for bioethanol production is carried out. This includes a preliminary cost analysis of the production process and an evaluation of the potential environmental benefits associated with converting agricultural residue into biofuel (Jamaludin, 2010).

1.5 RELEVANCE OF STUDY

Bioethanol remains the most widely produced and utilized biofuel globally, owing to its well-established process technology and the broad range of, raw materials suitable for its production. Beyond its energy value, bioethanol assists nations in developing strategic pathways toward reduced dependence on crude oil imports, while simultaneously enabling them to meet their targets for carbon dioxide (CO_2) emission reduction and contributing to job creation in rural agricultural communities (Gurjabn and Elvers, 2008).

The environmental consequences of continued reliance on fossil fuels have become increasingly evident on a global scale. Global CO_2 emissions from fossil fuel combustion witnessed a substantial escalation throughout the twentieth century, with fossil fuels accounting for approximately 90% of the world's total CO_2 emissions. Furthermore, fossil fuels and industrial processes collectively contributed roughly 78% of the overall growth in greenhouse gas (GHG) emissions between 1970 and 2011 (Talebi et al., n.d.; Uma et al., 2023).

In the Nigerian context, the cumulative impact of fossil fuel dependence on national carbon emissions underscores the urgent need for cleaner, renewable energy alternatives. In this regard, Nigeria occupies a strategically significant position at the intersection of palm oil production and bioenergy development. The country is actively exploring palm oil and its associated agricultural residues as potential feedstocks for clean, renewable bioenergy and biofuel production, with the dual objective of addressing national energy dependence and mitigating the environmental challenges associated with palm oil processing particularly waste generation and greenhouse gas emissions from Palm Oil Mill Effluent (POME) (Aworunse et al., 2023). Oil palm trunk (OPT), as an abundant lignocellulosic by-product of oil palm plantation management, represents a largely underutilized feedstock with considerable potential for bioethanol production. A biofuel program centered on the conversion of oil palm waste, including OPT biomass, into bioethanol, therefore offers a compelling proposition: the simultaneous production of a cleaner, economically viable, and renewable fuel, while reducing the environmental burden of agricultural waste disposal (Govindaswamy and Lane, 2010). This study is therefore relevant not only to Nigeria's renewable energy agenda but also to broader global efforts aimed at achieving sustainable biofuel production from non-food lignocellulosic biomass resources.

CHAPTER TWO

LITERATURE REVIEW

2.1 BRIEF HISTORY OF BIOETHANOL

The concept of using biological matter as fuel predates the modern biofuel industry. As early as 1925, Henry Ford envisioned plant-derived alcohol as the fuel of the future, famously asserting that fermentable vegetable matter could yield a superior and more affordable fuel than those known at the time. This vision was partially realized as early as 1908, when Ford developed the Model T, a vehicle engineered to operate on ethanol, gasoline, or blends of both (Bernton, Kovarik & Sklar, 1982).

Despite this early promise, bioethanol received little scientific or commercial attention through the early twentieth century, largely due to the low and stable prices of fossil energy resources. Interest was revived during the 1970s, when sharp rises in global petroleum prices, combined with the declaration of methyl tertiary butyl ether (MTBE), a common gasoline additive as a toxic environmental pollutant, created a compelling case for cleaner fuel alternatives. In response, the United States government initiated targeted research and development programs aimed at producing sustainable, cost-effective biofuels from renewable biomass. Between 1980 and 1990, these efforts were supported through farmer subsidies, tax exemptions, and dedicated bioethanol research funding. As a result of these policy interventions and technological advances, global biofuel production has grown substantially, rising from approximately 4.4 billion liters in 1980 to significantly higher volumes in subsequent decades. Today, biofuels, as a central component of green technology, are widely recognized as a promising and increasingly necessary alternative to fossil fuels, particularly in developing nations facing growing energy security challenges.

2.2 OVERVIEW OF BIOFUEL

Biofuels are defined as any fuel derived from organic material, including plant biomass, agricultural residues, crop by-products, and animal waste, and may be utilized in liquid, gaseous, or solid forms to meet diverse energy demands as substitutes for petroleum-derived fuels (Demirbas et al., 2016; Larson, 2008). As the world confronts the dual challenges of depleting fossil fuel reserves and escalating greenhouse gas (GHG) emissions, biofuels have emerged as a strategically important component of the global transition toward renewable and sustainable energy systems (Green & White, 2020).

Biofuels are broadly classified into three generations based on their feedstock and production technology. First-generation biofuels, derived from food crops such as corn and sugarcane, laid the technological foundation for the modern biofuel industry. However, concerns over food security, arable land competition, and feedstock sustainability prompted the development of second-generation biofuels, which utilize non-food lignocellulosic biomass such as agricultural residues and woody crops. More recently, third-generation biofuels derived from algae and other microorganisms have demonstrated considerable potential due to their high biomass yield and minimal land-use requirements (Mahapatra et al., 2021). Among liquid biofuels, bioethanol accounts for approximately 80% of global production, with biodiesel comprising the remainder. Liquid biofuels contribute to approximately 18% of primary energy consumption in the transportation sector (Demirbas et al., 2016; Wang et al., 2007).

Biofuels are chemically distinguished from conventional petroleum fuels by their elevated oxygen content, which ranges from 10% to 45%, compared to zero oxygen content in petroleum products. They also contain significantly lower levels of sulfur and nitrogen, contributing to cleaner combustion profiles and reduced atmospheric pollutant emissions. Despite these advantages, the biofuel industry continues to face challenges related to high production costs, land-use change, water resource management, and the scalability of biomass conversion technologies — areas that remain active frontiers of research and innovation (Renewable Fuel Standard, 2011; Thompson & Garcia, 2016).

2.2.1 TYPES OF BIOFUELS

Biofuels are broadly classified into three categories based on their physical state at ambient conditions: solid biofuels, liquid biofuels, and gaseous biofuels. Each category is distinguished by its feedstock composition, production pathway, and primary application domain.

2.2.1.1 Solid Biofuels

Solid biofuels consist of organic, non-fossil biomass of biological origin and find significant application in heat production, electricity generation, and industrial energy supply (Dahman et al., 2019). Common feedstocks for solid biofuel production include fuelwood, charcoal, wood pellets, wood residues, animal waste, and other renewable organic materials. Biochar is among the most notable examples of processed solid biofuels (Alalwan et al., 2019).

Wood and Lignocellulosic Biomass: Wood and other plant materials, including straw, grass, and agricultural residues, represent the most fundamental form of solid biofuel, utilized through direct combustion to generate heat and energy. Processed derivatives such as charcoal, sawdust, wood chips, and compressed pellets offer improved handling and energy density characteristics. These materials are widely employed for heating and cooking, particularly in rural communities and developing regions where modern energy infrastructure remains limited.

Pellet Fuels: Pellet fuels are densified biomass particles produced by compressing recycled wood waste, agricultural residues, or other organic biomass materials into uniform cylindrical forms. The densification process significantly improves the material's energy density, making it considerably easier to transport, store, and utilize in a variety of thermal systems, including stoves, furnaces, and industrial boilers.

2.2.1.2 Liquid Biofuels

Liquid biofuels are designed to serve as direct alternatives to conventional fossil-based liquid fuels such as gasoline and diesel, with primary applications in the transportation sector as well as in domestic cooking and heating. Compared to solid and gaseous biofuels, liquid biofuels offer considerable advantages in terms of high energy density, ease of storage, and compatibility with existing fuel infrastructure and engine technologies (Zhou et al., 2016). Liquid biofuels are broadly sub-classified into two feedstock-based categories: triglyceride-based biofuels, which include vegetable oils, biodiesel, hydrogenated oils, and bio-gasoline; and lignocellulosic-based biofuels, which encompass bio-oils, biomass-to-liquid (BTL) diesel, and drop-in biofuels. The principal examples of liquid biofuels are described as follows.

Bioethanol: Bioethanol (CH_3CH_2OH) is a renewable alcohol fuel produced through the microbial fermentation of fermentable sugars and starches derived from biomass feedstocks such as corn, sugarcane, and wheat, as well as from lignocellulosic materials such as agricultural residues and woody crops. It is commonly blended with gasoline in varying proportions (for example, E10 and E20 blends) to power internal combustion engines, increase octane rating, and reduce carbon monoxide and other smog-forming emissions. Bioethanol is the subject of the present study, with oil palm trunk serving as the lignocellulosic feedstock.

Biodiesel: Biodiesel is produced through transesterification, a chemical reaction between vegetable oils, animal fats, algal lipids, or recycled cooking grease and an alcohol (typically methanol) in the presence of a catalyst. The resulting product can be used in diesel engines either in pure form or blended with petroleum diesel at various ratios, most commonly at 20% (B20).

Biodiesel is both renewable and biodegradable, offering lower particulate and hydrocarbon emissions compared to conventional petroleum diesel.

Biobutanol: Biobutanol is produced from biomass feedstocks including corn, sugarcane, wheat, and cellulosic materials through microbial fermentation, most commonly employing bacterial strains such as *Clostridium acetobutylicum* or *Clostridium beijerinckii* via the acetone-butanol-ethanol (ABE) fermentation pathway, which yields biobutanol alongside acetone and ethanol as co-products. Biobutanol possesses a higher energy density than bioethanol and can serve as a direct substitute for gasoline in existing internal combustion engines without requiring engine modification (Biomass Energy in the Middle East, EcoMENA, 2023).

Bio-oil: Bio-oil is produced primarily through fast pyrolysis, a process in which biomass feedstocks such as crop residues, wood, algae, or municipal organic waste are rapidly heated in a low-oxygen environment. The resulting product is a dark brown, energy-dense liquid that can be utilized directly for heat and power generation or, following further chemical upgrading, as a potential substitute for petroleum-derived transportation fuels.

Methanol: Methanol is a clean-burning liquid fuel produced from biomass through high-temperature gasification, which converts solid biomass into a synthesis gas (syngas) that is subsequently catalytically converted into methanol. It finds application in specialized engine systems and as a chemical intermediate in biodiesel transesterification reactions.

2.2.1.3 Gaseous Biofuels

Gaseous biofuels are characterized by their low density and gaseous state under ambient conditions. They are produced from organic waste streams through thermochemical processes such as pyrolysis and gasification, or through biological processes such as anaerobic digestion. Notable examples include biogas, biohydrogen, and bio-syngas. Gaseous biofuels are commonly utilized in Otto cycle engines connected to electricity generators for combined heat and power (CHP) production. The principal types are described as follows.

Biogas: Biogas is generated through the anaerobic digestion of organic matter, including agricultural waste, animal manure, food waste, and landfill material. It is composed predominantly of methane (CH_4), typically at concentrations of 50 to 70%, with the remainder consisting largely of carbon dioxide (CO_2). Biogas can be utilized directly for heating and electricity generation or, after purification and upgrading to biomethane, as a vehicle fuel and natural gas substitute.

Syngas (Synthesis Gas): Syngas is a gaseous mixture of hydrogen (H_2) and carbon monoxide (CO) produced by the high-temperature gasification of biomass under controlled oxygen or steam conditions. It can be utilized directly for electricity generation or converted into liquid transportation fuels through Fischer-Tropsch synthesis and other catalytic processes, making it a versatile intermediate in the broader bioenergy value chain.

2.2.2 GENERATION OF BIOFUELS

Biofuels are classified into generations based on the nature of their biomass feedstock and the technology employed in their production. This generational framework reflects the progressive evolution of biofuel technology toward greater sustainability, efficiency, and reduced competition with food systems.

2.2.2.1 First-Generation Biofuels

First-generation biofuels are derived primarily from food crops and animal feed materials, including sugarcane, corn, vegetable oils, and animal fats. Their production relies on well-established processing technologies such as fermentation, distillation, and transesterification, and they are accordingly referred to as conventional biofuels (Jeswani et al., 2020; Oumer et al., 2018). Common examples include bioethanol produced from corn and sugarcane, and biodiesel produced from vegetable oils and animal fats.

The United States and Brazil collectively account for approximately 90% of global bioethanol output, with India ranking as a distant third-largest producer and consumer. The European Union leads global biodiesel production (Singh et al., 2024). While Nigeria possesses considerable agricultural resources and some existing bioethanol production capacity, it does not yet rank among the major global producers relative to these leading nations.

Despite their technological maturity, first-generation biofuels are constrained by significant sustainability concerns, including competition with food supply chains, pressure on arable land, and limited net greenhouse gas emission reductions. These limitations have driven research toward more advanced biofuel generations.

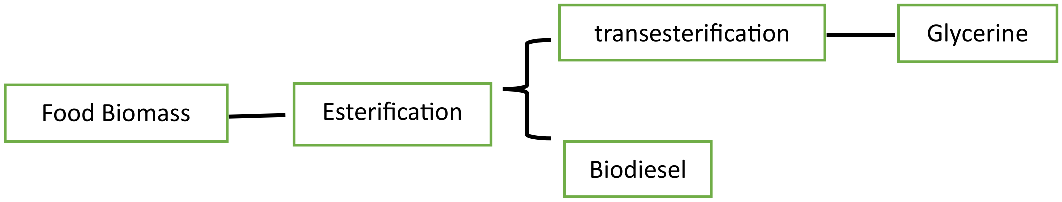

Figure 2.1: First-generation biofuel (Singh et al., 2024, fig.2).

2.2.2.2 Second-Generation Biofuels

Second-generation biofuels are produced entirely from non-food feedstocks, including dedicated energy crops, lignocellulosic plant materials, agricultural residues, forest residues, and organic waste streams (Jeswani et al., 2020). By utilizing non-food biomass sources, second-generation biofuels directly address the food-versus-fuel dilemma that constrains the sustainability of first-generation systems (Thompson and Garcia, 2016).

Compared to first-generation processes, second-generation biofuel production prioritizes both increased fuel recovery and the co-production of valuable secondary raw materials. Unlike first-generation systems, which focus solely on fuel output and discard non-fuel fractions as waste, second-generation processes are designed to minimize energy consumption, reduce waste generation, and improve process economics through biorefinery integration (Oumer et al., 2018). Advanced techniques such as membrane filtration and integrated biorefinery configurations are employed to enhance biofuel yield and process efficiency, utilizing a range of mesophilic and thermophilic microorganisms in both batch and continuous reaction systems.

Notable examples include cellulosic ethanol, derived from crop residues and wood chips through enzymatic hydrolysis and fermentation, and Fischer-Tropsch diesel, synthesized from biomass-derived syngas via thermochemical gasification. Lignocellulosic feedstocks such as oil palm trunk (OPT), rice straw, and sugarcane bagasse are abundantly available, low-cost, and do not compete with food supply chains, making second-generation bioethanol a particularly promising pathway for sustainable large-scale biofuel production.

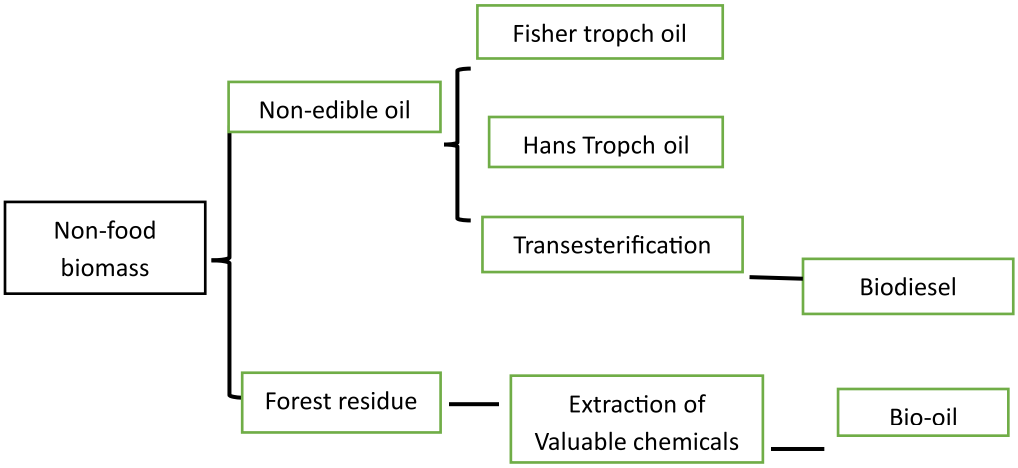

Figure 2.2 Second-generation biofuel (Singh et al., 2024, fig 3)

2.2.2.3 Third-Generation Biofuels

Third-generation biofuels are derived primarily from algae and other microorganisms through processes such as transesterification or hydrotreatment of algal oil (Jeswani et al., 2020). Unlike first- and second-generation feedstocks, algal biomass does not compete with food crops for arable land or water resources and can be cultivated under diverse environmental conditions using non-potable water and waste nutrient streams. Primary feedstock sources include microalgae, waste cooking oil, animal fats, and fish oil, all of which are readily available and do not interfere with food supply chains (D. Singh et al., 2020; Tariq et al., 2012).

Third-generation biofuels offer significantly higher biomass productivity per unit area compared to conventional food crops (Hafeez et al., 2020b). Algae-based production systems additionally offer environmental benefits through the valorization of nutrient-rich wastewater streams, thereby reducing water pollution and alleviating pressure on waste treatment facilities. Promising examples include algal biodiesel produced from microalgae cultivated in open pond or photobioreactor systems, and biohydrogen generated through algal bio-photolysis.

Both second- and third-generation biofuels remain largely in the research, pilot, and demonstration phases of development and are collectively referred to as advanced biofuels, reflecting their technological sophistication and departure from food-based feedstocks (Jeswani et al., 2020). Continued investment in advanced biofuel research is essential to overcoming existing technical and economic barriers.

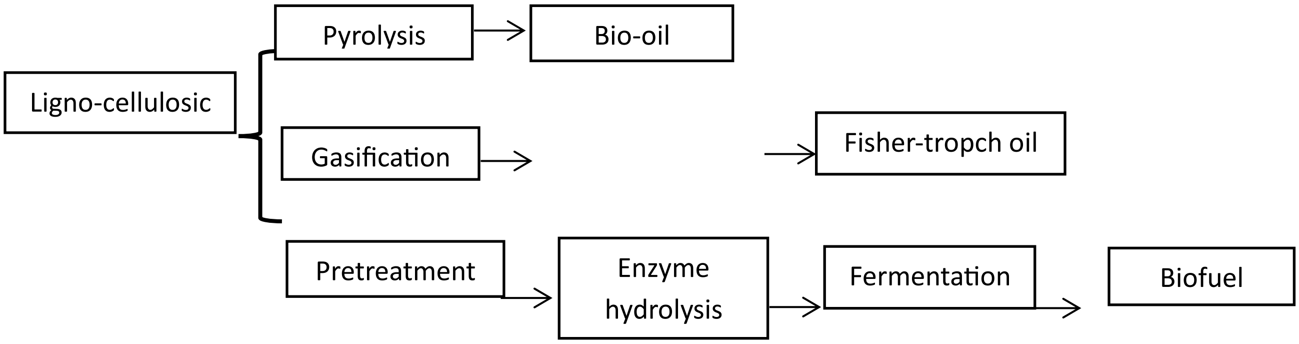

Fig 2.3 Figure 2.3Third-generation biofuel (Singh et al., 2024, fig.4)

2.2.2.4 Fourth-Generation Biofuels

Fourth-generation biofuels represent the most technologically advanced frontier in biofuel development, utilizing genetically modified organisms (GMOs), particularly engineered microalgae, alongside photobiological solar fuels and electro-fuels as primary production platforms (Abdullah et al., 2019; D. Singh et al., 2019). The defining characteristic of fourth-generation biofuels is their capacity to achieve net-negative carbon outputs through the capture and sequestration of carbon dioxide during the production process itself, positioning them as a potentially transformative tool in global climate change mitigation.

Genetic engineering of microalgal biomass enables significant improvements in photosynthetic efficiency, light penetration within cultivation systems, and oil extraction through induced cellular autolysis and product secretion pathways. The solar-based raw materials utilized in fourth-generation systems are widely available, economically accessible, and essentially inexhaustible (Abdullah et al., 2019). Genome editing technologies employed in this regard include zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), and the clustered regularly interspaced short palindromic repeats and associated protein 9 system (CRISPR/Cas9). These tools enable precise manipulation of microbial metabolic pathways to enhance lipid accumulation, improve stress tolerance, and optimize biofuel yields.

Despite their considerable potential, fourth-generation biofuels remain at the experimental and early developmental stage. Significant scientific, regulatory, and ethical considerations surrounding the open-environment deployment of genetically modified organisms must be resolved before large-scale commercial application becomes feasible.

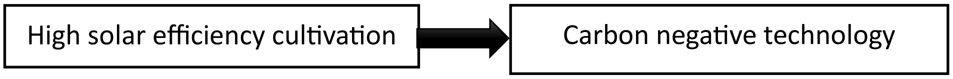

Figure 2.4 Fourth-generation biofuel (Singh et al., 2024, fig.5)

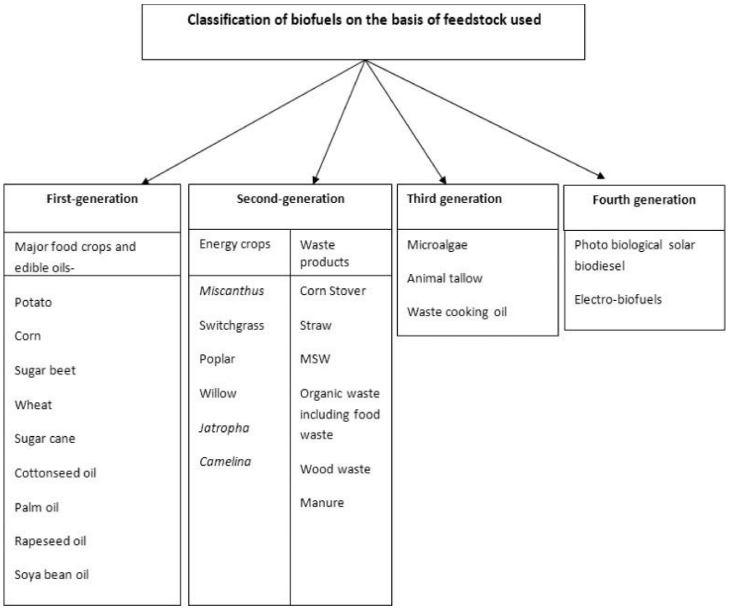

Figure 2.5 Summary of the classification of biofuels based on the feedstock used for production (Mahapatra et al., 2021, fig. 2)

2.2.3 SIGNIFICANCE OF BIOFUEL

Biofuels offer a range of technical, environmental, economic, and strategic advantages that underscore their growing importance as alternatives to conventional fossil fuels. The key areas of significance are discussed as follows.

I. Fuel Efficiency and Engine Compatibility

Biofuels possess superior lubrication properties and a higher cetane number compared to conventional petroleum diesel, resulting in more efficient combustion and reduced engine wear. They are largely compatible with existing internal combustion engine designs without requiring significant modification. When utilized as combustible fuels, particularly in the form of biodiesel, they have been shown to enhance engine durability and operational performance across a wide range of operating conditions (Singh et al., 2024).

II. Reduced Environmental Impact

Biofuels produce significantly lower net carbon emissions than conventional fossil fuels, as the carbon dioxide (CO_2) released during combustion is largely offset by the carbon absorbed by the biomass feedstock during its growth cycle. This closed-loop carbon cycle positions biofuels as a cleaner energy alternative with considerable potential for greenhouse gas (GHG) emission reduction in the transportation and energy sectors.

III. Renewability of Feedstocks

Unlike finite fossil fuel reserves, biofuel feedstocks, including agricultural residues, dedicated energy crops such as switchgrass and soybeans, animal manure, and crop waste, are renewable and can be continuously replenished through replanting and organic waste recovery. This inherent renewability ensures a long-term and sustainable supply of raw materials for biofuel production (Singh et al., 2024).

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V. Economic Stimulation and Rural Development

Biofuel production generates significant economic opportunities, particularly within rural and agricultural communities. The establishment of biofuel manufacturing facilities creates employment across the full value chain, encompassing feedstock cultivation, harvesting, processing, and distribution. Increased demand for biofuel crops further provides an additional and reliable revenue stream for the agricultural sector, stimulating rural economic development and supporting smallholder farming communities (Singh et al., 2024).

2.2.4 PRESENT STATUS OF BIOFUEL

Bioenergy has emerged as an increasingly dominant component of the global renewable energy landscape, contributing to electricity generation, industrial heat supply, and transportation fuel production. According to a report by the Renewable Energy Association (REA), bioenergy constitutes the United Kingdom's largest renewable energy source, contributing approximately 96.4% of non-domestic renewable electricity, 11% of non-domestic renewable heat, and 7.4% of total national energy consumption (REA, 2025). These figures reflect the maturity of bioenergy infrastructure in developed economies and the central role of biomass-derived biofuels in sustaining this growth trajectory.

In contrast, Nigeria's renewable energy sector has developed at a considerably slower pace. While cumulative primary energy consumption in Nigeria has risen steeply over recent decades, the growth of renewable energy capacity and generation has remained relatively modest and inconsistent with global trends (Renewable Energy Agency, 2019; Knoema, 2019; REN21 Secretariat, 2019). Nevertheless, since 2008, the share of renewable energy sources in Nigeria's energy mix, including biopower, solar photovoltaics (PV), and wind power, has appreciated noticeably, reflecting growing national awareness of and investment in sustainable energy development.

At the global level, the contribution of biofuels to power generation has exhibited a consistently increasing trend. Biofuels produced from diverse biomass feedstocks remain among the core drivers of renewable energy growth in both developed and developing economies. Their expanding share of global energy supply underscores the critical role that biofuel technology, including second-generation lignocellulosic bioethanol production as investigated in the present study, will play in achieving international climate change mitigation and energy security targets.

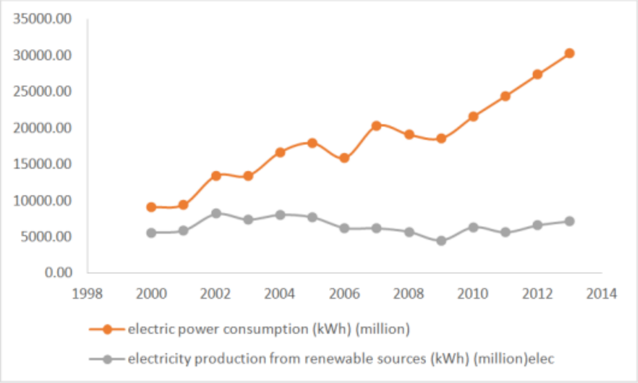

Fig. 1. Electricity consumption and renewable energy production in Nigeria from 2000 to 2014) [15]

Figure 2.6 View of Biofuel Development in Nigeria: Production and Policy Challenges

Fig. 2. Nigeria total primary energy consumption
Source (Knoema, 2019) [16]

Figure 2.7 View of Biofuel Development in Nigeria: Production and Policy Challenges

2.2.5 ADVANCEMENTS IN BIOFUEL PRODUCTION

2.2.5.1 Technological Advancements

Recent advances in enzyme engineering have significantly enhanced the enzymatic breakdown of lignocellulosic biomass into fermentable sugars, improving the efficiency and cost-competitiveness of cellulosic ethanol production. In parallel, the development of bio-electrochemical systems, including microbial fuel cells, has demonstrated promising potential for the direct conversion of organic waste streams into biofuels and electrical energy (Robinson and Clark, 2020). These technological innovations are progressively narrowing the economic gap between biofuel and fossil fuel production.

2.2.5.2 Feedstock Diversification

The range of viable biomass feedstocks for biofuel production has expanded considerably beyond traditional food crops such as corn and sugarcane to encompass non-food energy crops, agricultural residues, and algal biomass. Microalgae have attracted significant research interest owing to their exceptionally high lipid productivity, reported at up to 30 times greater fuel yield per unit area than conventional terrestrial crops, coupled with minimal land-use requirements. Advances in genetically engineered algal strains have further enhanced lipid accumulation and improved the commercial viability of algal biofuels.

2.2.5.3 Novel Conversion Technologies

Innovative thermochemical conversion methods, including pyrolysis and gasification, have expanded the range of biomass types that can be efficiently converted into biofuels and high-value chemical co-products. Recent studies have demonstrated that integrating biochar production into biofuel processing systems can simultaneously improve soil health and sequester atmospheric carbon, providing additional environmental co-benefits beyond fuel production alone (Singh et al., 2024).

2.2.6 CHALLENGES IN BIOFUEL PRODUCTION

2.2.6.1 Feedstock Availability and Competition with Food Production

A principal challenge confronting the biofuel industry is competition for feedstock resources with the food production sector. The utilization of food crops for biofuel production has raised serious concerns regarding food security and upward pressure on food prices. The availability of suitable arable land for dedicated energy crop cultivation is further constrained by competing demands from food production and biodiversity conservation. Logistical complexities associated with the harvesting, transportation, and storage of bulky, low-density biomass feedstocks add considerable cost to the overall supply chain (Singh et al., 2024). These limitations collectively underscore the importance of developing non-food lignocellulosic feedstocks, such as oil palm trunk (OPT), as sustainable and viable alternatives.

2.2.6.2 Technological and Economic Viability

Despite significant technological progress, many biofuel production processes remain economically uncompetitive relative to fossil fuel production. High capital and operational costs, inefficient biomass conversion processes, and comparatively low energy yields continue to hinder widespread commercial adoption. The underdevelopment of biofuel production and distribution infrastructure further compounds these economic constraints (Renewable Fuel Standard, 2011). Sustained investment in research and development, focused on improving conversion efficiency and reducing unit production costs, remains essential to overcoming these barriers.

2.2.6.3 Environmental Impacts and Sustainability

Although biofuels are widely promoted as a cleaner alternative to fossil fuels on account of their lower net greenhouse gas (GHG) emissions, the broader environmental consequences of biofuel production, including land-use change, high water consumption, and biodiversity loss, must be carefully evaluated on a case-by-case basis. The expansion of oil palm plantations for biodiesel production, for instance, has been associated with large-scale deforestation and habitat destruction in tropical regions. Comprehensive life cycle assessments (LCA) and the adoption of sustainable land management practices are therefore essential to ensuring that the environmental benefits of biofuels are not negated by the ecological costs incurred during their production.

2.2.6.4 Social and Political Challenges

Beyond technical and economic barriers, social and political factors present significant obstacles to the broader adoption of biofuels. Public perception and acceptance vary considerably across regions, influenced by cultural norms, media narratives, and environmental advocacy. The biofuel industry is further subject to complex and often uncertain regulatory frameworks, which can undermine investor confidence and distort market dynamics. Addressing these challenges requires transparent, inclusive, and science-informed policymaking, underpinned by coordinated collaboration among governments, industry stakeholders, and civil society organizations.

2.2.7 FUTURE PERSPECTIVES IN BIOFUEL DEVELOPMENT

2.2.7.1 Emerging Technologies and Ongoing Research

Advances in genetic engineering, synthetic biology, and bioprocess optimization are actively enhancing the efficiency and scalability of biofuel production systems. Researchers are engineering novel microbial strains capable of more efficiently converting lignocellulosic biomass into bioethanol, while the continued development of electrochemical and thermochemical conversion technologies is expanding the range of viable feedstocks and improving overall energy yields. These emerging technologies hold considerable promise for overcoming the current technical and economic limitations of biofuel production at a commercial scale.

2.2.7.2 Role of Policy and Economic Incentives

Policy frameworks and economic incentives will play a decisive role in shaping the future trajectory of the biofuel industry. Government mandates, including renewable fuel standards that require minimum proportions of transportation fuels to be sourced from renewable feedstocks, alongside targeted subsidies and tax incentives, constitute critical instruments for stimulating private sector investment in biofuel research, development, and commercialization. The long-term effectiveness of such policies, however, is contingent upon their alignment with broader sustainability objectives and their capacity to adequately address associated social and environmental concerns.

2.2.7.3 Sustainability Considerations

Achieving genuine environmental sustainability in biofuel production necessitates a holistic, supply-chain-wide approach that encompasses feedstock cultivation, conversion processing, and end-use consumption. Priority areas include minimizing land-use change, preserving natural ecosystems, and reducing the water and energy intensity of production processes. The promotion of advanced biofuels with demonstrably lower environmental footprints, such as cellulosic ethanol and algae-based biodiesel, represents a critical pathway toward mitigating the ecological impacts associated with conventional first-generation biofuel systems. Achieving this goal requires coordinated engagement among policymakers, industry stakeholders, and environmental organizations to develop the regulatory and operational frameworks necessary for sustainable large-scale biofuel production.

2.3 THE CONCEPT OF FOOD WASTE ASSOCIATED WITH BIOFUEL PRODUCTION

Food waste generation represents a significant and globally recognized socioeconomic challenge, with far-reaching implications for water resources, energy systems, environmental quality, and social equity (Kibler et al., 2018). The diversion of food crops toward biofuel production further intensifies this challenge by creating direct competition between fuel feedstock demand and human food supply chains, thereby exacerbating food insecurity and increasing the volume of food resources consumed by non-nutritive applications (Moioli et al., 2018).

Addressing food waste in the context of biofuel production requires coordinated interventions across multiple levels of governance. At the individual level, attention must be directed toward consumer behavior and household waste reduction practices. At the local level, effective governance frameworks and waste management systems must be monitored and enforced. At the national and international levels, appropriate policy instruments, regulatory investments, and institutional coordination are required to drive the systemic changes necessary for meaningful waste reduction. While the complete elimination of food waste, particularly where it intersects with biofuel feedstock demand, remains a long-term and ambitious target, significant reductions are achievable through the combined application of advanced waste management technologies and deliberate public policy interventions (Kibler et al., 2018).

These concerns have provided a strong impetus for the development of second- and third-generation biofuels, which utilize non-food biomass feedstocks, thereby decoupling biofuel production from food supply competition and offering a more sustainable and socially responsible production pathway.

2.3.1 BIOFUEL POTENTIAL IN NIGERIA

Nigeria's vast landmass of over 900,000 km^2, favorable tropical climate, and abundant agricultural resources position it as a nation with considerable potential for sustainable biofuel production. Despite this potential, certain feedstock categories, particularly marine-based biomass such as algae and aquatic macrophytes, remain largely underexplored in the national bioenergy discourse.

2.3.1.1 Aquatic Biomass: Water Hyacinth

Water hyacinth (*Eichhornia crassipes*) is a free-floating invasive aquatic macrophyte that has spread extensively across Nigerian waterways, having entered through the Porto-Novo creek from the Benin Republic via the Badagry creek. It is among the most prolific invasive plants documented, capable of growing up to 1 m in height with a leaf breadth of approximately 15 cm, doubling its population within 12 days, and remaining submerged for at least six months without adverse physiological effect (Wang et al., 2007). Of the 65 invasive species identified in Nigeria by the Global Invasive Species Database, water hyacinth is the most abundant, having infested lakes, ponds, rivers, marshes, and wetland habitats across more than 30 of Nigeria's 36 states, including the Federal Capital Territory, as reported by the National Institute for Freshwater Fisheries Research (NIFFR).

The ecological consequences of water hyacinth infestation are wide-ranging and severe, encompassing the obstruction of sunlight availability to native subaquatic plants and invertebrates, excessive dissolved oxygen depletion, alteration of water pH, restriction of water flow and navigability leading to flooding and transportation difficulties particularly in the Niger Delta region, stunted growth and mortality of fish populations, destruction of aquatic habitats, and loss of biodiversity and recreational resources. Various eradication strategies have been employed, including the introduction of over 3,000 *Neochetina* weevils for biological control since 1992, mechanical containment using floating barriers, and chemical treatment with glyphosate, the latter of which was subsequently outlawed due to its associated aquatic toxicity. Despite these interventions, water hyacinth populations have continued to proliferate, as existing control approaches are either cost-prohibitive or insufficiently sustainable at the required scale.

Notwithstanding its ecological nuisance, water hyacinth has demonstrated considerable beneficial potential. It has been shown to remove 24 to 80% of heavy metals from leachate at total metal concentrations of approximately 3 mg/L (Mahmood et al., 2010) and to eliminate over 90% of total nitrogen and phosphorus from agricultural effluent (Hadad et al., 2006). Most significantly for Nigeria's renewable energy sector, water hyacinth holds substantial potential for sustainable biofuel production, including fuel ethanol and biogas through anaerobic digestion, and offers the additional practical advantage of relative ease of harvest (Fermoso et al., 2016). The exploitation of this invasive weed as a bioenergy feedstock, therefore, presents a compelling dual benefit, namely the mitigation of its ecological impact and a meaningful contribution to Nigeria's renewable energy supply.

2.4 FEEDSTOCKS FOR BIOETHANOL PRODUCTION

The selection of an appropriate feedstock is a fundamental determinant of the technical feasibility, economic viability, and environmental sustainability of any bioethanol production process. Bioethanol feedstocks are broadly categorized into three groups based on their biochemical composition and the complexity of their conversion pathway: sugar-based feedstocks, including sugarcane and sugar beet, which provide directly fermentable sugars; starch-based feedstocks, including corn and cassava, which require enzymatic hydrolysis before fermentation; and lignocellulosic feedstocks, including agricultural residues, forest biomass, and dedicated energy crops, which necessitate pretreatment and saccharification before fermentable sugars can be recovered (Balat, 2011). Each feedstock category presents distinct advantages and limitations with respect to sugar yield, land-use requirements, processing cost, and competition with food supply chains.

Of the three categories, lignocellulosic feedstocks have attracted the greatest research interest in recent years, owing to their abundant availability, low cost, and independence from food supply chains. Oil palm trunk (OPT), the primary feedstock investigated in the present study, belongs to this category and represents a particularly promising second-generation bioethanol feedstock, as discussed in detail in subsequent sections.

2.4.1 OIL PALM TRUNK (OPT) AS A BIOETHANOL FEEDSTOCK

Oil palm trunk (OPT) is an abundant bioresource for bioethanol production, particularly its sap, which contains high levels of soluble sugars. While the sap can be directly fermented by *Saccharomyces cerevisiae*, the fibrous component of the trunk is lignocellulosic biomass and requires pretreatment methods such as alkali pretreatment to break down its recalcitrant structure. This process releases fermentable sugars, including glucose and xylose, for fermentation. Research indicates that storing felled trunks increases sugar content. Efficient use of both sap and fiber can be achieved through integrated approaches such as simultaneous saccharification and fermentation (SSF). (JIRCAS, 2008).

2.4.1.1 Advantages of OPT as a Feedstock

1. Abundant waste resource. Felled old oil palm trunks are considered waste and are readily available, especially in countries such as Malaysia and Indonesia, making them a sustainable feedstock for bioethanol.
2. High sugar content. Oil palm sap is rich in soluble sugars, including glucose, sucrose, and fructose. Storage of the trunks can further increase these sugar levels, providing a direct substrate for fermentation.
3. High cellulose content in fiber. The fibrous fraction of the trunk contains high cellulose content, which can be converted into fermentable sugars following appropriate pretreatment.

2.4.1.2 Composition and Potential

Oil palm trunk biomass is a lignocellulosic residue composed primarily of cellulose, hemicellulose, and lignin. It has potential applications in biofuel production, bio-composite materials, and biochar synthesis, as well as agricultural use as mulch and nutrient source. The high carbohydrate content of OPT makes it suitable for various biorefining processes, although the high moisture content is a key consideration for its utilization (Khalil et al., 2012).

Composition

Lignocellulosic material. OPT is rich in cellulose at approximately 50%, hemicellulose at about 25%, and lignin at about 25% (Khalil et al., 2012).

1. High moisture content. The biomass contains significant moisture, which can affect handling and processing (Eom et al., 2015).
2. Ash content. OPT also contains a small percentage of ash composed of inorganic mineral elements such as potassium, silicon, and aluminum (Evaluation of Nigerian Oil Palm Frond Biomass Potential as a Feedstock for Bioenergy Generation, 2023).

Potentials and Applications

1. Bioenergy production. The high lignocellulosic content makes OPT a valuable feedstock for thermochemical conversion methods, including combustion and torrefaction to produce bioenergy and biochar (Evaluation of Nigerian Oil Palm Frond Biomass Potential as a Feedstock for Bioenergy Generation, 2023).
2. Bio-composite materials. OPT fibers can be used in producing composite materials for construction, furniture, and transportation due to their anatomical and physical properties.
3. Bioethanol production. The carbohydrate-rich structure of OPT allows bioethanol production through fermentation and other biochemical processes.
4. Agricultural applications. Felled OPT can be used as mulch or fertilizer, although its slow decomposition may create a breeding ground for pests.
5. Biochar for catalyst synthesis. The lignocellulosic structure can be converted into high surface area mesoporous materials that show promise as solid acid catalysts for reactions such as the transesterification of used cooking oil.
6. Paper production. OPT fibers can serve as a source material for paper production.

7. Nutrient release. Carbohydrates in OPT undergo microbial degradation to release micro and macronutrients into the soil, thereby enhancing soil fertility.

2.4.1.3 Processing Considerations

1. Pretreatment methods such as steam explosion, acid hydrolysis, or alkaline hydrolysis are required to break down lignin and release sugars.
2. Enzymatic hydrolysis converts cellulose and hemicellulose into glucose and xylose for fermentation.

A major challenge involves optimizing pretreatment conditions to maximize sugar yield while minimizing the formation of inhibitory compounds.

2.4.2 COMPOSITION OF OIL PALM TRUNK (OPT)

Oil palm trunk is primarily composed of holocellulose, which includes cellulose and hemicellulose, along with starch, lignin below 20%, and trace quantities of free sugars. The raw material composition of OPT has been reported to contain 65 to 80% holocellulose and 18 to 21% lignin (Williams & Darwin Reese, 1950). The compositional distribution within the trunk is structurally heterogeneous. The vascular bundles are relatively enriched in alpha cellulose, while the parenchyma cells contain higher concentrations of starch and lignin. The inner regions of the trunk characteristically retain high moisture content. This substantial polysaccharide composition makes OPT a versatile and promising lignocellulosic feedstock for second-generation bioethanol production, as well as for applications in cattle feed and food-grade processing (Rabelo et al., 2011).

The high carbohydrate content of OPT makes it a particularly suitable feedstock for bioethanol production. The sap extracted from OPT is rich in fermentable sugars, predominantly glucose and sucrose, which can be efficiently converted to ethanol through microbial fermentation. Fermentable sugar concentrations in the trunk sap have been reported to exceed 85 g/L of glucose in certain trunk regions (Williams & Darwin Reese, 1950). Under optimized fermentation conditions using *Saccharomyces cerevisiae*, ethanol yields of up to 0.54 g per gram of sugar have been documented, corresponding to final ethanol concentrations of approximately 30 g/L and a product purity of nearly 95%. Furthermore, residual lignin and fermentation stillage generated during OPT processing represent viable secondary bioenergy streams, including solid fuel and methane-rich biogas, thereby supporting an integrated and sustainable biorefinery model.

2.5 METHODS OF BIOMASS CONVERSION

Biomass conversion into energy products is achieved through two principal process categories: thermochemical conversion and biochemical conversion. Both pathways yield three primary product streams: biofuels, heat, and electrical power. The choice of method depends on the nature of the feedstock, desired products, and process economics.

2.5.1 THERMOCHEMICAL CONVERSION

Thermochemical conversion employs heat and chemical processes to decompose biomass into energy-rich products. It is subdivided into the following methods.

2.5.1.1 Pyrolysis

Pyrolysis involves the thermal decomposition of biomass feedstock under anaerobic conditions in the absence of oxygen, breaking long-chain organic molecules into short-chain gaseous, liquid, and solid products. Fast pyrolysis has demonstrated particular promise in producing concentrated bio-oils and recovering liquid biofuels with medium to low calorific value. A key operational advantage of pyrolysis is the conversion of solid biomass into gases and vapors that are more convenient to handle, transport, and store. However, the process requires significant energy input to sustain the endothermic reactions necessary for syngas production (Ruan et al., 2019).

2.5.1.2 Carbonization

Carbonization, commonly referred to as slow pyrolysis, is primarily employed for charcoal production. The process is conducted through three principal methods: internal heating of raw materials with controlled combustion; external heating using fuelwood or fossil fuels; and the use of hot circulating gases for chemical production in retort or converter systems (Ruan et al., 2019).

2.5.1.3 Combustion

Combustion involves the direct burning of biomass in the presence of air to convert chemical energy into heat, mechanical power, or electricity. Process equipment employed includes stoves, furnaces, boilers, steam turbines, and turbo generators. Combustion products such as heat, power, or combined heat and power (CHP) are generated through consecutive heterogeneous and homogeneous reactions. Key process variables include feedstock particle size, combustion temperature, and atmospheric composition. The principal limitation of combustion is the relatively high emission of carbon dioxide, nitric oxide, particulate matter, and ash (Hassan et al., 2020).

2.5.1.4 Gasification

Gasification is a thermochemical process that converts biomass into energy rich gaseous products, primarily syngas and fuel gas, through partial oxidation at elevated temperatures. Syngas derived from gasification serves as a precursor for methanol and hydrogen production, both of which are valuable transportation fuels. The Biomass Integrated Gasification Combined Cycle (BIG/CC) system represents an important advancement in gasification technology, enabling gas cleaning prior to turbine combustion and resulting in a more compact and efficient energy conversion system (Patel et al., 2016).

2.5.1.5 Liquefaction

Liquefaction converts biomass or organic material into stable liquid hydrocarbons under conditions of low temperature and high hydrogen pressure. The high-pressure liquefaction of biomass yields bio-oils comprising complex mixtures of volatile organic acids, alcohols, aldehydes, ethers, esters, ketones, furans, phenols, and hydrocarbons. Catalytic liquefaction improves product energy density through the use of catalysts or elevated hydrogen partial pressures. However, significant technical challenges have limited the broader industrial application of this process (Ruan et al., 2019).

2.5.2 BIOCHEMICAL CONVERSION

Biochemical conversion utilizes microbial and enzymatic activity to convert biomass into biofuels and other value-added products. The two principal biochemical conversion pathways are fermentation and anaerobic digestion.

2.5.2.1 Fermentation

Fermentation is an anaerobic process widely employed for the commercial production of ethanol from sugar and starch-based crops, including sugarcane, sugar beet, and wheat. The process involves the enzymatic hydrolysis of saccharides into simple sugars, followed by their microbial conversion into ethanol, with subsequent purification by distillation. Solid residues generated during fermentation are not discarded but are valorized as cattle feed, boiler fuel, or gasification feedstock. Sugarcane is particularly preferred owing to its high productivity and residue energy potential (Ruan et al., 2019). For lignocellulosic biomass such as wood and grasses, the more complex polysaccharide structures necessitate acid or enzymatic pretreatment before fermentation to release fermentable sugars (Hassan et al., 2020).

2.5.2.2 Anaerobic Digestion

Anaerobic digestion involves the microbial decomposition of organic feedstocks in the absence of oxygen, generating biogas comprising primarily methane and carbon dioxide along with heat and hydrogen sulfide as byproducts. The process is conducted in sealed bioreactor tanks under controlled conditions over several days. Solid digestate remaining after digestion is applied as a soil conditioner and fertilizer, while the biogas is utilized as fuel for heat and electricity generation. Anaerobic digestion is widely regarded as one of the most energy-efficient and environmentally sound technologies for simultaneous waste treatment and renewable energy recovery (Adams et al., 2017; Ruan et al., 2019).

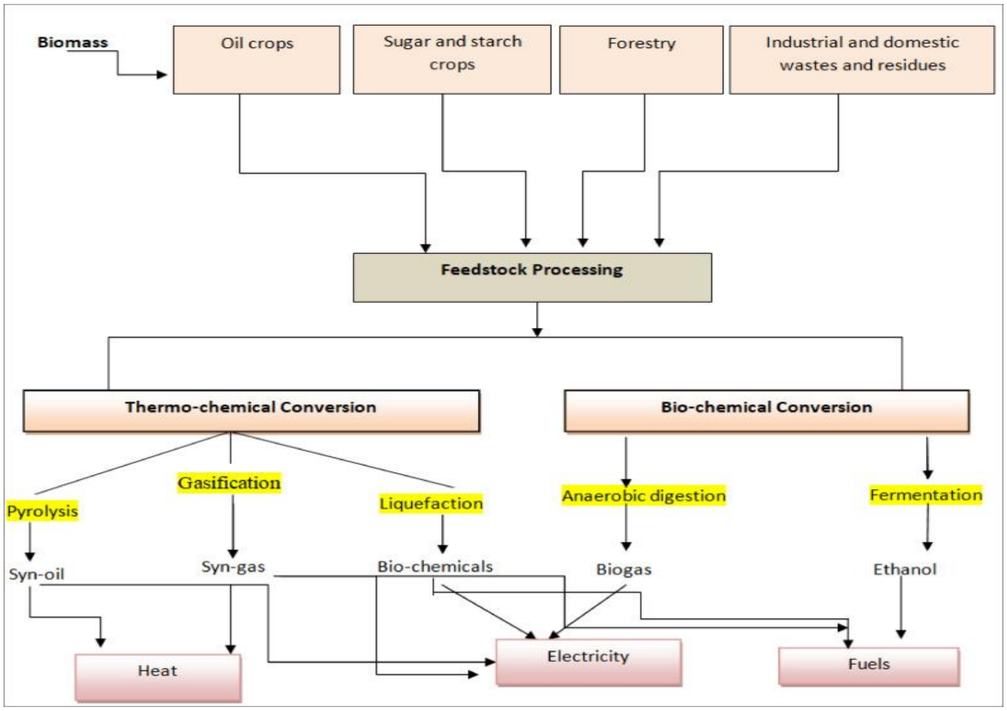

Figure 2.8 Overview of major biomass conversion (Mahapatra et al., 2021, fig. 5)

2.6 PRETREATMENT OF LIGNOCELLULOSIC BIOMASS

Pretreatment, also referred to as pre-hydrolysis, is the process through which the cellulose component of lignocellulosic biomass is exposed and rendered more susceptible to enzymatic hydrolysis. Pretreatment involves a synergistic interaction among thermal action, medium pH, and process exposure time, resulting in a reduction of cellulose crystallinity and consequently improving its accessibility to cellulase enzymes (Lynd et al., 2002). As a critical step in lignocellulosic bioethanol production, pretreatment serves to disrupt the complex architecture of plant cell walls, composed primarily of cellulose, hemicellulose, and lignin, thereby enhancing cellulose accessibility for enzymatic hydrolysis and subsequent fermentation. The effectiveness of the pretreatment step directly determines the fermentable sugar yield and the overall efficiency of the bioethanol production process.

2.6.1 CATEGORIES OF PRETREATMENT METHODS

Pretreatment methods are broadly classified into four categories: physical pretreatment, chemical pretreatment, physicochemical pretreatment, and biological pretreatment (Amin et al., 2017).

2.6.1.1 Physical Pretreatment

Physical pretreatment methods encompass mechanical operations, various forms of irradiation, and ultrasonic pretreatment, all of which are employed to enhance the accessibility of hydrolysable polymers within lignocellulosic materials. The primary objective of physical pretreatment is to reduce biomass particle size and cellulose crystallinity, thereby increasing the surface area available for enzymatic attack and improving mass transfer efficiency during subsequent processing stages. Among physical methods, mechanical pretreatment is the most widely applied, particularly for agricultural residues, crop residues, and forestry biomass.

Mechanical Milling and Grinding: Mechanical pretreatment of lignocellulosic biomass enhances bioconversion effectiveness by improving particle size distribution, increasing enzymatic accessibility, augmenting surface area, improving flow properties, and increasing bulk density and porosity, without generating toxic inhibitory byproducts (Barakat et al., 2014). A variety of milling equipment is employed to reduce particle size and cellulose crystallinity, including ball mills, hammer mills, knife mills, attrition mills, centrifugal mills, colloid mills, vibratory mills, pin mills, and extruders (Cheng and Timilsina, 2011). While mechanical milling is effective in improving biomass digestibility, it is associated with high energy consumption, which represents its principal operational limitation and a key consideration in process economics.

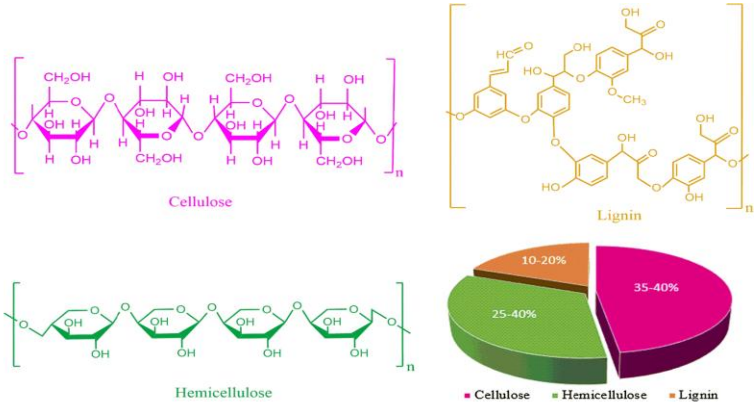

Figure 2.10 Lignocellulose composition: cellulose, hemicellulose, and lignin (Amin et al., 2017, fig.1)

Milling reduces crystallinity, particle size, and degree of polymerization of cellulose. For wheat straw, these structural changes, particularly the reduction in crystallinity, have been linked to increased digestibility during hydrolysis, as described by the following equation (O'Dwyer et al., 2008).

$$\text{Digestibility} = 2.04(\text{Specific surface area})^{0.99} \times (100-\text{Crystallinity index}) \times (\text{Lignin content})^{-0.39}$$

Eq.2.1

2.6.1.1.1 Thermal Treatment

Thermal treatment is a physical pretreatment approach that employs elevated temperatures to disrupt the complex structural architecture of lignocellulosic biomass, including agricultural residues, woody materials, and oil palm trunk, thereby facilitating subsequent enzymatic hydrolysis and fermentation for bioethanol production. The principal thermal treatment methods are described as follows.

Steam Explosion: Steam explosion involves exposing biomass to high-pressure steam at temperatures between 160°C and 260°C for a defined period, followed by rapid and sudden depressurization. The explosive decompression physically disrupts the lignocellulosic matrix, significantly improving biomass accessibility to hydrolytic enzymes.

Hydrothermal Liquefaction: Hydrothermal liquefaction is particularly suited to wet feedstocks such as algae and aquatic biomass. The process employs moderate temperatures in the range of 200°C to 350°C under elevated pressure in the presence of water to convert biomass directly into a liquid bio-crude oil (Hydrothermal Pretreatment of Biomass, n.d.).

Torrefaction: Torrefaction is a mild thermal treatment conducted at temperatures between 200°C and 300°C in a low-oxygen environment. The process removes moisture and volatile organic compounds from the biomass, yielding a brittle, hydrophobic solid product with improved grindability and transportability relative to raw biomass.

Pyrolysis: Pyrolysis involves the rapid thermal decomposition of biomass at temperatures between 300°C and 700°C in the complete absence of oxygen. The process yields three product streams: bio-oil, syngas, and solid biochar (How Biofuel Works, 2021).

2.6.1.1.2 Ultrasonication

Ultrasonication employs high-frequency sound waves to disrupt plant cell walls, facilitating the extraction and separation of hemicellulose, cellulose, and lignin from lignocellulosic biomass (Fernandes et al., 2023). While this method has demonstrated potential as a pretreatment tool, research on the susceptibility of lignocellulosic materials to enzymatic hydrolysis following ultrasonic treatment remains limited, and further investigation is required to establish its technical and economic viability for large-scale bioethanol production.

2.6.1.2 Chemical Pretreatment

Chemical pretreatment employs reactive chemical agents to disrupt the natural recalcitrance of lignocellulosic biomass, which arises from the rigid and protective role of lignin within the plant cell wall matrix. By breaking down this structure, chemical pretreatment renders cellulose and hemicellulose accessible to hydrolytic enzymes and fermenting microorganisms, enabling their conversion into fermentable sugars for bioethanol production. Common chemical agents employed in this regard include sulfuric acid (H_2SO_4), hydrochloric acid (HCl), acetic acid (CH_3COOH), sodium hydroxide (NaOH), potassium hydroxide (KOH), lime ($Ca(OH)_2$), aqueous ammonia ($NH_3 \cdot H_2O$), and hydrogen peroxide (H_2O_2) (González et al., 2005; Us and Perendeci, 2012).

2.6.1.2.1 Acid Pretreatment

Acid pretreatment involves the application of dilute or concentrated acid solutions, including acetic acid, hydrochloric acid, and sulfuric acid, to hydrolyze lignocellulosic biomass and improve the accessibility of cellulose for enzymatic saccharification (Monlau et al., 2013; Pakarinen et al., 2011). The process disrupts inter-component bonds within the biomass matrix, solubilizes hemicellulose, and reduces cellulose crystallinity, collectively resulting in enhanced enzymatic hydrolysis efficiency and increased fermentable sugar release (Li et al., 2009). The primary reaction involves the hydrolysis of hemicellulose, particularly xylan, which may generate inhibitory compounds such as furfural and hydroxymethylfurfural (HMF) as degradation products (Hendriks and Zeeman, 2008). Acid pretreatment is most effective at acid concentrations below 4% by weight, with dilute

H_2SO_4 at 2% and HCl at 2% reported to yield the highest methane outputs under optimized conditions (Song et al., 2014).

The reaction kinetics of acid pretreatment are critical for the selection of appropriate reactor configurations and operating conditions. Hemicellulose is converted to xylose via a first-order reaction, which subsequently degrades to furfuraldehyde and acetic acid at temperatures above 180°C. Complete biomass conversion with maximized sugar yield and minimized furfural formation is typically achieved through a two-stage process: slow hydrolysis of hemicellulose at low temperature (approximately 90°C) using concentrated acid, followed by rapid hydrolysis of the remaining biomass fraction at higher temperatures in the range of 120°C to 130°C (Amin et al., 2017b). The reaction pathways are represented as follows:

Hemicellulose → Xylose → Degradation Products (Furfuraldehyde) (Equation 2.2)

Fast hydrolyzing hemicellulose → Xylose → Degradation Products (Furfuraldehyde) (Equation 2.3)

Slow hydrolyzing hemicellulose (Equation 2.4)

Both inhibitor formation and the extent of lignocellulose hydrolysis are governed by pretreatment severity, which is quantified using the Combined Severity Factor (CSF). The CSF is influenced by acid concentration, temperature, and residence time, and is calculated as follows (Amin et al., 2017b):

$CSF = \log R_o - pH$ (Equation 2.5)

where: $R_o = t \times e^{((T_R - T_H) / 14.75)}$

Higher pretreatment severity increases the susceptibility of acid-pretreated biomass to cellulase activity, leading to improved glucose yields. For corn straw, increasing the CSF from 0.5 to 2.2 raised glucose release from 32% to 57% (Lloyd and Wyman, 2005). However, the generation of fermentation inhibitors in dilute acid-pretreated hydrolysates can compromise downstream fermentation performance. Additional limitations include the relatively high cost of dilute acid pretreatment compared to alternative methods, the corrosive and hazardous nature of acid reagents requiring specialized and costly reactor materials, and the added secondary treatment costs associated with acid recovery (Kumar et al., 2009; Mosier et al., 2004). Furthermore, the use of H_2SO_4 or HNO_3 has been reported to negatively impact methane yields in anaerobic digestion applications (Hendriks and Zeeman, 2008).

2.6.1.2.2 Alkali Pretreatment

Alkali pretreatment involves the addition of alkaline agents to lignocellulosic biomass, causing swelling of the internal structure, reduction in cellulose polymerization and crystallinity, disruption of lignin, and cleavage of linkages between lignin and other structural polymers (Badiei et al., n.d.). This method is most effective for biomass feedstocks with relatively low lignin content (Sun et al., 2002). Sodium hydroxide (NaOH) and potassium hydroxide (KOH) are the most widely employed alkali agents for improving biomass digestibility. NaOH pretreatment has demonstrated consistent effectiveness in enhancing digestibility and increasing methane yield (Zheng et al., 2009), while KOH offers a potential advantage in applications where sodium discharge into effluent streams is a concern (Jaffar et al., 2016).

2.6.1.2.3 Organosolvent Pretreatment

The organosolvent process employs organic or aqueous organic solvent mixtures, with or without inorganic acid catalysts, to selectively extract lignin from lignocellulosic biomass. Solvents commonly used include methanol, ethanol, acetone, ethylene glycol, triethylene glycol, and tetrahydrofurfuryl alcohol, while organic acids such as oxalic, acetylsalicylic, and salicylic acid may serve as catalysts at elevated temperatures (Sarkanen, 1980; Zhao et al., 2009). Glycerol-based autocatalytic organosolvent pretreatment of wheat straw achieved removal of 70% of hemicellulose and 65% of lignin, with 98% cellulose retention (Sun et al., 2008). A modified ethanol-based organosolvent method combined with hydrogen peroxide post-treatment of horticultural waste yielded a hydrolysate containing 26.9 g/L reducing sugars, from which fermentation using *Saccharomyces cerevisiae* produced 11.69 g/L ethanol (Geng et al., 2012). The combination of alcohol-based organosolvent treatment with ball milling (BM) has been reported to synergistically improve the enzymatic digestibility of Japanese cypress (*Chamaecyparis obtusa*) while reducing the severity of treatment required (Hideno et al., 2013). The organosolvent process is capable of achieving approximately 90% enzymatic hydrolysis efficiency due to effective lignin removal, while simultaneously yielding high-quality lignin as a value-added co-product.

The principal limitation of this process is the high cost of organic solvents and catalysts. Solvent recovery through distillation can partially offset operational costs; however, the flammability of organic solvents necessitates stringent safety measures, adding to process expenditure. Furthermore, residual organic solvents act as inhibitors of enzymatic hydrolysis and must be thoroughly removed before downstream processing (Mosier et al., 2004; Sun and Cheng, 2002).

2.6.1.2.4 Ozonolysis

Ozonolysis employs ozone (O_3), a powerful oxidizing agent, to selectively degrade lignin by attacking its aromatic ring structures, while leaving cellulose and hemicellulose largely unaffected. It has been applied to a range of lignocellulosic materials, including wheat straw, sugarcane bagasse, pine, peanut, cotton straw, rye straw, and poplar sawdust (García-Cubero et al., 2009; Sun and Cheng, 2002). The process is typically conducted at ambient temperature and pressure and does not generate toxic residues that would inhibit subsequent hydrolysis or fermentation. Ozone gas is passed through a reaction vessel containing the biomass substrate, which may be configured as packed beds, fixed beds, or stirred semi-batch reactors.

Biomass moisture content significantly influences ozonolysis efficiency. Miura et al. (2012) reported that moisture content exceeding 40% reduced ozone consumption and resulted in diminished delignification of Japanese cedar (*Cryptomeria japonica*). Combined application of ozonolysis and wet disk milling (WDM) increased glucose and xylose yields to 68.8% and 43.2%, respectively, without adversely affecting mannose yield. The principal limitation of ozonolysis is the large quantity of ozone required, which renders the process costly at scale (Sun and Cheng, 2002).

2.6.1.2.5 Ionic Liquid Pretreatment

Ionic liquid (IL) pretreatment employs ionic liquids at a biomass-to-solvent ratio of 1:10 (w/w) and temperatures between 100°C and 150°C to dissolve and fractionate lignocellulosic biomass. Ionic liquids are salts composed of large organic cations and small inorganic anions that exist as liquids at relatively low temperatures. The anions present in ILs, including chloride, formate, acetate, and alkyl phosphonate, form hydrogen bonds with cellulose at elevated temperatures, disrupting its crystalline structure and enabling high cellulose digestibility of over 90% (Lee et al., 2009). Following dissolution, antisolvents such as water, methanol, or ethanol are used to regenerate the solubilized biomass, which is subsequently subjected to enzymatic hydrolysis to produce fermentable sugars.

Despite their considerable potential, IL pretreatments are associated with several limitations. Residual ILs remaining in the pretreated biomass can inhibit hydrolytic enzyme activity and impair downstream fermentation performance. Recovery of ILs from antisolvents by flash distillation is feasible but energy-intensive, and the development of cost-effective recycling methods remains a prerequisite for large-scale application. Toxicity toward cellulase enzymes and fermentative microorganisms must also be thoroughly assessed before industrial deployment (Zhao et al., 2009; Maurya et al., 2015).

2.6.1.3 Physicochemical Pretreatment

Physicochemical pretreatment methods combine physical and chemical mechanisms to achieve more effective disruption and fractionation of lignocellulosic biomass than either approach can accomplish independently.

2.6.1.3.1 Steam Explosion (Autohydrolysis)

Steam explosion is among the most widely applied and extensively studied pretreatment methods for lignocellulosic biomass (Babel et al., 2007; Singh et al., 2015). The process involves treating chipped biomass with high-pressure saturated steam at temperatures of 160°C to 260°C for durations ranging from a few seconds to approximately 20 minutes, followed by sudden depressurization. The combined mechanical and chemical effects of steam explosion arise from the physical disruption caused by explosive decompression and the autohydrolysis of hemicellulose acetyl groups, which generates acetic acid at elevated temperatures and promotes further hemicellulose solubilization (Quiévy et al., 2010; Pan et al., 2005). The severity of the process is quantified using the severity parameter (R_0):

$$R_0 = t \times \exp[(T - 100) / 14.75]$$

where t is the reaction time in minutes, and T is the hydrolysis temperature in degrees Celsius. Optimal conditions for maximum sugar yield correspond to severity parameter values in the range of 3.0 to 4.5 (Alfani et al., 2000).

Key process parameters include biomass particle size, temperature, residence time, and moisture content. Steam explosion offers several advantages over alternative pretreatment technologies, including significant improvement in enzymatic hydrolysis efficiency, lower environmental impact, reduced capital investment, and high sugar recovery (Avellar and Glasser, 1998). It is recognized as one of the most cost-effective pretreatment options for hardwoods and agricultural residues; however, it is less effective for softwoods due to their low acetyl group content. The addition of SO_2 or H_2SO_4 has been proposed to improve performance on softwood substrates (Berlin et al., 2006; Kumar et al., 2012).

The principal limitations of steam explosion include partial hemicellulose degradation, the generation of fermentation inhibitors such as furfural and hydroxymethylfurfural (HMF), and high energy consumption associated with achieving the required chip size before treatment (Mosier et al., 2004; Hamelinck et al., 2005). Where inhibitor concentrations are significant, a dedicated detoxification step, such as treatment with activated charcoal, over-liming, or ion exchange, may be necessary, thereby increasing overall process cost (Schmidt and Thomsen, 1998; Yang and Wyman, 2008).

2.6.1.3.2 Ammonia Fiber Explosion (AFEX)

Ammonia fiber explosion (AFEX) is a physicochemical pretreatment in which lignocellulosic biomass is treated with liquid ammonia at temperatures of 90°C to 100°C for 30 to 60 minutes, followed by rapid pressure release (Kim et al., 2011). The sudden depressurization causes rapid expansion of liquid ammonia, resulting in swelling, physical disruption of biomass fibers, and partial de-crystallization of cellulose. AFEX effectively modifies or reduces cellulose crystallinity and the lignin fraction of lignocellulosic materials (Laureano-Perez et al., 2005) without generating fermentation inhibitors, thereby eliminating the need for a water wash step before downstream processing.

AFEX is most effective for herbaceous and agricultural residue feedstocks and demonstrates limited performance on woody biomass and high-lignin feedstocks. AFEX-pretreated corn stover achieved 70% glucan conversion after 72 hours of hydrolysis, while ethanol fermentation at 6% w/w glucan loading yielded 93% theoretical ethanol yield (Teymouri et al., 2005; Uppugundla et al., 2014). Under optimized conditions of ammonia loading, temperature, moisture content, and residence time, AFEX can achieve over 90% conversion of cellulose and hemicellulose to fermentable sugars across a broad range of lignocellulosic feedstocks. The high volatility of ammonia facilitates its recovery and recycling after pretreatment (Sendich et al., 2008), though ammonia recovery costs remain a significant consideration for commercial-scale application (Mosier et al., 2004).

A related ammonia-based method, ammonia recycled percolation (ARP), passes aqueous ammonia at concentrations of 5 to 15 wt% through a packed bed reactor containing biomass at temperatures of 140°C to 210°C for 90 minutes. ARP effectively solubilizes and removes both hemicellulose and lignin as liquid-phase products (Yang and Wyman, 2008). Soaking in aqueous ammonia (SAA) at lower temperatures of 40°C to 90°C for extended reaction times has been employed to preserve glucan and xylan fractions for subsequent simultaneous saccharification and co-fermentation (SSCF) (Kim et al., 2008).

2.6.1.3.3 CO_2 Explosion

CO_2 explosion employs supercritical carbon dioxide as a pretreatment medium, exploiting its gas-like mass transfer properties and liquid-like solvating capacity to enhance biomass delignification and improve enzymatic digestibility. Supercritical CO_2 pretreatment has been shown to effectively remove lignin from both hardwood and softwood substrates, and its performance can be further improved by the addition of co-solvents such as ethanol (Kim and Hong, 2001). In aqueous conditions, CO_2 dissolves to form carbonic acid, which increases the hydrolysis rate of hemicellulose. The molecular size of CO_2 is comparable to that of water and ammonia, enabling penetration into small biomass pores and increasing the accessible surface area for enzymatic attack (Schacht et al., 2008). Compared to steam explosion, CO_2 explosion generates fewer fermentation inhibitors, and compared to ammonia explosion, it is more cost-effective (Zheng et al., 1998).

2.6.1.3.4 Oxidative Pretreatment

Oxidative pretreatment involves the addition of an oxidizing agent, most commonly hydrogen peroxide (H_2O_2) or peracetic acid ($C_2H_4O_3$), to water-suspended biomass to solubilize lignin and hemicellulose and improve cellulose accessibility. Treatment with 1 to 2% H_2O_2 at 25°C to 30°C has been shown to dissolve approximately 50% of lignin and most of the hemicellulose, with solubilization efficiency reported to be approximately five times greater than that achieved by NaOH treatment alone (Chaturvedi and Verma, 2013). Dilute alkaline peroxide pretreatment of rice hulls achieved near-complete conversion (96%) of the substrate to fermentable sugars following enzymatic hydrolysis (Saha and Cotta, 2007).

Wet oxidation is a related approach suited to high-lignin feedstocks, in which biomass is treated with water and air or oxygen at temperatures above 120°C for a minimum of 3 minutes (Varga et al., 2004). At temperatures above 170°C, the process becomes exothermic and self-sustaining. Wet oxidation promotes hydrolytic cleavage of hemicellulose into low molecular weight soluble sugars, oxidative cleavage and solubilization of lignin, and partial degradation of cellulose, rendering it highly susceptible to enzymatic hydrolysis (Schmidt and Thomsen, 1998). The addition of alkaline agents such as sodium carbonate assists hemicellulose solubilization and minimizes the formation of furan-based inhibitory degradation products (Ahring et al., 1996).

Studies on common reed (*Phragmites australis*) demonstrated solubilization of 51.7% hemicellulose and 58.3% lignin, with 87.1% cellulose retention in the solid fraction. Optimized wet oxidation conditions at 185°C for 12 minutes increased cellulose digestibility more than threefold, achieving 82.4% cellulose-to-glucose conversion, and simultaneous saccharification and fermentation yielded an ethanol concentration of 8.7 g/L at 73% of theoretical yield (Szijártó et al., 2009). The principal limitations of wet oxidation are the high temperature and pressure requirements, the use of strong oxidizing agents, and the associated costs of large-scale reactor maintenance and oxygen supply.

2.6.1.3.5 Microwave Pretreatment

Microwave pretreatment employs microwave irradiation to rapidly and homogeneously heat lignocellulosic biomass, degrading lignin and hemicellulose and increasing the enzymatic susceptibility of cellulose. Residence times are typically in the range of 5 to 20 minutes (Zhu et al., 2006). NaOH is the most effective alkali reagent for microwave-assisted pretreatment, with sugar yields of 70 to 90% reported for switchgrass under optimized conditions (Hu and Wen, 2008). Microwave pretreatment of wheat straw under an orthogonal experimental design yielded 148.93 g ethanol per kg of wheat straw, significantly exceeding the yield of untreated material at 26.78 g/kg (Lindroos, 2011). Treatment of oil palm empty fruit bunch fiber under microwave-assisted alkaline conditions resulted in 74% lignin removal, 24.5% holocellulose loss, and a total reducing sugar yield of 41% (Nomanbhay et al., 2013).

The principal advantages of microwave pretreatment are its short reaction times, uniform heating of the reaction mixture, and potential for minimizing inhibitor generation. The microwave approach can be further enhanced through combination with chemical additives to improve sugar yield, and is considered one of the most promising pretreatment methods for altering the native lignocellulosic structure and improving enzymatic saccharification efficiency (Lu et al., 2011).

2.6.1.4 Biological Pretreatment

Biological pretreatment is considered an environmentally benign and cost-effective alternative to chemical and physicochemical pretreatment methods, requiring no chemical inputs and operating under mild environmental conditions (Wan and Li, 2012). It employs cellulolytic and hemicellulolytic microorganisms, predominantly filamentous fungi isolated from soil, living plants, or lignocellulosic waste materials, to selectively degrade lignin and improve biomass digestibility (Vats et al., 2013).

White-rot fungi are the most effective microorganisms for biological pretreatment, producing lignin-degrading enzymes including lignin peroxidases, manganese peroxidases, and laccases (Kumar and Wyman, 2009). Species such as *Phanerochaete chrysosporium*, *Ceriporia lacerata*, *Cyathus stercoreus*, *Ceriporiopsis subvermispora*, *Pycnoporus cinnabarinus*, and *Pleurotus ostreatus* have demonstrated high delignification efficiency across various lignocellulosic substrates (Kumar and Wyman, 2009; Shi et al., 2008). *Ceriporiopsis subvermispora*, acting through the combined activity of manganese peroxidase and laccase, achieved glucose yields of 24.2 to 56.5% during enzymatic hydrolysis, representing a two to threefold improvement over untreated raw materials (Wan and Li, 2012). Biological pretreatment of rice husks using *Phanerochaete chrysosporium* yielded 44.7% reducing sugars (Potumarthi et al., 2013). Among white-rot basidiomycetes evaluated for wheat straw pretreatment, *Trametes versicolor* demonstrated superior performance in enzymatic hydrolysis of holocellulose (Pinto et al., 2012). *Streptomyces griseus* has also been reported as effective for the treatment of both hardwood and softwood feedstocks (Thakur et al., 2020).

At high substrate concentrations, saccharification and fermentation processes may generate elevated concentrations of inhibitory compounds, including furan derivatives and phenolic compounds. Treatment with laccase enzymes has been proposed as a strategy to mitigate inhibitor formation under such conditions (Alvira et al., 2010). The key advantages of biological pretreatment include low capital cost, minimal energy requirements, absence of chemical inputs, and operation under mild conditions. The principal limitation is the slow rate of hydrolysis, which constrains process throughput and limits industrial applicability (Sun and Cheng, 2002). Continued screening of basidiomycete fungal isolates for rapid and efficient delignification capacity remains an important area of ongoing research.

2.6.2 PRETREATMENT METHODS FOR OIL PALM TRUNK (OPT)

Pretreatment of oil palm trunk (OPT) is a critical processing step necessary to overcome its inherent lignocellulosic recalcitrance, thereby enabling efficient enzymatic hydrolysis and conversion of structural polysaccharides into fermentable sugars for bioethanol production (Wardani et al., 2021). The primary pretreatment methods investigated for OPT, along with their mechanisms, effectiveness, and associated limitations, are discussed as follows.

2.6.2.1 Alkaline Peroxide Combined with Autoclave Treatment

In this method, dried and ground OPT biomass, with the vascular bundle (VB) fraction isolated by sieving through an 80-mesh screen, is mixed with a 5% (v/v) hydrogen peroxide (H_2O_2) solution. The pH is adjusted to approximately 11.5 using sodium hydroxide (NaOH) to establish an alkaline peroxide environment. The mixture is incubated at room temperature for approximately 3 days to allow reaction with lignin and hemicellulose, after which it is autoclaved at 121°C and 1 atm for 15 minutes to enhance delignification and further disrupt the biomass structure. The treated biomass is subsequently washed with distilled water to neutral pH and dried at 105°C for 48 hours prior to enzymatic hydrolysis. Simultaneous saccharification and co-fermentation (SSCF) is then performed using a co-culture of *Saccharomyces cerevisiae* and *Pichia stipitis* to ferment both hexose and pentose sugars into ethanol.

This pretreatment has demonstrated lignin removal of up to 83.26%, cellulose retention of approximately 80.74%, and fermentable sugar yields of up to 93.22% following enzymatic hydrolysis. Scanning electron microscopy (SEM) analysis has confirmed significant structural disruption of the OPT matrix, improving enzyme accessibility. Key advantages include effective lignin removal under moderate temperature and pressure conditions, environmental compatibility of H_2O_2 as an oxidant, as it decomposes into water and oxygen, and the production of a substrate directly suitable for SSCF. Limitations include the extended 3-day room temperature incubation period, the requirement for precise pH and peroxide concentration control, the generation of wastewater requiring treatment, and challenges associated with industrial-scale autoclave operation.

2.6.2.2 Steam Explosion Combined with Alkaline Extraction

This two-step pretreatment combines the physical disruption of steam explosion with the chemical delignification of alkaline extraction to enhance the enzymatic digestibility of OPT biomass. In the first step, OPT chips are subjected to high-pressure saturated steam at approximately 210°C for approximately 4 minutes, followed by rapid depressurization. The explosive decompression physically disrupts the biomass structure, primarily removing hemicellulose and partially breaking down lignin to increase cellulose accessibility. In the second step, the steam-exploded biomass is treated with a NaOH solution at a concentration of 15% at 90°C for 60 minutes, achieving further delignification of up to 71.67% and reducing residual lignin content to approximately 6.13%, while increasing the alpha-cellulose content to up to 87.14%.

The combined pretreatment significantly improves enzymatic hydrolysis efficiency and ethanol yield compared to either untreated biomass or single-step pretreatment. Advantages include high delignification efficiency, reduced fermentation inhibitor formation relative to acid pretreatments, and rapid processing times. Limitations include the high energy demand of steam generation, significant NaOH consumption requiring recovery or neutralization, the need for specialized high-pressure reactor systems, and the complexity of integrating two sequential pretreatment steps at an industrial scale.

2.6.2.3 Ammonia Soaking (Soaking in Aqueous Ammonia)

Soaking in aqueous ammonia (SAA) is an alkaline pretreatment method employed to selectively remove lignin from OPT biomass and enhance its susceptibility to enzymatic hydrolysis. Crushed and sieved OPT particles of 125 to 1000 µm are soaked in a 7% (w/w) aqueous ammonia solution at a solid-to-liquid ratio of 1:12, and incubated in a water bath at 80°C for 8 hours with occasional agitation. Following incubation, the solids are recovered by filtration, washed thoroughly with distilled water to remove residual ammonia, and subjected to enzymatic hydrolysis using commercial cellulase preparations such as Accellerase 1000 at loadings of 60 FPU/g glucan at 50°C for 96 hours. The resulting sugars are fermented using *Saccharomyces cerevisiae* D5A to produce ethanol.

SAA pretreatment achieves lignin removal in the range of 39.1 to 56.5%, with significant release of glucose and xylose, including up to 5.6 g/L xylose. Advantages include energy efficiency due to low temperature and atmospheric pressure operation, selective lignin removal with minimal cellulose and hemicellulose degradation, and reduced inhibitor formation relative to acid pretreatments. The principal limitations include the requirement for efficient ammonia recovery and recycling to manage operational costs and environmental impact, generation of ammonia-containing wastewater, potential loss of up to half of the biomass mass during pretreatment, and restricted throughput arising from the extended soaking duration.

2.6.2.4 Hydrothermal Treatment

Hydrothermal treatment employs hot compressed water, through autoclaving or steam treatment, to disrupt the OPT biomass structure, with particular effectiveness on the starch-rich parenchyma (PA) fraction. The process involves chopping and drying OPT to reduce moisture content, followed by autoclaving at 121°C for a defined duration to solubilize starch and partially disrupt the lignocellulosic matrix prior to enzymatic hydrolysis. This method may be combined with mechanical fractionation of OPT into its parenchyma and vascular bundle (VB) fractions, enabling simplified starch hydrolysis from the PA fraction and more intensive pretreatment of the fiber-rich VB fraction.

Hydrothermal treatment efficiently extracts and hydrolyzes starch from OPT, yielding high fermentable sugar concentrations free from chemical inhibitors such as furfural or HMF. The absence of acid or alkali reagents eliminates the need for detoxification and extensive washing steps, reducing operational complexity and capital costs. Limitations include the requirement for pressure vessel equipment, susceptibility of starch-rich hydrolysates to microbial contamination during processing, insufficient lignin removal from vascular bundles when applied as a standalone treatment, and the need for careful optimization of temperature, residence time, and solid loading to maximize sugar yield.

2.6.2.5 Combined Pretreatment Methods

For improved biomass deconstruction and sugar yield, several combined pretreatment strategies have been investigated for OPT, including alkaline peroxide combined with autoclave treatment, microwave-assisted alkali pretreatment, and two-step steam explosion followed by alkaline extraction. These integrated approaches leverage the complementary mechanisms of physical disruption and chemical delignification to maximize cellulose accessibility and overall bioethanol production efficiency, as discussed in the preceding sub-sections.

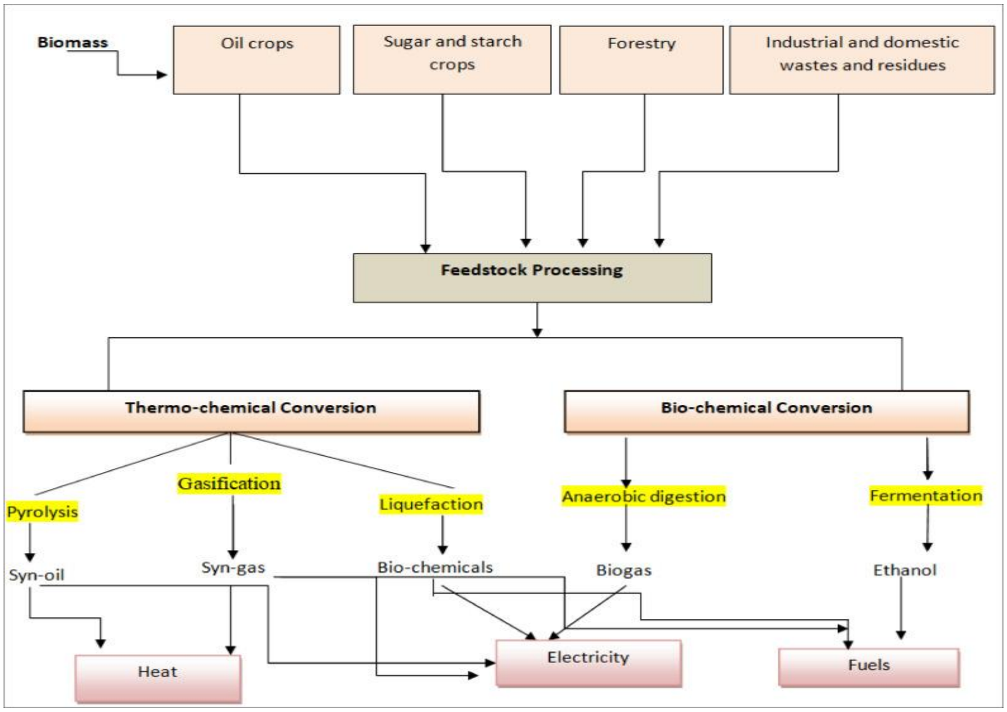

Figure 2.8 Overview of major biomass conversion (Mahapatra et al., 2021, fig. 5)

2.7 BIOREFINERY SYSTEM

The production of biofuels from biomass is most efficiently pursued within a biorefinery framework, which maximizes resource utilization and minimizes waste generation in an environmentally sustainable manner. The concept of a biorefinery was formally defined by the National Renewable Energy Laboratory (NREL) in 1990 as the utilization of biomass for the integrated manufacture of fuels, energy, and a range of bioproducts (Uçkun Kiran et al., 2016). A biorefinery system encompasses the full value chain of biomass utilization, including feedstock production, conversion processing, and end-product recovery. Numerous biorefinery models have attracted considerable research interest owing to their demonstrated capacity to reduce carbon dioxide emissions and lower overall production costs (Naik et al., 2010). The biorefinery approach has additionally reduced the unit cost of biofuel manufacturing while enabling the concurrent recovery of diverse value-added co-products, including antioxidants, natural dyes, nutraceutical supplements, and cosmetic ingredients (De Bhowmick et al., 2018).

2.7.1 TYPES OF BIOREFINERY

Several distinct biorefinery configurations have been developed, each differentiated by the nature of the biomass feedstock and the processing technologies employed.

2.7.1.1 Green Biorefinery

Green biorefineries process fresh, wet biomass, including grasses, green crops, and leafy plant materials, collectively referred to as green biomass, and are inherently multi-product systems. In the primary processing step, green biomass undergoes wet fractionation to yield two principal output streams. The first is a fiber-rich press cake containing cellulose, starch, pigments, crude pharmaceutical compounds, and other organic materials, which can be further processed into green feed pellets or converted into syngas and synthetic fuels. The second is a nutrient-rich green juice containing free amino acids, enzymes, hormones, pigments, and minerals, from which high-value biochemicals can be extracted (Abels et al., 2013).

2.7.1.2 Forest and Lignocellulosic Biorefinery

Lignocellulosic biorefineries process hard, fibrous plant materials sourced from timber, agricultural residues, or municipal solid waste. Lignocellulosic biomass is structurally composed of three primary chemical fractions: hemicellulose, comprising predominantly pentose sugar molecules; cellulose, a glucose-based polysaccharide; and lignin, a complex phenolic polymer. The plant material is cleaned and subjected to enzymatic and chemical digestion to separate these three fractions (Abels et al., 2013). Cellulose and hemicellulose are subsequently hydrolyzed into simpler fermentable sugars and valuable intermediate products through the following transformation reactions:

$$\text{Lignocellulose} + H_2O \rightarrow \text{Lignin} + \text{Cellulose} + \text{Hemicellulose}$$

$$\text{Hemicellulose} + H_2O \rightarrow \text{Xylose } (C_5H_{10}O_5)$$

$$\text{Xylose} + \text{Acid catalyst} \rightarrow \text{Furfural } (C_5H_4O_2) + 3H_2O$$

$$\text{Cellulose } (C_6H_{10}O_5)_n + H_2O \rightarrow \text{Glucose } (C_6H_{12}O_6)$$

Furfural, an important intermediate product of hemicellulose hydrolysis, finds application as a solvent in lubricating oil refining, as a precursor in the synthesis of certain plastics, and as a cleaning agent in liquid fuel systems (Uçkun Kiran et al., 2016). This biorefinery type is of direct relevance to the present study, as oil palm trunk (OPT) is a lignocellulosic feedstock processed through analogous fractionation and hydrolysis pathways.

2.7.1.3 Whole-Crop Biorefinery

Whole-crop biorefineries utilize cereal crops, including wheat, rye, triticale, and maize, as their primary feedstocks. Processing begins with the mechanical separation of the harvested crop into grain and straw fractions. The straw fraction enters a lignocellulosic processing pathway for syngas production, which is subsequently used to synthesize fuels and methanol. The grain fraction is converted into starch, which may be further transformed through chemical modification, plasticization, or biotechnological conversion into a range of value-added products (Abels et al., 2013; Uçkun Kiran et al., 2016).

2.7.1.4 Integrated Biorefinery

Integrated biorefineries combine multiple conversion technologies, including thermochemical, biochemical, and physicochemical processes, within a single production system to simultaneously minimize economic costs and maximize product yield and diversity. For example, flour milling operations, which typically achieve grain-to-flour conversion yields of 70 to 80% and generate starch-rich fractions containing approximately 25 to 30% starch, can be integrated with biochemical conversion platforms to produce high-value chemicals such as succinic acid from the residual starch content.

2.7.1.5 Algae Biorefinery

Algae biorefineries utilize microalgal biomass as their primary feedstock. Algae and cyanobacteria are autotrophic microorganisms capable of synthesizing organic matter through photosynthesis using sunlight, carbon dioxide, and water (Kamm et al., 2004). Within an algae biorefinery, microalgal biomass cultivated in open raceway ponds or closed photobioreactor systems is processed for oil extraction, biodiesel production, and mitigation of carbon dioxide emissions from industrial point sources. The oil content of certain microalgal species can exceed 80% of their dry weight, making them among the most lipid-productive organisms known (Uçkun Kiran et al., 2016). The algae biorefinery model is therefore particularly relevant to third- and fourth-generation biofuel production systems discussed in the preceding sections of this review.

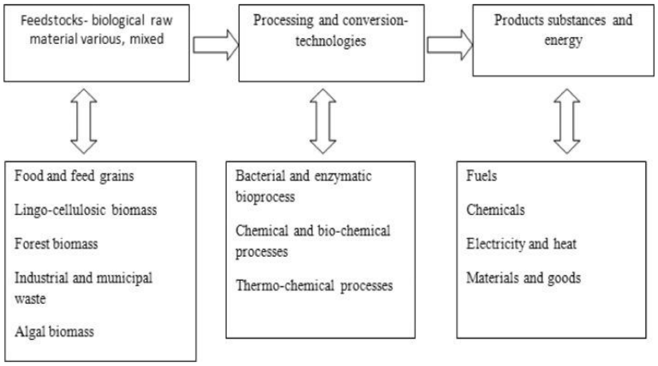

Figure 2.9 Overview of basic principles of biorefinery (Mahapatra et al., 2021)

2.8 APPLICATION OF BIOETHANOL

Bioethanol is a versatile renewable chemical and energy carrier with a wide range of applications spanning the fuel, chemical, pharmaceutical, and industrial sectors. The principal applications of bioethanol are discussed as follows.

2.8.1 Transportation Fuel and Gasoline Blending

The most widespread application of bioethanol is as a transportation fuel, either blended with conventional gasoline or used in its pure form. Bioethanol is blended with gasoline in varying proportions, including E5 and E10 blends containing 5% and 10% ethanol respectively, E20 and E25 blends commonly used in Brazil, and E85 blends containing 85% ethanol for use in flexible-fuel vehicles. These blends improve fuel combustion efficiency owing to the high-octane rating of ethanol, reduce tailpipe emissions of carbon monoxide (CO), unburned hydrocarbons, and particulate matter, and decrease net greenhouse gas (GHG) emissions relative to pure gasoline (Balat, 2011). In its pure form, designated E100, bioethanol is used as the primary transportation fuel in countries such as Brazil, where dedicated ethanol-powered engine technology is well established.

2.8.2 Combined Heat and Power Generation

Bioethanol can be utilized as a fuel in combined heat and power (CHP) systems, where it is combusted to simultaneously generate electricity and useful thermal energy. Its relatively high energy content, clean combustion profile, and compatibility with gas turbine and internal combustion engine-based CHP configurations make it a viable and flexible energy source for decentralized power generation applications, particularly in rural and off-grid settings where biomass feedstocks are locally available.

2.8.3 Chemical Feedstock and Industrial Solvent

Bioethanol serves as an important raw material and solvent in the chemical industry. As a feedstock, it is used in the synthesis of a broad range of organic chemicals, including ethylene, acetaldehyde, acetic acid, ethyl acetate, and diethyl ether, all of which are precursors to plastics, synthetic rubber, and other polymer materials. As a solvent, bioethanol finds extensive application in the formulation of paints, varnishes, lacquers, adhesives, and cleaning agents, owing to its ability to dissolve both polar and moderately non-polar compounds (Uçkun Kiran et al., 2016).

2.8.4 Pharmaceutical and Cosmetic Industry

In the pharmaceutical industry, bioethanol is widely used as a solvent for the extraction and formulation of active pharmaceutical ingredients (APIs), tinctures, and medicinal preparations. Its antimicrobial properties also make it a key component in the manufacture of hand sanitizers, antiseptic solutions, and disinfectants, a use that gained heightened global prominence during the COVID-19 pandemic. In the cosmetic industry, bioethanol is employed as a solvent and carrier in the production of perfumes, colognes, deodorants, hairsprays, and skin care formulations.

2.8.5 Food and Beverage Industry

Food-grade bioethanol is utilized as a preservative, solvent for flavor extraction, and carrier for food additives and colorants in the food processing industry. It is also the primary fermentation product in the production of alcoholic beverages, including beer, wine, and spirits, representing one of the oldest and most established applications of ethanol fermentation technology.

2.8.6 Hydrogen Production

Bioethanol has attracted growing research interest as a feedstock for hydrogen production through catalytic steam reforming, a process in which ethanol reacts with steam at elevated temperatures over a catalyst to yield hydrogen-rich syngas. Bioethanol-derived hydrogen is considered a promising clean energy carrier for fuel cell applications and represents a potentially carbon-neutral pathway to hydrogen production, as the CO_2 released during reforming is offset by the carbon absorbed during biomass feedstock growth (Balat, 2011).

2.8.7 Environmental and Sustainability Benefits

Beyond its direct applications, bioethanol production and utilization contribute several broader environmental and sustainability benefits. The substitution of fossil-derived fuels and chemicals with bioethanol reduces net lifecycle GHG emissions, as CO_2 released during combustion is largely reabsorbed by the biomass feedstock during growth, constituting a closed carbon cycle. The utilization of agricultural residues and lignocellulosic waste streams, such as oil palm trunk (OPT) as investigated in the present study, for bioethanol production additionally contributes to agricultural waste valorization, reduces open burning of crop residues, and supports the transition toward a circular bioeconomy (Wardani et al., 2021).

CHAPTER THREE

MATERIALS AND METHODS

MATERIALS

3.1 SAMPLE COLLECTIONS

Oil palm trunk (OPT) used in this study was obtained from a young plant (Elaeis guineensis) at Agbonkina's oil palm house around Idogbo by-pass, Benin City, Nigeria. This feedstock was brought to the laboratory (Luco Chemicals Laboratory Ltd), where the research was done.

3.1.1 REAGENTS, EQUIPMENT, AND THEIR USES

The raw material and equipment used for this research study are tabulated below.

Table 3.1: Materials, reagents, and their uses

SN	MATERIAL/REAGENTS	MODEL
1	Oil palm trunk	Feedstock
2	HCl (99% pure AR)	MF: HCl MW: 36.46 Assay (acid-metric): Min 35% Weight/mL at 20 °C: About 1.18g Non-volatile matter: Max 0.01%
3	NaOH (99% pure AR)	
	Glucose	99% Pure
4	KOH PELLET	Net. Wt.: -500gm

		MW: 56.11 Assay: Min. 85%
5	DNS reagent (3,5 – Dinitrosalicylic acid)	Testing sugars
6	Potassium Tetrahydrate	

Table 3.2: Equipment used for the experiment

SN	EQUIPMENTS	MODEL
1	Laboratory drying oven	DHG-9053A Techmel &Techmel USA.
2	Crusher	
3	500 mics standard test sieves	New standard test sieve
4	Digital measuring scale	Name: Atom Electronic compact scale
5	Beakers	SCHOTT DURAN Made in Germany
	Conical flask	G.G - 17
	Glass tubes	JINOTECH 25X150
	Reagent bottle	JINOTECH
6	Glass rod	
7	Heating Mantle	Model: ZDHW – 1000ML

		Voltage: 220V Power: 300W Max working temperature: 380 ºC JINOTECH INSTRUMENTS
8	pH paper	
9	Measuring cylinder	
10	Temperature gauge	
11	Magnetic stirrer (MS300 &MS400)	
12	Autoclave	Model: YX – 280A; Vol: 18L; Pressure: 0.14MPa – 0.165MPa; DES TEMP: 126 ºC
13	UV-VIS spectrophotometer	Model: 752N, PEC Medical USA.
14	Rhetort stand	
	Refractometer	Name: SNDWAY SW – 593 Range: 0 – 55% Battery: 2x1.2V AA Ni-mh

3.1.2 PREPARATION OF FEEDSTOCK

The oil palm trunk (OPT) used in this study was obtained from a young plant at Agbonkina's oil palm house around Idogbo by-pass, Benin City, Nigeria.

plate 3.1: feedstock (OPT)

These feedstocks were brought to the laboratory (Luco Chemicals Laboratory Ltd), chopped into smaller pieces, and dried at 134 °C with the use of a laboratory oven due to the moisture content of the OPT.

plate 3.2: laboratory oven

The crushed feedstocks were sieved in the laboratory using a 500-micron standard sieve to separate the particle sizes. The retained particles were used as the raw material for ethanol production.

Plate 3.3 sieving process

1.2 METHODS

3.2.1 CHEMICAL PRETREATMENTS

3.2.1.1 Optimization of Pretreatment

10% of the feedstock (crushed OPT) was pretreated with dilute sodium hydroxide (NaOH) at a concentration of 20% NaOH solution. Then, the mixture was heated using a magnetic stirrer until it began to bubble, a process that took approximately 45 minutes. The mixture was diluted with water and was decanted continuously for 4 days until its pH became neutral. Finally, it was dried in the oven at 105°C for 24h. Three factors, temperature (ºC), concentration of sodium hydroxide (NaOH) (%), time (minutes), and Response sugar yield (mg/g), were observed for their influence on the breakdown of lignocellulosic and lignin fibers.

Build Information

Design	Info
File Version	13.0.1.0
Study Type	Response Surface
Design Type	Box-Behnken
Design Model	Quadratic
Build Time (ms)	16.00
Subtype	Randomized
Runs	17.00
Blocks	No Blocks

Design Factors

Name	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A: Acid conc. (%)	1.0000	6.00	-1 ↔ 1.00	+1 ↔ 6.00	3.50	1.77
B: Time (minute)	10.00	120.00	-1 ↔ 10.00	+1 ↔ 120.00	65.00	38.89
C: Temperature (°C)	30.00	120.00	-1 ↔ 30.00	+1 ↔ 120.00	75.00	31.82

Plate 3.4 Decanting process of the pretreated mixture.

3.2.1.2 Optimization of Ethanol Production Efficiency

The optimization of ethanol production efficiency in this study was carried out using **Response Surface Methodology (RSM),** comprising 17 experimental runs. This statistical approach was employed to evaluate and optimize the effects of key process variables on ethanol yield from oil palm trunk biomass.

The main objective was to determine the optimal fermentation conditions that would yield the highest ethanol concentration and fermentation efficiency from the pretreated oil palm trunk. The optimization focused on identifying the best combination of process parameters that influence ethanol production during **SSCF**.

Optimization Procedure

i. Pretreated oil palm trunk samples were hydrolyzed under varying combinations of acid concentration, temperature, and reaction time according to the 17-run RSM design.
ii. The resulting hydrolysates were subjected to simultaneous saccharification and co-fermentation (SSCF) using Saccharomyces cerevisiae and Pichia stipitis.
iii. Ethanol concentration and sugar yield were determined spectrophotometrically (absorbance at 610 nm), while the fermentation efficiency was computed from the ratio of ethanol yield to theoretical yield.
iv. Data obtained were analyzed using Design-Expert software, which generated regression equations, 3D response surface plots, and contour diagrams to determine the optimal point.

Three independent variables were selected based on preliminary trials and literature data. These factors were optimized using an RSM design matrix.

Table 3.3 Data obtained for optimization

Factor	Symbol	Range tested	Description
Acid concentration	A	1% - 6%	Concentration of dilute acid used during pretreatment
Reaction time	B	10 – 20 minutes	Duration of the hydrolysis reaction
Temperature	C	30 – 120°C	Reaction temperature during pretreatment and fermentation

This experiment was designed to investigate both the individual and interaction effects of these variables on ethanol yield and fermentation efficiency.

The results presented in Table 4.4 revealed 17 experimental runs under varied acid concentration (1-6%), temperature (30-120 minutes). Among the 17 RSM combinations, maximum ethanol yield occurred at 120°C and 2 hours. This condition achieved optimal saccharification and co-fermentation efficiency.

Optimization of saccharification and co-fermentation was done based on the central composite design response surface method. Experimental data results were analyzed using the Design-Expert program to predict regression (statistical model) response data. The independent variables, that is, cell ratio, temperature, and fermentation time, were optimized, whereas the observed response is ethanol and fermentation efficiency.

3.2.2 ENZYMATIC HYDROLYSIS

The treated OPT was hydrolyzed using a reasonable amount of yeast (Saccharomyces cerevisiae). Enzymatic digestibility of treated OPT was performed at 50°C using a 250 mL flask containing 50g of the feedstock and an HCl solution on a magnetic stirrer with a working time of 10 minutes. The released reducing sugar concentration was analyzed based on the amount of liberated reducing sugars using the 3,5-dinitrosalicylic acid (DNS) method according to the Miller method. (chemistry & 1959, 1959). The alkaline-treated OPT fibers give a substantial glucose concentration from enzyme hydrolysis.

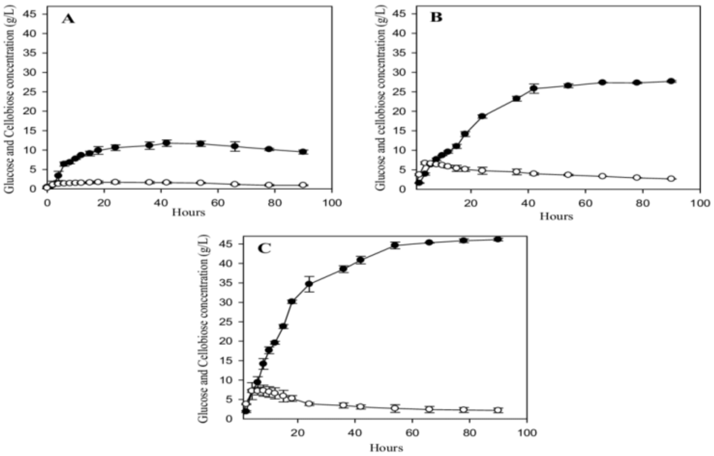

Figure 3.1 (Tareen et al., 2020)

3.2.3 FERMENTATION PROCESS

1. Feedstock preparation

50g of feedstock was measured, and 17.5 mL of KCl was diluted with water to prepare a 500 mL solution. The KCl solution was added to the feedstock and heated for 65 minutes.

2. Neutralization and Enzyme Preparation

The heated solution was neutralized with NaOH solution and maintained at a low temperature. The mixture was then adjusted to 46 ºC before the enzyme was added.

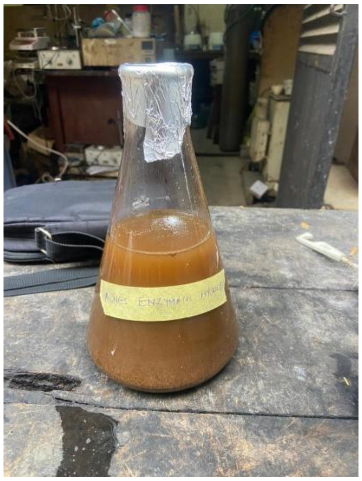

Plate 3.5: Enzyme feedstock is prepared for the fermentation process.

3. Fermentation

After 24 hours, 1g of yeast was dissolved in 50 ml of warm water and added to the enzymatic solution.

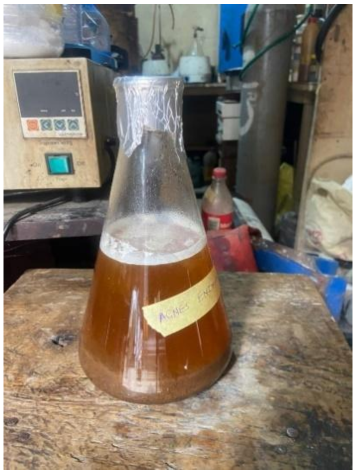

Plate 3.6: The third day of fermentation.

4. Sugar content testing

2ml of the enzymatic solution was mixed with 1 mL of DNS. The mixture was heated for about 10 minutes to test the sugar content in the solution using a 610 wavelength.

The sugar test was conducted for 4 days, and it was observed that the sugar content reduced in every daily check (from 3.8 to 0.405).

Plate 3.7 Enzymatic solution mixed with DNS

Plate 3.8 Sugar testing with UV-spectrophotometer

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Separation and composition

The OPT contained 29-45% cellulose, 12-29% hemicellulose, and 18-23% lignin, confirming its suitability as a lignocellulose feedstock.

The separation of the OPT involved cutting, drying, size reduction, drying, and sieving. Darwis et al. (Darwis et al., 2013) Observed a higher density of the vascular bundle in the central part of the trunks compared to the bottom. The standard chemical composition of Nigerian palm trunks is analyzed below in Table 2.

Table 4.1. Typical Composition Ranges (Said et al., 2021)

Cellulose	**29-45%**
Hemicellulose	12-29%
Lignin	18-23%
Extractives	4-11%
Ash	2-3%

i. **Fermentation observation:** Sugar concentration dropped from 3.8 mg/g to 0.404 mg/g over four days, indicating progressive sugar utilization by yeast for ethanol formation.

Table 4.2. progressive sugar utilization

Days of fermentation	Sugar concentration (mg/g)
Day 1	3.8
Day 2	1.560
Day 3	0.606
Day 4	0.405

4.2 RESPONSE SURFACE METHODOLOGY (RSM)

The Response Surface Methodology (RSM) using a Box–Behnken design was applied to optimize the effects of acid concentration (A), pretreatment time (B), and temperature (C) on sugar yield from oil palm trunk (OPT). Seventeen experimental runs were conducted, and the results were fitted to a quadratic model (as shown in Table 4.3).

The ANOVA showed that the model was statistically significant with an F-value of 115.99 and a p-value < 0.0001, confirming a strong correlation between experimental and predicted values. The R^2 (0.9933) and Adjusted R^2 (0.9848) indicate excellent model accuracy and reliability. The Adequate Precision ratio (28.27) exceeded the standard threshold of 4, confirming a strong signal-to-noise ratio. Among the factors studied, acid concentration (A), time (B), and temperature (C)—along with their interactions (AB, BC) and quadratic terms (A^2, B^2, C^2)—were statistically significant ($p < 0.05$). This shows that all three parameters had a strong effect on sugar yield.

The model predicted the optimum pretreatment condition at 3.5% acid concentration, 120°C, and 120 minutes, which produced the maximum sugar yield of 553.54 mg/g. This condition represents the best balance between lignin breakdown and cellulose preservation, ensuring high sugar recovery without excessive degradation.

Table 4.3 RSM experimental runs for optimization of Ethanol production efficiency

RUN	**Acid conc. (%)**	**Time (minutes)**	**Temperature (℃)**	**Abs @ 610**	Sugar yield (mg/g)
1	1	65	120	0.878	246.452
2	6	120	75	1.232	348.719
3	3.5	120	120	0.814	227.963
4	3.5	65	75	1.941	553.542
5	3.5	65	75	1.941	553.542
6	3.5	10	120	0.871	244.43
7	3.5	65	75	1.941	553.542
8	6	65	120	0.84	235.474
9	3.5	65	75	1.941	553.542
10	1	10	75	0.662	184.052
11	1	120	75	0.631	175.096
12	6	65	30	0.716	199.652

13	1	65	30	0.496	136.096
14	3.5	10	30	0.389	105.185
15	3.5	120	30	0.759	212.074
16	6	10	75	0.768	214.674
17	3.5	65	75	1.941	553.542

Table 4.4. **Sugar yield ANOVA for the Quadratic Model**

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4.527E+05	9	50301.29	115.99	< 0.0001	significant
A-Acid conc	8244.77	1	8244.77	19.01	0.0033	
B-Time	5805.67	1	5805.67	13.39	0.0081	
C-Temperature	11348.64	1	11348.64	26.17	0.0014	
AB	5112.29	1	5112.29	11.79	0.0109	
AC	1388.82	1	1388.82	3.20	0.1167	
BC	3804.18	1	3804.18	8.77	0.0211	
A^2	1.050E+05	1	1.050E+05	242.23	< 0.0001	
B^2	1.146E+05	1	1.146E+05	264.19	< 0.0001	
C^2	1.539E+05	1	1.539E+05	354.85	< 0.0001	
Residual	3035.62	7	433.66			

Lack of Fit	3035.62	3	1011.87			
Pure Error	0.0000	4	0.0000			
Cor Total	4.557E+05	16				

The Model F-value of 115.99 indicates that the model is statistically significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case, A, B, C, AB, BC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Predicted R^2** of 0.8934 is in reasonable agreement with the **Adjusted R^2** of 0.9848 as shown in the table below; i.e., the difference is less than 0.2.

Table 4.5. **Fit Statistics**

R^2	**0.9933**
Adjusted R^2	0.9848
Predicted R^2	0.8934
Adeq Precision	28.2733
Std. Dev.	20.82
Mean	311.62
C.V. %	6.68

Adequate Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Your ratio of 28.273 indicates an acceptable signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors

$$\begin{aligned} Sugar\ yield\ (\%) &\\ &= 553.54 + 32.10A + 26.94B + 37.66C + 35.75AB - 18.63AC - 30.84BC \\ &- 157.95A^2 - 164.95B^2 - 191.17C^2 \end{aligned}$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1, and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

4.3 RESPONSE SURFACE PLOTS

4.3.1 EFFECT OF ACID CONCENTRATION AND TIME ON SUGAR YIELD

These plots show that sugar yield initially **increases** with both **acid concentration** and **pretreatment time,** reaching an optimal region before declining slightly. At **low acid concentration and short time,** lignin removal is incomplete, limiting cellulose accessibility and resulting in **low sugar yield.** As both factors increase moderately, **hydrolysis efficiency improves,** allowing more cellulose and hemicellulose to convert into fermentable sugars.

However, at **very high acid levels or prolonged exposure,** sugar degradation and inhibitor formation (e.g., furfural and HMF) occur, reducing the yield.

The optimum zone occurs at a **moderate acid concentration (≈3.5%) and time (~65-120 mins)**, producing the highest sugar yield around 553.5 mg/g.

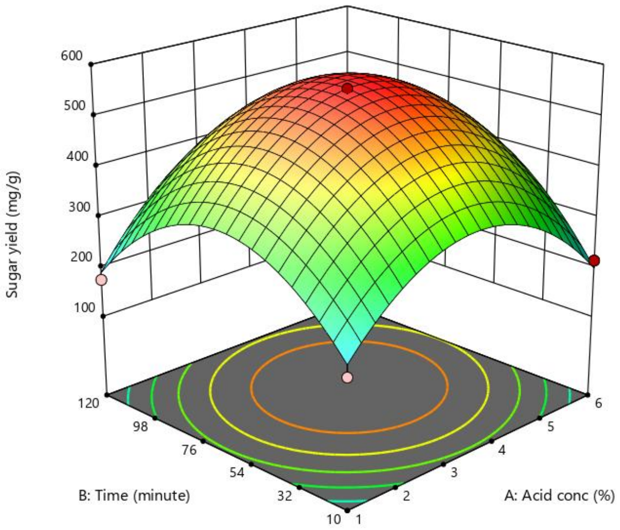

Figure 4.1. The effect of concentration and time on sugar yield

1.3.2 EFFECT OF ACID CONCENTRATION AND TEMPERATURE ON SUGAR YIELD

This plot indicates that temperature has a significant influence on the effectiveness of acid hydrolysis. At **low temperature (30-60 ºC)**, cellulose breakdown is slow, resulting in lower sugar yield even when the acid concentration is increased. **Rising temperature** up to around 100-120 ºC enhances lignin solubilization and cellulose hydrolysis, improving sugar recovery.

Beyond this optimum temperature, sugar degradation begins, leading to decreased yield despite higher acid concentration. The **best sugar yield** is achieved at **moderate acid concentration (3-4%) and high temperature (≈ 120 ºC)**, validating that heat improves pretreatment efficiency but must be carefully controlled.

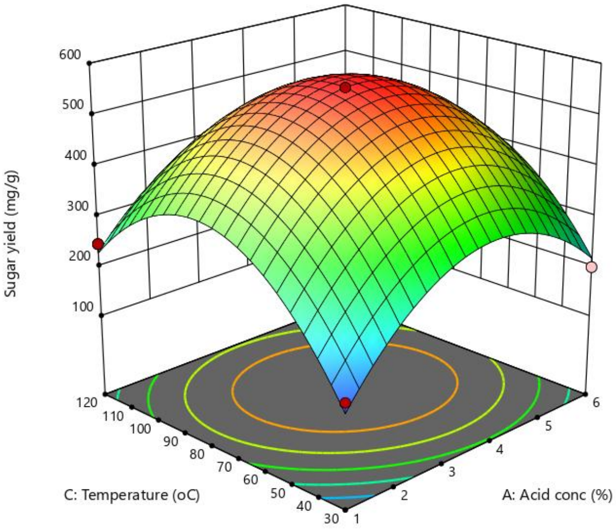

Figure 4.2 Plot of the effect of acid concentration and temperature on sugar yield

4.3.3 EFFECT OF TEMPERATURE AND TIME ON SUGAR YIELD

This plot reveals a strong interaction **between reaction temperature and residence time.**

Short time and low temperature produce poor hydrolysis because the reaction energy is insufficient to disrupt lignin-cellulose bonds. As both **temperature and time increase**, sugar yield rises sharply due to enhanced diffusion and reaction kinetics. However, **excessively high temperature combined with prolonged time** leads to sugar decomposition and inhibitor formation, which reduces the yield.

The optimum condition is found around 120 ºC and 2 hrs, giving the **maximum sugar yield and ethanol potential**, as confirmed in this experiment.

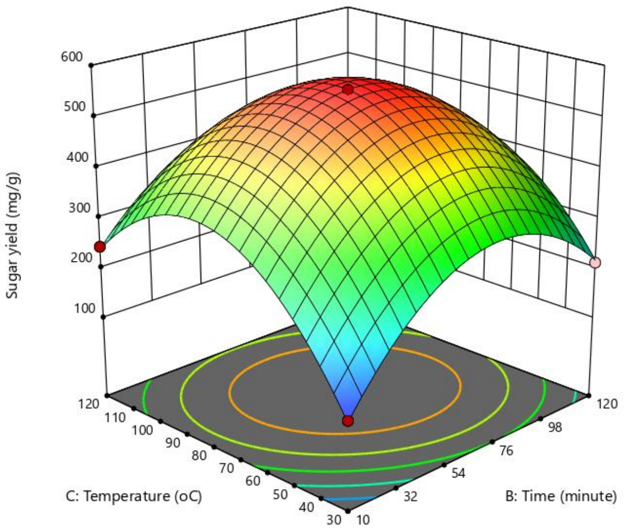

Figure 4.3 Plot of the effect of temperature and time on sugar yield

4.4 OPTIMUM CONDITION OF PRETREATMENT

The pretreatment stage is a critical step in bioethanol production because it determines how effectively cellulose and hemicellulose in the OPT are broken down into fermentable sugars. From the optimization results obtained using **RSM**, **the optimum pretreatment condition** for maximizing sugar yields was found to be at a **temperature of 120 ^{o}C, acid concentration of 3.5%**, and a **reaction time of 2 hrs.**

Under these conditions, the hydrolysis of the lignocellulosic structure was the most efficient, resulting in the **highest sugar yield of approximately 553.5 mg/g.** This indicates that the chosen parameters effectively disrupted the lignin-cellulose matrix and enhanced the release of fermentable sugars without causing excessive degradation. At **lower temperatures or weaker acid concentrations**, delignification and hemicellulose hydrolysis were incomplete, limiting the accessibility of enzymes to cellulose and leading to reduced sugar output. Conversely, when the temperature or acid concentration was increased beyond the optimum, the sugar yield began to decline. This reduction is attributed to the **formation of inhibitory compounds** such as furfural and HMF, which occur when sugars are degraded under harsh acidic and thermal conditions.

Therefore, the combination of **moderate acid concentration (3.5%), high temperature (120 ^oC), and controlled reaction time (2 hours)** represents the most balanced condition. It allows maximum sugar recovery while minimizing degradation and chemical loss. These conditions are consistent with previous studies on lignocellulosic biomass pretreatment, which also emphasize that **temperature and acid strength** are the most influential factors in optimizing hydrolysis efficiency.

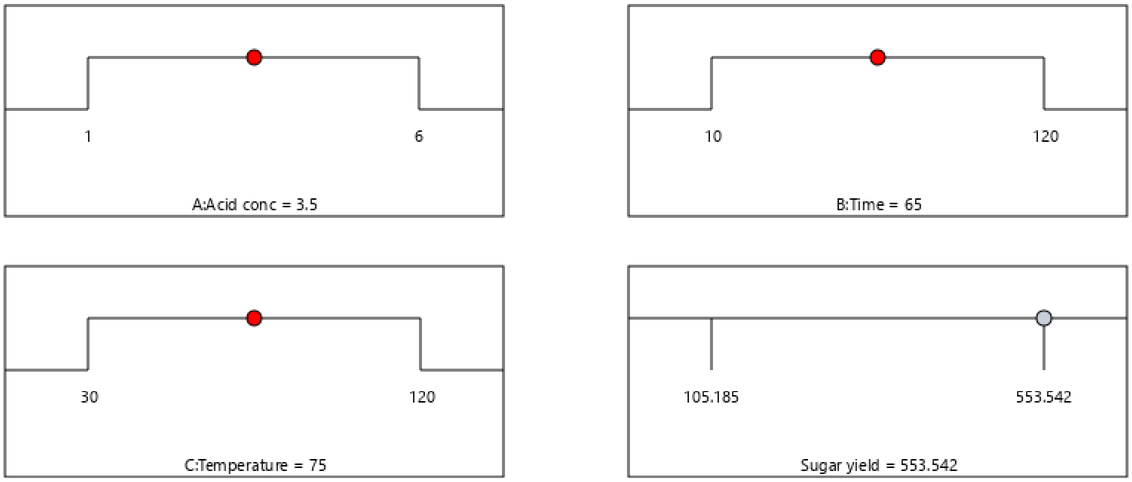

Figure 4.4 Optimum condition of pre-treatment

4.4.1 LINK BETWEEN PRETREATMENT RESULTS AND FERMENTATION PERFORMANCE

The success of the fermentation stage directly depends on the effectiveness of the pretreatment process. Under the optimum condition of 3.5% acid concentration, 120°C temperature, and 2 hours reaction time, the pretreatment yielded the highest concentration of fermentable sugars. This provided a rich substrate for the yeast (Saccharomyces cerevisiae) during fermentation, resulting in a steady conversion of sugars to ethanol.

Throughout the four-day fermentation period, the sugar concentration decreased progressively from 3.8 mg/g to 0.405 mg/g, indicating active microbial metabolism and efficient sugar utilization. Correspondingly, ethanol yield increased significantly, confirming that the optimized pretreatment effectively enhanced cellulose accessibility and improved enzymatic hydrolysis efficiency.

The strong relationship between pretreatment efficiency and ethanol yield demonstrates that an appropriately balanced pretreatment condition not only maximizes sugar release but also minimizes the formation of inhibitory compounds that could hinder yeast performance. Thus, the optimized parameters established in this study—120°C, 3.5% acid, and 2-hour duration—represent the most favorable condition for achieving high ethanol output from oil palm trunk biomass.

1.4.2 FTIR OF OPT

The temperature versus wavelength graph below illustrates that absorbance increases with rising temperature up to the optimum point (around 120°C), indicating improved breakdown of lignocellulosic material and higher sugar release. Beyond this temperature, absorbance decreases, suggesting thermal degradation of sugars and formation of inhibitory by-products. This pattern confirms that temperature strongly affects the pretreatment efficiency and that 120°C is the most effective condition for maximum sugar yield.

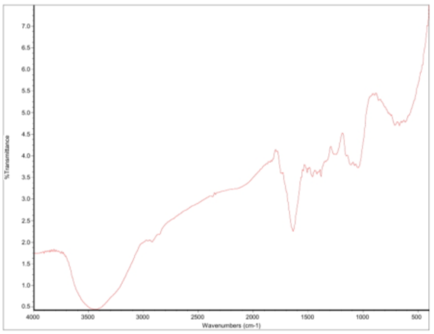

Figure 4.5 FTIR of OPT

1.4.3 CHARACTERIZATION OF OPT

The characterization plot for oil palm trunk in the experiment illustrates the relationship between the predicted and actual sugar yield values obtained from the optimization model. The data points in the plot closely align along the 45° line, confirming strong agreement between predicted and experimental results. This high correlation is supported by statistical parameters — $R^2 = 0.9933$, Adjusted $R^2 = 0.9848$, and Adequate Precision = 28.27 — indicating excellent model accuracy and reliability. The plot demonstrates that the quadratic model effectively predicts sugar yield from oil palm trunk, validating the experimental design and confirming that the process parameters were accurately optimized.

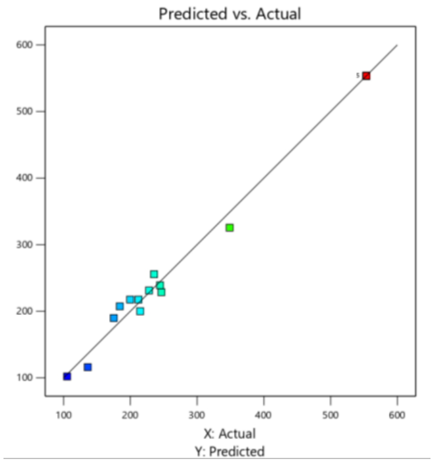

Figure 4.6. characterization plot

4.5 DISCUSSION

The results demonstrate that Oil Palm Trunk is a non-food biomass for bioethanol production. The gradual reduction in sugar content throughout fermentation confirmed effective enzymatic hydrolysis and yeast activity. High cellulose content favored glucose release, while controlled acid pretreatment enhanced substrate accessibility by breaking the lignin-cellulose bond.

The maximum ethanol yield was achieved at 120 ºC and 2 hours, aligning with previous findings. (Prawitwong et al., 2012), which reported improved sugar conversion under similar thermal and acidic conditions. However, excessively high temperatures or prolonged exposure could generate inhibitory compounds such as furfural, explaining reduced yields at extreme parameters.

The study also shows that simultaneous saccharification and co-fermentation improved overall conversion efficiency by minimizing product inhibition and shortening process time. The use of *Saccharomyces cerevisiae* proved effective, consistent with its known ethanol tolerance and high fermentation rate.

In comparison with conventional feedstock like sugarcane or maize, OPT offers an advantage due to its abundance and non-competition with food resources. Its dual sugar (sap) and fiber content make it suitable for integrated biorefinery systems. Nonetheless, challenges such as moisture content, pretreatment cost, and inhibitor formation remain areas for process optimization.

Overall, the findings validate the technical feasibility of producing **bioethanol from OPT,** supporting its potential as a renewable energy resource that can reduce dependence on fossil fuels and promote sustainable waste management in palm-producing regions.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

1.1 CONCLUSION

This research successfully demonstrated that Oil Palm Trunk (OPT) is a promising lignocellulosic biomass for sustainable bioethanol production. The study showed that OPT contains high amounts of cellulose and hemicellulose, which can be effectively converted into fermentable sugars through acid pretreatment and enzymatic hydrolysis. The fermentation results revealed a progressive decrease in sugar concentration and an increase in ethanol yield, confirming efficient microbial activity and substrate conversion.

Optimization process parameters, such as acid concentrations, temperature, and reaction time, revealed that the **best ethanol yield** was obtained at **120 ^oC and 2 hours,** conditions that promoted effective lignin breakdown and enhanced saccharification. The use of *Saccharomyces cerevisiae* proved efficient for ethanol fermentation, indicating its suitability for industrial-scale production.

Overall, the study established that bioethanol production from OPT waste is technically feasible, environmentally beneficial, and economically attractive. Utilizing OPT as a feedstock not only supports renewable energy generation but also promotes effective waste management in oil-palm producing regions, reducing environmental pollution and contributing to Nigeria's drive towards energy diversification and sustainability.

1.2 RECOMMENDATIONS

Based on the findings of this research, the following recommendations are proposed:

1. Future studies should focus on optimizing pretreatment techniques that reduce energy consumption and chemical usage, possibly incorporating environmentally friendly methods such as enzymatic or biological pretreatments.
2. Integration of bioethanol production into existing palm oil processing systems should be encouraged to promote a **biorefinery approach**, where residues like OPT, fronds, and empty fruit bunches are converted into valuable bio-products.
3. Further pilot-scale and industrial-scale trials should be carried out to validate the laboratory results and evaluate the economic viability of large-scale bioethanol production from OPT.
4. Genetically engineered or co-cultured microorganisms capable of fermenting both hexose and pentose sugars should be explored to improve overall ethanol yield.
5. A comprehensive life-cycle and cost-benefit analysis should be conducted to evaluate the environmental impact and financial sustainability of using OPT as a bioethanol feedstock.
6. Policies and incentives should be developed to encourage investment in biofuel technologies, particularly those utilizing agricultural residues such as oil palm trunk, to reduce dependence on fossil fuels and foster green energy initiatives.

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