OPTIMIZATION OF TERNARY FEEDSTOCK (CASSAVA PEELS, COCONUT HUSK, SAWDUST) FOR BIOETHANOL PRODUCTION USING SIMPLEX LATTICE DESIGN

BY

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UNIVERSITY OF BENIN

EDO STATE, NIGERIA

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A PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMICAL ENGINEERING, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF ENGINEERING IN CHEMICAL

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CERTIFICATION

This is to certify that this research project was carried out by **ABAZIE CHIDIMMA MIRACLE** with matriculation number **ENG2006140** in the Department of Chemical Engineering at the University of Benin, Benin city, Edo state Nigeria.

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DEDICATION

This project work is dedicated to the Almighty God, the source of my life, who has guided me this far in my academic journey. A special thanks goes out to **Mr. and Mrs. ABAZIE**, the world's greatest parents and my siblings who have always been a source of inspiration for me academically and have contributed and supported me financially, spiritually, and morally.

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I wish to express my profound gratitude to my project supervisor **PROF. (Mrs) C. OKIEIMEN** for her help, support and the bank of knowledge which she was willing to instill during the course of the project.

I extend my sincere appreciation to the HOD, Department of Chemical Engineering **PROF. (Mrs) E. A. OYEDOH** has shown awesome, great and tenacious leadership and guidance throughout the course of this project

I'm also grateful to the lecturers for their tutoring which made my stay here a great one. I will also want to appreciate Luco chemical laboratory limited,for it's support during this experiment.

I would like to express my profound gratitude to my family, to my parents, **Mr & Mrs ABAZIE** for their financial, moral, spiritual, and constant support all through my stay in school and my siblings whose love and prayer kept me going.

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ABSTRACT

Given Nigeria's abundant agro-industrial wastes, the study focused on optimizing a ternary blend of cassava peels (CP), coconut husk (CH), and sawdust (SD) to maximize bioethanol yields. Unlike previous studies that examined these feedstocks individually, this work investigated their co-processing potential to overcome disposal challenges and enhance their utilization.

The characterization of the feedstocks revealed diverse compositions: CP was rich in hemicellulose, CH presented a balanced composition, and SD was cellulose-rich but highly recalcitrant due to its high lignin content. Utilizing a {3,2} Simplex Lattice Design (SLD) across 15 experimental runs, a Special Quartic model was developed to elucidate the relationship between blend ratios and sugar yield. This model demonstrated high significance (F-value = 88.93, $p < 0.0001$) and an excellent fit ($R^2 = 0.9916$), highlighting substantial synergistic interactions, especially between CP and CH.

The optimized blend, consisting of 66.7% CP, 16.7% CH, and 16.7% SD, yielded an impressive experimental sugar yield of 370.31 mg/g, which significantly surpassed the yields from individual feedstocks. Subsequent validation of this optimized blend involved acid pretreatment, enzymatic hydrolysis, and fermentation using Saccharomyces cerevisiae, resulting in an experimental ethanol yield of 0.0644 g ethanol/g biomass. This achievement represents 85.4% of the theoretical yield, confirming a high fermentation efficiency and validating the strategic blending as an effective waste-to-wealth strategy for sustainable bioenergy production.

CHAPTER ONE

INTRODUCTION

BACKGROUND OF STUDY

The transition from conventional fossil fuels to renewable energy sources for residential and industrial applications has become one of the most critical problem. According to Onoji et al. (2016) and Saini et al. (2020), the rapid depletion of finite crude oil reserves, the extreme volatility of global fossil fuel markets, and the significant environmental degradation brought on by the extraction, processing, and burning of fossil fuels are the three main factors driving this transition. Fossil fuel combustion has been categorically identified by the Intergovernmental Panel on Climate Change (IPCC, 2021) as the main source of human-generated greenhouse gas (GHG) emissions, which accelerates global climate change and its related effects, such as extreme weather events, rising sea levels, and biodiversity loss.

As a result, the whole community is working hard to move toward a circular and sustainable bioeconomy. This approach to change is being led by renewable energy sources like biomass, geothermal, wind, and solar. Among these, biomass is special since it is the only renewable carbon source that can be transformed straight into liquid fuels, which is necessary to decarbonize the transportation industry (Baeyens et al., 2015). According to Kurian et al. (2013), bioenergy, which is made from biological materials, includes a variety of fuels such as biogas, biodiesel, biohydrogen, and bioalcohols like bioethanol.

More than 85 percent of biomass energy has historically been used as solid fuels (charcoal and fuelwood, for example) for cooking, heating, and lighting, frequently with poor efficiency and serious health effects from indoor air pollution (FAO/GBEP, 2007). In many impoverished countries, this "traditional bioenergy" is the most prevalent. To provide refined energy carriers

for use in homes, businesses, and transportation, "modern bioenergy" uses effective conversion processes to create solid fuels (pellets, briquettes), gaseous fuels (biogas, syngas), and liquid biofuels (biodiesel, bioethanol).

The potential carbon neutrality of burning biomass is a major benefit; the amount of carbon dioxide emitted during energy conversion is roughly equal to the amount that the plants take in from the environment during their growth phase (Sarkar et al., 2012). This establishes biomass as a necessary and sustainable substitute feedstock to satisfy the world's future energy needs.

In terms of production and consumption, bioethanol (ethanol made from biomass) is the most popular biofuel worldwide. Since Henry Ford and Alexander Graham Bell saw the promise of turning plentiful plant sugars into renewable fuel, it has been used for more than a century (Neelakandan et al., 2009). According to the European Commission (2017), bioethanol makes up 20% of all biofuel usage and is currently a major source of renewable energy used in the transportation industry as an additive or gasoline substitute.

The benefits of bioethanol are numerous. It greatly lessens reliance on fossil fuels and improves energy security as a 100% biological energy source (Berevolanu, 2021). When compared to traditional gasoline, bioethanol can lower greenhouse gas emissions by between 30 to 85%, depending on the feedstock and production method (Fulton, 2004; Saini et al., 2015). Additionally, compared to gasoline, bioethanol has better fuel qualities, such as a higher octane number, wider flammability limitations, and a higher heat of vaporization. These features enable internal combustion in engines to operate more efficiently, which reduces emissions of carbon monoxide, unburned hydrocarbons, and carcinogens (Balat, 2007; Rozenfelde et al., 2017). In addition to lowering the fuel's sulfur content, blending ethanol with gasoline helps minimize sulfur oxide emissions, which are the main contributor to acid rain (Fao.org, 2008).

Based on its feedstock, bioethanol is divided into generations. Corn and sugarcane are examples of food crops that are used to make first-generation bioethanol. Despite being economically established, it has generated the controversial "food vs. fuel" argument because it is competing for water and arable land, which could lead to an increase in food costs (Naik et al., 2010).

This crucial constraint sparked the creation of second-generation (2G) bioethanol, which is made from lignocellulosic biomass that is inedible. The most prevalent organic polymer on Earth is lignocellulose, which is found in plants as structural material. It includes forestry debris (such as sawdust), agricultural residues (such as rice husks and cassava peels), and crops grown specifically for energy (Sarkar et al., 2012). By turning low-value industrial and agricultural byproducts into high-value energy, the use of these materials signifies a perspective shift towards waste valorization. This strategy supports the ideas of a circular economy by addressing waste management issues and avoiding the food vs fuel problem (Hansen et al., 2021).

The intrinsic recalcitrance of lignocellulosic biomass prevents 2G bioethanol from being widely commercialized, despite its potential. The intricate cross-linked matrix of cellulose, hemicellulose, and lignin that shields plant cell walls from enzymatic and microbial degradation is the cause of this resistance (Mosier et al., 2005). Therefore, in order to break down this structure, eliminate lignin, and make cellulose available to hydrolytic enzymes, a necessary and frequently expensive pretreatment step is needed.

Single-feedstock systems have been the subject of the majority of research. Suboptimal sugar and ethanol yields are caused by the compositional constraints of certain feedstocks, such as the low cellulose content of various agricultural leftovers or the unusually high lignin concentration of coconut husk (Owuama, 2021). One creative way to get around these restrictions is by co-processing or feedstock blending.

The idea is based on synergy; the compositional characteristics of various biomass kinds complement one other. For example, whilst one feedstock may have a high cellulose concentration, another may be rich in readily available hemicellulose. A more balanced and processable feedstock mixture that increases overall sugar recovery, boosts process efficiency, and possibly lowers the concentration of inhibitors produced during pretreatment can be developed by constructing strategic ternary (three-component) blends (Wahid et al., 2019). However, systematic optimization of these multi-feedstock ratios is still a poorly studied topic.

Nigeria, as a predominantly agrarian economy, generates millions of tons of lignocellulosic waste annually. Cassava peels, a by-product of the massive garri production industry, are often discarded, causing environmental pollution. Coconut husk, a fibrous residue from coconut processing, and sawdust, from sawmills and wood workshops, are similarly underutilized. These materials represent a significant, untapped national resource for sustainable bioenergy production. Therefore, this study looks into the best combination of cassava peels, coconut husk, and sawdust for the synthesis of bioethanol in an effort to address both an energy deficiency and an environmental waste problem. In order to unlock the mutually beneficial potential of these ternary feedstocks and provide a scientific foundation for a sustainable, waste-to-wealth biofuel strategy that is suited to the Nigerian context and comparable agro-industrial economies, this research will employ the systematic optimization power of a Simplex Lattice Design.

Problem statement

Resource depletion, price volatility, and serious environmental effects like greenhouse gas emissions fueling climate change are only a few of the major problems associated with the world's continued reliance on fossil fuels (IPCC, 2022). As a result, bioethanol has become a viable liquid biofuel that may be used in place of or in addition to gasoline. Although first-

generation bioethanol is technically advanced and made from food crops like corn and sugarcane, it is hampered by the controversial "food-versus-fuel" controversy and restrictions on the usage of arable land (Naik et al., 2010). Therefore, by utilizing plentiful and inexpensive agricultural and forestry waste, second-generation (2G) bioethanol which is produced from non-food lignocellulosic biomass offers a sustainable substitute.

Like many other developing countries, Nigeria produces enormous amounts of lignocellulosic waste, such as sawdust, cassava peels, and coconut husks, which are frequently misused and lead to disposal problems as well as environmental challenges. Even though these feedstocks' individual potential for producing bioethanol has been somewhat investigated, using them alone poses substantial financial and technological challenges. Despite their abundance, cassava peels have been shown to have a high lignin concentration and a low cellulose content, which results in low glucose yields (Sanni et al., 2019). Because of its high lignin content, coconut husk is very resistant and necessitates rigorous pretreatment (Owuama, 2021). Despite being high in cellulose, sawdust can vary in composition and can not offer a balanced nutritional profile for effective fermentation.

The deliberate mixing of several feedstocks is a viable but understudied method to get beyond these particular restrictions. The idea of blending feedstocks can have a synergistic effect, in which the advantages of one component balance out the disadvantages of another. To make a more balanced sugar platform, for example, a cellulose-rich feedstock, such as sawdust, could be mixed with a hemicellulose-rich feedstock. This could also dilute inhibitors or increase the biomass matrix's porosity for improved reagent penetration (Kumar et al., 2016). The ideal blending ratio for these ternary mixes is a complicated, multifaceted issue that is not immediately apparent.

Thus, the main issue this study attempts to solve is the absence of a methodical framework for maximizing the production of bioethanol by optimizing the blend ratio of sawdust, coconut husk, and cassava peels. The intricate relationships between the constituents of a mixture where the aggregate must equal 100% cannot be captured by traditional one-variable-at-a-time (OVAT) experimental techniques, which are also ineffective and time-consuming. A statistically sound experimental design that can effectively simulate the response surface and determine the best formulation within the limited design space of a ternary mixture is desperately needed

Aim and Objectives.

This study aim to optimize the ternary blend ratio of cassava peels, coconut husk, and sawdust for enhanced bioethanol production using surface lattice design and to validate the optimized blend through experimental fermentation and yield analysis.

Objectives of this study are:

1. To characterize the raw cassava peels, coconut husk, and sawdust through lignocellulosic compositional analysis to determine the proportions of cellulose, hemicellulose, and lignin.
2. To design and conduct experimental runs using a {3,2} Simplex Lattice Design (SLD) to model and optimize the ternary mixture for maximum sugar yield.
3. To pretreat, hydrolyze, and ferment the feedstock blends according to the experimental design and quantify the sugar and ethanol yields.
4. To validate the predictive model by comparing the experimental sugar and ethanol yields with the model's predictions and determine the fermentation efficiency.
5. To calculate and compare the experimental and theoretical ethanol yields to assess process efficiency and identify potential areas for improvement.

SCOPE OF STUDY

This research is a laboratory scale study to optimize ternary feedstock that includes cassava peels, coconut husk, sawdust using simplex lattice design for optimized blend ratio with focus on acid/alkali-assisted pretreatments using reducing sugar yield(DNS assay) as performance metrics. It focuses on small scale and only provide initial indications of future potential rather than a thorough technological or financial analysis of the feasibility of producing bioethanol.

Significance of study

This study tackles important issues related to the production of second-generation bioethanol, which is a major advancement in the fields of sustainable bioenergy and waste management. This work is significant in many ways, including methodological, economic, and environmental developments.

By turning low-value agricultural and wood-processing wastes such as cassava peels, coconut husks, and sawdust into a high-value energy product, this work directly advances the ideas of a circular economy. These wastes are frequently burned in the open or allowed to break down in landfills in many poor countries, which results in soil erosion, air pollution, and greenhouse gas emissions (Sarkar et al., 2012). This research contributes to the reduction of these environmental contaminants and encourages the sustainable management of organic waste streams by offering a feasible route for their use. This trash valuation is in line with international initiatives to lessen dependency on fossil fuels and the energy sector's carbon impact (Naik et al., 2010).

The presentation of feedstock mixing as an optimization tool is a significant methodological advance. This study methodically examines the synergistic impacts of a ternary mixture rather than depending on a single, frequently suboptimal feedstock. This method can get around the drawbacks of each biomass separately. For example, the high cellulose content of sawdust can

make up for the low cellulose level of cassava peels, while the hemicellulose from cassava peels can enhance sawdust's cellulose content. Because bio-refineries are not reliant on a single feedstock type, this approach can result in a more reliable and accessible supply chain, improving operational resilience (Liu et al., 2016).

Additionally, the Simplex Lattice Design (SLD) offers a strong and effective statistical framework for multi-component mix optimization. Traditional one-variable-at-a-time (OVAT) experimentation is unable to capture component interactions; this methodology goes beyond that (Cornell, 2002). Without conducting time-consuming and expensive experimental experiments, academics and industry practitioners can use the prediction model produced by this work to identify the ideal blend ratio for their particular biomass sources. This study provides a reproducible model for improving alternative multi-feedstock systems, like biogas or bio-oil, in the manufacture of biofuel.

Lastly, the study's conclusions have favorable socioeconomic implications, especially for rural and agricultural economies. For farmers and small business owners engaged in cassava and coconut agriculture, as well as sawmill activities, this technology can create new revenue streams by establishing a market for what was previously regarded as waste (Ochieng et al., 2020). Additionally, the possibility of setting up small-scale, decentralized bioethanol production facilities can reduce the need for imported fossil fuels by improving energy security in rural regions, creating jobs locally, and producing clean cooking fuel or a blendstock for gasoline.

CHAPTER TWO

LITERATURE REVIEW

2.1. INTRODUCTION TO LIGNOCELLULOSIC BIOMASS FOR BIOFUELS

The search for sustainable and renewable energy alternatives has accelerated due to the global energy crisis, which is being fueled by the depletion of fossil fuel sources, geopolitical instability, and the pressing need to counteract climate change (Naik et al., 2010). Biofuels have become a viable option among these substitutes, especially for the transportation industry, which contributes significantly to greenhouse gas emissions. The most popular liquid biofuel in the world is bioethanol, which can be used in modified engines or mixed with gasoline (Sarkar et al., 2012).

First-generation bioethanol has been commercially marketed. It is made from crops that are high in sugar (sugarcane, sugar beet) and starch (corn, wheat). Nonetheless, it is heavily criticized for competing with food supply and arable land, which raises moral and financial questions about the "food versus fuel" controversy (Naik et al., 2010). The creation of second-generation bioethanol, which is made from inedible lignocellulosic biomass, has been accelerated by this restriction. The most plentiful and sustainable organic resource on Earth is lignocellulosic biomass, which includes forestry wastes, agricultural residues, energy crops, and the organic portion of municipal solid trash (Saini et al., 2015). An enormous and underutilized resource for the production of renewable energy that does not jeopardize food security is provided by the estimated billions of tons of biomass produced annually worldwide (Saini et al., 2015)

Given there are so many agro-industrial wastes in Nigeria and many other tropical and sub-tropical countries, there is a huge potential for second-generation bioethanol. About 71.2 million hectares of fertile arable land are Nigeria's blessing, which makes it easier to grow crops high in

carbohydrates like sorghum, yam, and cassava (Ohimain, 2013). According to Elemike et al. (2015), using the leftovers from these crops such as peels, husks, and stalks to produce bioethanol offers a calculated chance to solve waste management problems, make money from waste, and add to the country's energy mix without interfering with the food supply chain.With an emphasis on the potential of a ternary blend of cassava peels, coconut husk, and sawdust,three plentiful and complementary wastes in tropical regions. This chapter offers a thorough overview of the science and technology underlying the manufacture of lignocellulosic bioethanol.

2.2. The Structural Composition of Lignocellulosic Biomass

The intricate and diverse structural framework of lignocellulosic biomass is a key factor in determining how well it converts to biofuels. Because of a phenomena called biomass recalcitrance, this architecture, which gives plants mechanical strength and protection, poses serious obstacles to the generation of biofuel (Zhao et al., 2012). Developing successful deconstruction techniques requires an understanding of this complex make-up.

2.2.1. The Three-Polymer Matrix

The three polymeric components of lignocellulosic biomass; cellulose, hemicellulose, and lignin combine to produce a complex, cross-linked matrix. Plant species, age, and growing conditions all have a substantial impact on the quantity of these components (Bajpai, 2016).

A. CELLULOSE

The most prevalent organic polymer on Earth is cellulose, which makes up between 40 and 50 percent of the dry weight of lignocellulosic biomass and is the main structural element of plant cell walls (McKendry, 2002). The intricate hierarchical structure of this amazing biological polymer contributes to its remarkable mechanical capabilities as well as its formidable resistance to degradation.

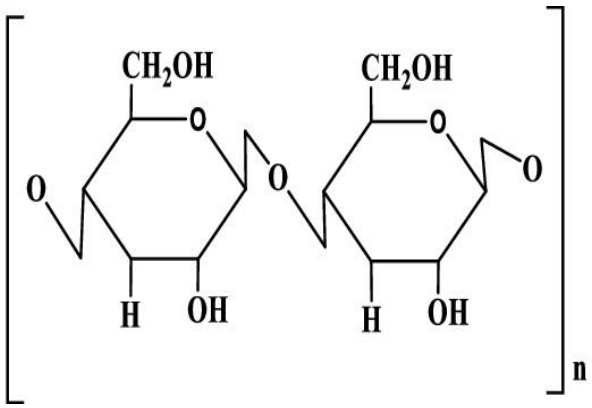

Figure 2.1: Chemical Structure of Cellulose (Madivoli, 2023)

Molecular Structure and Chain Organization: In terms of molecular structure, cellulose is a high molecular weight, linear homopolymer made up solely of D-glucose units joined by β-(1,4)-glycosidic linkages. The disaccharide cellobiose is the repeating unit in this particular linking structure, which produces a rather straight chain molecule with each glucose unit rotated 180° with respect to its neighbors (Bajpai, 2018). A cellulose chain has two chemically different ends: the reducing end has a reducing hemiacetal group at one end, while the non-reducing end has an extra secondary hydroxyl group at the other end (Alam et al., 2014). Given that distinct cellulase enzymes are selective for unique chain ends, this structural asymmetry has significant ramifications for enzymatic breakdown.

Different biomass sources fluctuate greatly in their degree of polymerization (DP); cellulose from native wood usually has DP values greater than 10,000 glucose units, whereas agricultural leftovers often have lower DP values between 3,000 and 5,000 units. The biomass's mechanical characteristics and susceptibility to deterioration are both impacted by this difference in chain length.

Supramolecular Organization and Crystalline Structure: The ability of cellulose to create highly structured crystalline structures through intra- and intermolecular hydrogen bonding is its most distinctive feature. The fundamental structural framework of the plant cell wall is provided

by the basic fibrils that are created when individual cellulose chains align in parallel orientations and bundle together. These elementary fibrils then grow into bigger microfibrils (Bajpai, 2018). A composite material with both highly ordered crystalline and less ordered amorphous regions is the consequence of this hierarchical arrangement. Depending on the plant species, tissue type, and development conditions, the precise value of the crystallinity index of cellulose in native wood can vary from 50% to 70% (Poletto et al., 2012). Cellulose I, the natural form present in plant biomass, is one of the numerous polymorphs that define the crystalline areas. Iα and Iβ are the two allomorphs of cellulose I, the latter of which is more thermodynamically stable and more common in higher plants.

Thermal and Chemical Stability: The crystalline structure and vast hydrogen-bonding network provide exceptional heat stability. Despite the relative lability of its glycosidic linkages, cellulose exhibits significant chemical resistance to destruction. The inaccessibility of these connections inside the crystalline regions is the cause of this seeming paradox. Although cellulose's hydroxyl groups can be chemically modified, the supramolecular structure has a significant impact on the rate and magnitude of reactions, with amorphous regions reacting more readily than crystalline domains.

THE CHALLENGE OF CELLULOSE RECALCITRANCE

Although cellulose can be readily converted to glucose in theory by enzymatic or acid-catalyzed cleavage of its glycosidic linkages, recalcitrance makes this conversion difficult in practice. There are several reasons for this resistance (Zhao et al., 2012):

1. The crystal structure: Enzyme accessibility is restricted by a physical barrier formed by the dense packing of cellulose chains in crystalline areas. Cellulase enzymes have difficulty penetrating crystalline domains but can easily hydrolyze bonds in amorphous areas.

2. Macromolecular Organization: The aggregation of cellulose into microfibrils with diameters of 3-5 nm creates struct to cellulose. The decomposition temperature of cellulose generally ranges between 315°C and 400°C, depending on factors including crystallite size, degree of crystallinity, and DP (Poletto et al., 2012). Kim et al. (2010) Showed that larger crystallites and higher crystallinity indices are associated with increased thermal stability, as more energy is required to disrupt the well-ordered hydrogen-bonding network.

3. Interactions in the matrix: The complex matrix of hemicellulose and lignin that surrounds cellulose in native biomass further restricts enzyme access by physically obstructing enzymes and preventing them from attaching to lignin in a useful way.

4. Pore Structure:.The limited porosity of native biomass restricts the diffusion of enzymes into the cell wall structure, creating mass transfer limitations that slow the hydrolysis process.

One significant technological and financial obstacle to the conversion of lignocellulosic biomass into biofuels and bioproducts is the recalcitrance of cellulose. Therefore, in addition to removing hemicellulose and lignin, effective pretreatment techniques must also alter the structure of cellulose to increase its accessibility to hydrolytic enzymes. Knowing the basic characteristics of cellulose is essential for creating biomass conversion technologies that are more effective and can overcome the difficulties presented by this extraordinary natural polymer.

B. HEMICELLULOSE

One significant technological and financial obstacle to the conversion of lignocellulosic biomass into biofuels and bioproducts is the recalcitrance of cellulose. Therefore, in addition to removing hemicellulose and lignin, effective pretreatment techniques must also alter the structure of cellulose to increase its accessibility to hydrolytic enzymes. Knowing the basic characteristics of

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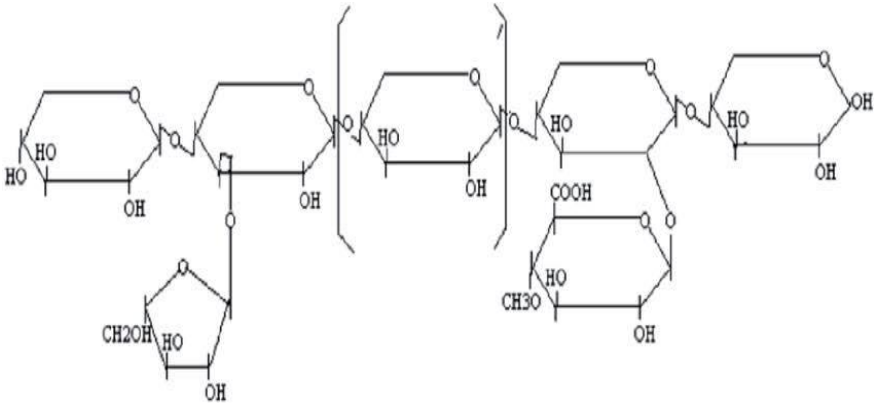

Figure 2.2: Chemical Structure of Hemicellulose (Samir & Ghadbane, 2021)

Chemical Heterogeneity and Structural Diversity: A critical structural feature of many hemicelluloses, particularly xylans, is the presence of acetyl groups attached to the sugar backbone. The degree of acetylation can reach 3-13% of the hemicellulose dry weight, and these groups play a significant role in the polymer's properties and behavior during pretreatment (Sørensen et al., 2011).

The fundamental difference between hemicellulose and cellulose lies in its heterogeneous nature. It is a branched, amorphous heteropolymer composed of multiple sugar monomers, creating a complex and varied structure across different plant species. Based on their primary sugar composition and linkage patterns, hemicelluloses are classified into four main categories (Kang et al., 2014):

1. Xylans: The most abundant hemicellulose type, particularly in hardwoods and agricultural residues. The backbone consists of β-(1,4)-linked xylopyranose units, often substituted with arabinofuranose, glucuronic acid, and acetyl groups.

2. Mannans: Predominant in softwoods, featuring a backbone of β-(1,4)-linked mannose and glucose units, with varying degrees of galactose substitution.

3. Xyloglucans: Found in primary cell walls of higher plants, consisting of a cellulose-like β-(1,4)-glucan backbone with frequent xylose substitutions.

4. Mixed-Linkage β-Glucans: Present in grasses and cereals, characterized by alternating β-(1,3) and β-(1,4) linkages between glucose units.

Architectural Role in the Cell Wall Matrix: As a molecular link between cellulose microfibrils and the lignin polymer, hemicellulose is an essential structural element of the plant cell wall. Hemicellulose creates a thick, cross-linked network that covers the cellulose microfibrils through covalent bonds (mostly ferulic acid bridges) and strong hydrogen bonds (Sørensen et al., 2011). This complex network forms a physical barrier that prevents access to cellulose while also making a substantial contribution to the cell wall's structural integrity.

Hemicellulose's amorphous structure and increased solubility are attributed to its comparatively low degree of polymerization (DP), which usually ranges between 100 and 200 sugar units as opposed to cellulose's thousands (Bajpai, 2018). Compared to highly crystalline cellulose, hemicellulose is more vulnerable to chemical and enzymatic attack due to its lower molecular weight and branching shape.

Behavior During Pretreatment and Processing: The processing of biomass is significantly impacted by hemicellulose's vulnerability to hydrolysis in mild environments. The glycosidic linkages in hemicellulose easily hydrolyze in an acidic environment at high temperatures, releasing monomeric sugars (mostly xylose from xylans). However, this process needs to be closely monitored because harsh circumstances can result in the following products of sugar degradation:

Furfural: Formed from the dehydration of pentose sugars (xylose, arabinose)

5-Hydroxymethylfurfural (HMF): Derived from hexose sugars (mannose, glucose, galactose)

On the other hand, alkaline pretreatments are better at breaking down ester linkages that contain acetyl groups and ferulic acid cross-links, which results in the solubilization of hemicellulose with less sugar degradation (Jönsson & Martín, 2016).

Impact on Enzymatic Hydrolysis and Fermentation

The removal of hemicellulose during pretreatment is crucial for several reasons:

1. Removal of Physical Barriers: Cellulase enzyme accessibility to their cellulose substrate is significantly increased by pretreatment, which dissolves hemicellulose and produces pores and voids in the biomass structure.

2. Inhibitor Formation: During pretreatment, the hemicellulose's acetyl groups are liberated as acetic acid, which, at concentrations as low as 2–5 g/L, functions as a strong inhibitor of fermentation (Jönsson & Martín, 2016). By disabling oxidative phosphorylation and acidifying the cytoplasm of cells, acetic acid interferes with microbial metabolism and drastically lowers fermentation-related ethanol production.

3. Enzyme binding that is not productive: Hydrolytic enzymes can be redirected from their intended cellulose targets by the adsorption of certain hemicellulose components and the breakdown products they produce, which lowers the total hydrolysis efficiency.

The need of effective pretreatment techniques is highlighted by hemicellulose's dual nature as a possible source of process inhibitors and a desirable supply of fermentable sugars. The optimal pretreatment should optimize the recovery of sugars produced from hemicellulose while reducing the production of degradation products that impede the subsequent fermentation and enzymatic hydrolysis. Therefore, it is crucial to comprehend the intricate chemistry and structural function of hemicellulose in order to create effective biomass conversion procedures that make full use of all of the carbohydrate components of lignocellulosic biomass.

C. LIGNIN

As the second most abundant organic polymer on Earth after cellulose, lignin is a complex, three-dimensional, amorphous heteropolymer that is crucial for the structural and defensive components of vascular plants. It makes up 15–30% of lignocellulosic biomass by weight, but it has a disproportionately large impact on biomass recalcitrance (Bajpai, 2018). This macromolecule is fundamentally different from cellulose and hemicellulose because it is made up of aromatic alcohols rather than sugars, which gives it unique chemical and physical properties that are essential to its biological function and its role as a major barrier in biorefining.

Monomeric Composition and Biosynthetic Origin: Three main monolignols, which are phenylpropanoid units, undergo enzymatic dehydrogenation and radical coupling to form lignin, a phenolic polymer:

p-Coumaryl alcohol: Upon polymerization, it forms the p-hydroxyphenyl (H) unit.

Alcohol conifer: creates the guaiacyl (G) unit, which is distinguished by the aromatic ring's solitary methoxy group.

Alcohol from sinapyl: creates the syringyl (S) unit, which is distinguished by the aromatic ring's two methoxy groups.

The relative proportion of these monomers varies significantly among different plant taxa, reflecting their evolutionary adaptation (Bajpai, 2018).

Softwoods, sometimes known as gymnosperms: Guaiacyl (G) units from coniferyl alcohol make up nearly all (>95%) of their lignin. Because of its very condensed structure and many carbon-carbon linkages, softwood lignin is especially resistant to change.

Dicotyledonous angiosperms, or hardwoods: Guaiacyl (G) and syringyl (S) units copolymerize to form its lignin, which normally has a S/G ratio between 2:1 and 5:1. A lignin structure with

more ether linkages and less condensation is produced by the abundance of S-units, which have two methoxy groups that sterically inhibit coupling at the 5-position. This makes the lignin more vulnerable to chemical cleavage.

All three units (H, G, and S) are present in considerable amounts in grass lignins (monocots), along with ferulic acid, which has the ability to cross-link lignin to hemicellulose and form an exceptionally strong and intricate network.

One important factor affecting the reactivity of lignin is the ratio of syringyl (S) to guaiacyl (G) units. According to Li et al. (2014), a higher S/G ratio often corresponds to a less condensed, more linear polymer that is simpler to depolymerize during pretreatment, which helps to reduce overall biomass recalcitrance.

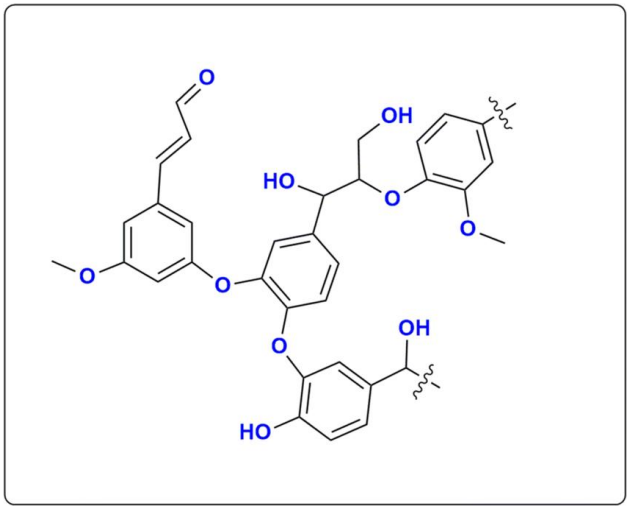

Figure 2.3: Chemical Structure of Lignin (Negi & Singh, 2020)

Structural Role and Recalcitrance Mechanisms: Lignin structural Iink is not merely a filler; it is intricately deposited within the spaces between cellulose microfibrils and hemicellulose chains, forming a hydrophobic, cross-linked, and chemically resistant seal. This lignification process provides the plant with rigidity and compressive strength to plant cell walls, enabling plants to achieve significant height. Furthermore, lignin's hydrophobic properties are essential for efficient

water and solute transport within the plant's vascular system. This waterproofing effect ensures that water moves effectively throughout the plant body. Finally, lignin acts as a protective barrier against various threats. It safeguards plants from microbial degradation and pest infestations by forming both a physical and chemical deterrent. (Li et al., 2014).

This very structure makes lignin the primary agent of biomass recalcitrance through two principal mechanisms:

1. The physical obstruction: The physical barrier that lignin creates around cellulose and hemicellulose significantly reduces the amount of access that hydrolytic enzymes have to their polysaccharide substrates. It serves as a "molecular gatekeeper" that must be gotten past by enzymes in order to get to their target bonds.

2. Enzyme binding that is not productive: The hydrophobic and aromatic surface areas of hydrolytic enzymes, especially cellulases, show a strong affinity for lignin. This causes a sizable portion of the enzymes to adsorb onto the lignin surface in an irreversible and ineffective manner. After binding, these enzymes are essentially deactivated and unable to hydrolyze cellulose, which significantly lowers the saccharification process's overall efficiency and raises the need for enzyme loading, which raises the process's cost (Li et al., 2014).

IMPLICATIONS FOR PRETREATMENT AND VALORIZATION

Because lignin is such a significant obstacle, effective pretreatment techniques created especially for its removal or redistribution are required. Because they cleave the ester and ether linkages within lignin and between lignin and hemicellulose, alkaline pretreatments (such as those involving sodium hydroxide or ammonia) are very effective at solubilizing lignin and causing the

biomass to swell. By dissolving lignin in organic solvents at high temperatures, the organosolv pretreatment produces a comparatively pure and perhaps profitable lignin stream.

Moreover, lignin's intrinsic complexity is no longer only seen as an issue. Lignin is becoming more and more acknowledged as a promising feedstock for the synthesis of value-added bio-based products and chemicals, including phenolic resins, carbon fibers, adhesives, and dispersants, in the framework of a contemporary biorefinery. This change in viewpoint from a waste product to a co-product is essential to enhancing the lignocellulosic biofuel production process's overall sustainability and profitability.

2.3. The Bioethanol Production Pathway from Lignocellulose

To overcome biomass recalcitrance, lignocellulosic biomass is converted to bioethanol through a multi-step biochemical process. Pretreatment, fermentation, hydrolysis (saccharification), and distillation/dehydration are the main phases. The success of the early pretreatment has a significant impact on the overall pathway's efficiency.

2.3.1. Pretreatment

The most important and frequently most costly unit operation is pretreatment, which accounts for 20–40% of the entire production cost (Mosier et al., 2005). Disrupting the strong lignocellulosic matrix is its main goal in order to make enzymatic hydrolysis easier. Several important goals must be met by the perfect pretreatment:

Disruption of the lignin seal and breakdown of the lignin-carbohydrate complex: The lignin-carbohydrate complex (LCC) is a complex network of covalent and hydrogen connections that is formed when cellulose and hemicellulose fibers are encased in a strong, waterproof glue. This establishes the main defense against enzymatic assault.

Hemicellulose solubilization: Hemicellulose is an amorphous, branching polymer that serves as a bonding agent between lignin and cellulose microfibrils. In order to create pores and blank spaces in the biomass structure, it must be removed.

Decrease in cellulose crystallinity: Linear glucan chains are densely packed together by strong hydrogen bonds in native cellulose, which is in a highly organized, crystalline state. Enzymatic degradation is extremely difficult to achieve with this crystalline structure.

Growth in biomass surface area and porosity: The natural biomass structure is dense and has a small specific surface area that enzymes can access. The material must physically "open up" during pretreatment.

Pretreatment methods are broadly classified into physical, chemical, physico-chemical, and biological methods.

1. Physical Pretreatment

Physical techniques seek to improve surface area, decrease cellulose crystallinity, and decrease biomass particle size. This comprises:

Mechanical Communication: Common but energy-intensive processes are milling and grinding. Compared to agricultural leftovers, woody biomass uses more energy (for example, 27.6 kWh/ton for switchgrass) (Barakat et al., 2014). Zhu et al. (2010) suggested chemical treatment prior to size reduction in order to decrease energy.

Extrusion: This method uses an extruder to heat, shear, and combine biomass. It is an industrially scalable, continuous process. Its effectiveness is influenced by variables such as temperature, screw speed, and solid loading. For increased efficiency, it can be used with ionic liquids (Da Silva et al., 2013; Han et al., 2020).

Microwave and Ultrasonic Pretreatment: Based on Kostas et al. (2017), microwave pretreatment provides quick and effective heating, but its industrial use is restricted due to a lack of mechanistic knowledge. Although ultrasonic pretreatment breaks down cell walls and expands surface area, it is still too young to be used on a broad scale (Zheng et al., 2014).

2. Chemical Pretreatment

Chemical methods use reagents to solubilize lignin and/or hemicellulose.These are classified into: Acid Pretreatment: Hydrolyzes hemicellulose and breaks down lignin using acids (such as HCl and H_2SO_4). Compared to concentrated acid, which corrodes equipment and produces significant amounts of inhibitors such furfural and 5-Hydroxymethylfurfural (HMF), diluted acid pretreatment shows greater promise for industrial scale applications (Solarte-Toro et al., 2019). Prior to fermentation, the hydrolysate frequently needs to be detoxified (Chandel et al., 2007)..

Alkaline Pretreatment: By saponifying ester linkages and causing biomass swelling, bases (such as NaOH, $Ca(OH)_2$, and NH_4OH) are used to efficiently remove lignin (Kim et al., 2016). Because agricultural leftovers contain less lignin than woody biomass, it works better on the former (Yang & Wyman, 2008). Time, temperature, and alkali concentration are important variables (Chen et al., 2015; Kim & Han, 2012).

Organosolv Pretreatment: Using organic solvents as the main delignifying agents, organosolv pretreatment is a potential physico-chemical technique for separating lignocellulosic biomass into its constituent cellulose, hemicellulose, and lignin. At high temperatures (generally 150–200°C) and pressures, this process normally uses solvents like ethanol, methanol, acetone, or ethylene glycol, frequently in conjunction with acidic (e.g., H_2SO_4, HCl) or alkaline catalysts. Borand & Karaosmanoħlu, 2018; Salapa et al., 2017).

Ionic Liquid (IL) Pretreatment: In mild circumstances, lignocellulosic components can be dissolved by ILs, which are non-volatile salts. Although they may be recovered and used again, they are costly despite their effectiveness (Yoo et al., 2017). Rice straw yields of sugar and ethanol were greatly increased by pretreatment with 1-ethyl-3-methylimidazolium acetate (Poornejad et al., 2014).

Pretreatment with Deep Eutectic Solvents (DESs): DESs are low melting point mixes of hydrogen bond donors and acceptors. Despite obstacles including high viscosity and hygroscopicity, they are becoming a more environmentally friendly delignification option (Chen & Mu, 2021; Yerizam et al., 2023).

3. Physico-chemical Pretreatment

These methods combine physical and chemical actions and includes:

Steam Explosion: After several minutes of treatment with high-pressure saturated steam (160–260°C), biomass is quickly decompressed. The procedure results in autohydrolysis, in which hemicellulose's acetyl groups catalyze additional hydrolysis by forming acetic acid (Baruah et al., 2018).

Ammonia fiber explosion (AFEX): This process is comparable to steam explosion but employs liquid ammonia under high pressure and at moderate temperatures (60–100°C). It efficiently breaks down cellulose and eliminates lignin without producing any noticeable inhibitors. Recovering and reusing ammonia is possible (Balan et al., 2009).

SC-CO_2, or supercritical CO_2 Explosion: The biomass structure is penetrated by CO_2 under supercritical circumstances. It bursts when depressurized quickly, expanding the surface area. This approach is non-toxic and environmentally friendly (Gu et al., 2013).

Liquid Hot Water (LHW): Biomass is heated to 160–240°C and then pressed with water to maintain its liquid state. It mostly solubilizes hemicellulose as oligomers with minimal inhibitor generation (Kim et al., 2009).

4. Biological Pretreatment

This process breaks down lignin and hemicellulose using microorganisms, mainly white-rot, brown-rot, and soft-rot fungi, or their enzymes (laccases, peroxidases) (Ghasemzadeh et al., 2017). Although it is a low-energy and environmentally benign technique, it is not as ideal for industrial-scale applications due to its slowness (sometimes taking weeks) and potential for partial carbohydrate loss (Velmurugan et al., 2020).

2.3.2. HYDROLYSIS (SACCHARIFICATION)

The solid residue, now enriched in cellulose, is hydrolyzed to convert the polysaccharides into fermentable sugars after pretreatment; enzymatic hydrolysis is the preferred method because of its higher specificity, milder conditions, and lack of corrosion and inhibitor formation problems (Taherzadeh & Karimi, 2007). Acid hydrolysis is equally an option.

Enzymatic hydrolysis employs a synergistic cocktail of enzymes:

Endoglucanases: These essential enzymes start the breakdown of cellulose by haphazardly severing the internal connections in its amorphous, disordered sections. In addition to shortening the chains, this activity gives the microfibril a large number of new beginning places. These new chain ends are crucial because they give exoglucanases the places they need to attach and start working, which drastically boosts the effective surface area for digestion and considerably accelerates the hydrolysis process as a whole (Payne et al., 2015; Horn et al., 2012).

Cellobiohydrolases: Also referred to as exoglucanases, these enzymes processively break down cellobiose units from the ends of cellulose strands. By traveling along a single cellulose chain

and producing the disaccharide cellobiose as their main product from both the reducing and non-reducing ends, they function as a threading mechanism (Payne et al., 2015; Horn et al., 2012). Cellobiose and other short-chain cello-oligosaccharides are hydrolyzed into glucose molecules by β-glucosidases. According to Taherzadeh and Karimi (2008), this is essential to avoid cellobiose buildup, which inhibits exoglucanases.

A variety of hemicellulases, including endoxylanases, β-xylosidases, and α-arabinofuranosidases, are necessary for the hydrolysis of hemicellulose because of its diverse structure (Verma & Satyanarayana, 2012). The effectiveness of the pretreatment is the most crucial factor that determines the efficiency of enzymatic hydrolysis, along with enzyme loading, temperature, pH, mixing, and solid loading.

2.3.3. Fermentation

Ethanol and carbon dioxide are the main byproducts of fermentation, a crucial biological process in which microorganisms, mostly yeasts, digest carbohydrates in an anaerobic environment to produce energy. This procedure serves as the primary conversion step in the production of bioethanol, turning the soluble sugar stream that results from hydrolysis into the required fuel molecule. The type of sugars present, the microbe used, and the fermenter's operating parameters all have a direct impact on the process's efficiency, yield, and rate (Taherzadeh & Karimi, 2007).

2.3.3.1 Hexose Fermentation

The fermentation of hexoses, or six-carbon sugars, like glucose, is a well-established and extremely effective process. Because of its high ethanol output, strong resistance to inhibiting circumstances (such as low pH and high ethanol concentration), and extensive history of safe

usage in industrial applications, the industry standard bacterium Saccharomyces cerevisiae performs very well in this position.

The Embden-Meyerhof-Parnas (EMP) pathway, also referred to as glycolysis, is the metabolic pathway responsible for this conversion. In this pathway, one glucose molecule undergoes ten enzymatic reactions to produce two pyruvate molecules. In aerobic conditions, pyruvate enters the mitochondria for complete oxidation, but in the anaerobic conditions required for ethanol production, pyruvate is first decarboxylated to acetaldehyde by the enzyme pyruvate decarboxylase, and then alcohol dehydrogenase (ADH) reduces acetaldehyde to ethanol while simultaneously regenerating NAD^+ from NADH, which is necessary for glycolysis to continue (Lin & Tanaka, 2006).

$C_6H_{12}O_6$ (Glucose) → 2 C_2H_5OH (Ethanol) + 2 CO_2 (Carbon Dioxide)

Hexose fermentation is the most dependable stage in the bioethanol production chain since this pathway is so well-optimized in industrial S. cerevisiae strains that they can obtain ethanol yields that are close to 90–95% of the theoretical maximum (Walker & Walker, 2018).

2.3.3.2 Pentose Fermentation

Although hexose fermentation is simple, the main difficulty in producing lignocellulosic bioethanol is using pentose sugars (such as xylose and arabinose) that are generated from hemicellulose. As was previously known, a sizable portion of the biomass hydrolysate is wasted because S. cerevisiae lacks the natural pathways necessary to metabolize these sugars.

Native Pentose-Fermenting Microorganisms

Certain yeasts and bacteria possess inherent pentose assimilation pathways.

Yeasts: Scheffersomyces stipitis and Candida shehatae are examples of native pentose-fermenting yeasts that use the Xylose Reductase-Xylitol Dehydrogenase (XR-XDH) pathway.

Using NADPH as a preferred cofactor, xylose reductase (XR) initially converts xylose to xylitol in this process. Xylitol dehydrogenase (XDH) then employs NAD^+ to convert xylitol to xylulose. This results in a redox cofactor imbalance, which frequently causes decreased ethanol yields and xylitol secretion. After being phosphorylated, xylulose moves into the pentose phosphate pathway's (PPP) non-oxidative phase, where it is transformed into glycolytic intermediates (Hahn-Hägerdal et al., 2007).

Bacteria: A more straightforward Xylose Isomerase (XI) pathway is used by bacteria such as Escherichia coli, which converts xylose to xylulose in a single, redox-neutral step. After being phosphorylated, xylulose is converted to xylulose-5-phosphate, which likewise enters the PPP. Although these organisms are naturally capable, their slow fermentation kinetics, limited ethanol tolerance, sensitivity to inhibitors, and (in the case of yeasts) necessity for precise micro-aeration to maintain redox balance make them unsuitable for industrial settings.

2.3.4. Distillation and Dehydration

Distillation is a widely used technique to purify ethanol from a fermented mixture. This process separates components of a liquid mixture based on their differing boiling points. In the context of ethanol production, a fermentation broth, often referred to as "beer," typically contains only 5-15% ethanol along with water, residual particles, and microbes. The primary goal of distillation in this scenario is to concentrate the ethanol to a higher purity. This concentration typically reaches close to its azeotropic point, which is approximately 95.6% ethanol by mass. The distillation process requires significant energy, with thermal energy in the form of steam accounting for about 90% of the total energy consumed in an ethanol facility (Kwiatkowski et al., 2006).

DISTILLATION PROCESSES FOR ETHANOL PURIFICATION

Various distillation methods are employed to overcome the azeotropic barrier and achieve higher purity ethanol:

Simple Distillation: This method involves heating the ethanol and water mixture to separate them, typically in a still. While effective for initial concentration, it cannot produce pure ethanol due to the azeotrope.

Extractive Distillation: This technique involves adding an entrainer, such as glycerol or ethylene glycol, to the ethanol-water mixture. The entrainer alters the relative volatilities of ethanol and water, allowing for further separation beyond the azeotropic point. This method can achieve high purity ethanol with relatively low energy consumption (Kiss & Suszwalak, 2012).

Azeotropic Distillation: This process involves introducing a third component, an azeotropic agent or entrainer (e.g., benzene or n-heptane), to form a new, lower-boiling azeotrope with water. This allows the water to be removed, leaving behind higher-purity ethanol (Black, 1980).

Membrane Distillation: This method investigates the separation of ethanol from solutions using membranes, where the ethanol flux is strongly affected by vapor pressure and ethanol concentration in the feed. It can achieve higher ethanol content in the permeate than in the broth (El-Bourawi et al., 2006).

Fractional Distillation: For initial purification, a fractional distillation column with numerous stages can purify ethanol to a concentration of 96.5% by volume.

Fuel-grade anhydrous ethanol, which must have a purity greater than 99.5%, requires an additional dehydration stage due to the ethanol-water azeotrope that limits purification by conventional distillation. Several techniques are employed to achieve this high level of purity, including molecular sieves, extractive distillation, and pervaporation.

Molecular Sieves: Molecular sieves are a highly effective method for dehydrating ethanol, preferentially adsorbing water molecules to produce anhydrous ethanol. Type 3A molecular sieves are particularly effective for this purpose, with some formulations designed specifically for fuel-grade ethanol dehydration. These sieves, such as Zeochem's Z3-03 and Hengye Inc.'s EthaDry, can reduce moisture content to below 10 ppm and achieve purities of up to 99.999% (Zeochem, 2024; Hengye, 2024)

Extractive Distillation: Extractive distillation is another widely used technique to overcome the ethanol-water azeotrope by introducing an entrainer that alters the relative volatilities of ethanol and water. This process can significantly reduce energy consumption compared to conventional distillation (Kiss & Suszwalak, 2012).

Entrainers: Common entrainers include glycols such as ethylene glycol, diethylene glycol, trimethylene glycol, triethylene glycol, and tetraethylene glycol, as well as glycerin, sulfolane, and 1,4-butanediol (Gil et al., 2009). Ethylene glycol is particularly effective and can be used in combination with salts like calcium chloride to further enhance separation efficiency and reduce energy usage. Cyclic carbonates derived from CO2 conversion processes have also been proposed as effective extractive agents for alcohol-water separation. Glycerin has shown to be a very competitive solvent, with varying energy consumption depending on the alcohol type and purity (Avci et al., 2019).

With a high calorific value, the leftover lignin and unfermented solids from the process often referred to as stillage, can be burned to produce power and process heat, enhancing the biorefinery's economics and energy balance (Hahn-Hägerdal et al., 2006).

2.4. REVIEW OF INDIVIDUAL FEEDSTOCKS FOR THE TERNARY BLEND

Understanding the unique properties of each component is essential for the effective execution of a mixed feedstock strategy. Cassava peels, coconut husks, and sawdust were chosen for this investigation because of their complimentary lignocellulosic characteristics and their prevalence as agro-industrial wastes in tropical areas.

2.4.1. CASSAVA PEELS

Over 300 million metric tons of cassava (Manihot esculenta Crantz) are produced worldwide each year, making it an essential staple crop in the tropics. With a predicted yearly production of 59.5 million tonnes, Nigeria is the largest producer in the world (FAO, 2020). With peels making up 10–15% of the raw root weight, processing cassava roots for food items like garri, fufu, and starch produces a significant amount of waste (FAO, 2020).

As a result, millions of tons of cassava peels are produced annually, and they are frequently thrown away carelessly. This acts as a haven for pathogens and contributes to environmental degradation through methane emissions during anaerobic decomposition (Sanni et al., 2019). Therefore, valuing this plentiful, inexpensive residue for the production of bioethanol helps to achieve renewable energy targets while addressing a significant waste management concern.

QUALITIES OF COMPOSITION AND VARIABILITY

Depending on the cassava species, soil type, harvest age, and peeling technique, the biochemical makeup of cassava peels is complicated and varies. Their notable residual starch content, which can vary from 20% to 50% on a dry weight basis, is one of their distinguishing characteristics (Adelekan, 2012). This starch hydrolyzes into glucose somewhat easily, making it a quickly fermentable sugar source that can produce high initial sugar yields with no advance processing. The peels have a characteristic lignocellulosic structure in addition to starch.

While hemicellulose, which mostly consists of pentosans like xylan, ranges from 15% to 25%, cellulose content typically ranges from 20% to 35% (Ongoen et al., 2021). According to reports, the lignin concentration ranges from 10% to 30%; however, initial evaluations in certain research suggest that it may occasionally be abnormally high, possibly as a result of the presence of outer skin layers or certain growing conditions. Accessing this lignocellulosic fraction necessitates thorough pretreatment since it is a secondary, more resistant source of fermentable sugars.

CHALLENGES IN UTILIZATION FOR BIOETHANOL

High Initial Moisture Content: Due to their high moisture content (about 70–80%), fresh cassava peels are extremely perishable and prone to microbial deterioration. To stabilize the biomass for processing and storage, this calls for quick, frequently energy-intensive sun-drying or mechanical drying (Nwokoro & Onyeze, 2019).

Recalcitrance of the Lignocellulosic Fraction: The lignin and hemicellulose in the peel form a protective layer around the cellulose, preventing the lignocellulosic fraction from readily converting the starch. To guarantee high total sugar yields from the complete biomass, not only the starch component, this lignocellulosic recalcitrance necessitates an efficient pretreatment approach (Mosier et al., 2005).

Cyanogenic Glycosides: When hydrolyzed, the cyanogenic glycosides lotaustralin and linamarin, which are found in cassava peels, can release the poisonous hydrogen cyanide (HCN). Although these chemicals can be greatly reduced by appropriate drying and heating during pretreatment, their presence needs to be watched because they may hinder fermenting microorganisms (Nwokoro & Onyeze, 2019).

PREVIOUS RESEARCH CONTEXT

The viability of using cassava peels to produce bioethanol has been confirmed by research. Early work by Neelakandan et al. (2009) indicated that acid hydrolysis followed by fermentation with Saccharomyces cerevisiae could provide large ethanol yields, highlighting the significance of the starch portion. More recent investigations have examined sophisticated pretreatment approaches.

For example, To improve sugar release, Ongoen et al. (2021) combined physical (size reduction) and biological (fungal pretreatment with Aspergillus niger) methods. They took advantage of the fungi's capacity to produce cellulases/hemicellulases to break down the fibrous structure and amylases to hydrolyze starch. A co-culture of Aspergillus niger (for simultaneous saccharification) and Saccharomyces cerevisiae (for fermentation) in a Single-Pot technique was used in another study by Sanni et al. (2019), which simplified the production workflow and increased efficiency.

Cassava peels are not considered a stand-alone feedstock in this study, but rather an essential part of a ternary blend, and their function is probably in two ways: they provide a burst of readily accessible sugars from starch and hemicellulose to initiate fermentation, and their fibrous structure adds to the overall physical matrix of the blended feedstock.

2.4.2. Coconut Husk

The coconut (Cocos nucifera L.), a perennial palm that is widely grown along tropical coasts, produces more than 62 million tons of coconuts a year worldwide. According to Rosa et al. (2021), the fruit's mesocarp, or outer husk, makes up 35–40% of its weight and is a substantial by-product. This fiber husk is regarded as agricultural waste in many producing countries and is frequently burned in open fields or allowed to decay, which increases greenhouse gas emissions

and pollutants in the environment. Therefore, its value addition for the manufacture of bioethanol is a crucial chance for waste-to-energy conversion in the circular bioeconomy.

COMPOSITIONAL CHARACTERISTICS AND STRUCTURAL RECALCITRANCE

The strong, fibrous texture of coconut husk is well known, and this is reflected in its distinct and difficult chemical structure. Among common agricultural leftovers, this makes it one of the most resistant lignocellulosic materials.

Lignin: Its unusually high lignin concentration, which is usually estimated to be between 30% and 45%, is its most distinctive characteristic (Owuama, 2021; Correia et al., 2020). Its recalcitrance is primarily caused by the high concentration of lignin, which creates a solid, chemically resistant, and hydrophobic seal. Its stability is further enhanced by the fact that the lignin is mainly of the guaiacyl-syringyl type and has a high degree of condensation (Rosa et al., 2021).

Cellulose and Hemicellulose: Although the cellulose content is moderate, often between 25% and 35%, it is difficult to remove due to its close connections with the lignin matrix. Xylan and mannan make up the majority of the relatively low (10–20%) hemicellulose content (Correia et al., 2020).

CHALLENGES IN UTILIZATION FOR BIOETHANOL

Extreme Recalcitrance: The dense structure and high lignin concentration need harsh pretreatment conditions, which result in higher energy and chemical consumption as well as longer reaction times (Binod et al., 2021).

Formation of Inhibitors: The severe pretreatment conditions needed might result in the significant production of fermentation inhibitors, such as furan derivatives (furfural, HMF) from

the breakdown of sugar and phenolic compounds from the breakdown of lignin (Jönsson & Martín, 2016).

High Ash Content: Minerals like potassium and sodium are present in the 2-5% ash that can be found in coconut husk. This may impact chemical balances during fermentation and hydrolysis and cause slagging in heat operations (Rosa et al., 2021).

PREVIOUS RESEARCH CONTEXT AND PRETREATMENT STRATEGIES

Although there isn't as much research on coconut husk for bioethanol as there is on other feedstocks, it has been shown that successful pretreatment is crucial. It has been determined that alkaline pretreatments are very appropriate. According to a study by Correia et al. (2020), pretreatment with alkaline hydrogen peroxide (AHP) greatly improved the enzymatic digestibility of coconut husk by delignifying it. In their assessment of different approaches, Binod et al. (2021) pointed out that for such resistant feedstocks, sequential pretreatment might be required. New techniques like Deep Eutectic Solvents (DES) have also been investigated in recent research.

Yerizam et al. (2023) achieved up to 65.81% lignin removal and an ethanol concentration of up to 14% (v/v) with the use of DES and Natural DES (NADES) for delignification. The most resistant ingredient in the ternary blend in the context of this investigation is coconut husk. Its function is complicated; although it presents a considerable obstacle, its fibrous nature may improve the physical structure of the blended feedstock by enhancing porosity and avoiding compaction during pretreatment, which may increase the accessibility of chemicals and enzymes to the other constituents.

2.4.3. SAWDUST

A common and plentiful lignocellulosic by-product produced worldwide by sawmills and the woodworking sector is sawdust. Although it is hard to measure exactly, estimations indicate that millions of tons are created each year as a direct result of the processing of lumber (López-Linares et al., 2015). Sawdust is an incredibly appealing and dependable feedstock for biorefining processes since it is a steady, year-round waste stream, unlike seasonally available agricultural residues. Despite this potential, a sizable amount is frequently wasted through low-value uses like open burning, landfilling, or combustion for low-grade heat, which results in a major loss of opportunity for sustainable value addition and contributes to environmental damage.

COMPOSITIONAL CHARACTERISTICS AND VARIABILITY

Depending on the type of tree (hardwood vs. softwood), the region of origin, and the particular sawmilling method, sawdust's chemical makeup varies greatly rather than being constant.

Cellulose: A high cellulose content, usually between 40% and 50% in hardwoods and 35% to 45%*in softwoods, is a characteristic of sawdust (Sun, 2010). It is a premium supply of glucose, the main sugar for traditional ethanol production, because to its high glucan content.

Hemicellulose: The content of hemicellulose varies significantly among wood species. Xylans (15–25%).make up the majority of hardwood hemicellulose, whereas mannans (15–20%) and galactans are abundant in softwood hemicellulose (Zhao et al., 2012). One important supplementary source of fermentable sugars is this percentage.

Lignin: According to Lopez-Linares et al. (2015), lignin content is significant and typically ranges from 20% to 30% for hardwoods and 25% to 35% for softwoods. The lignin structure plays a major role in recalcitrance; softwood lignin, which is primarily made up of guaiacyl units,

is frequently more condensed and resistant than the guaiacyl-syringyl lignin found in hardwoods, making breakdown more difficult.

CHALLENGES IN UTILIZATION FOR BIOETHANOL

Despite its high carbohydrate potential, the utilization of sawdust for bioethanol presents several unique challenges:

Intrinsic Recalcitrance: The dense, woody structure and high lignin content create a robust, cross-linked matrix that is highly resistant to enzymatic and microbial attack. This inherent recalcitrance often necessitates severe, energy-intensive pretreatment methods to achieve satisfactory sugar yields, despite sawdust's high carbohydrate potential (Zhu & Pan, 2010).

Variable Composition: The composition of sawdust is subject to variation due to its inherent heterogeneity, which frequently consists of a mixture of many wood species from a sawmill. A major obstacle to industrial-scale uniformity and economic predictability, this variability can lead to fluctuating sugar and ethanol production and hinder the optimization of biorefinery processes (López-Linares et al., 2015).

Presence of Extractive and Contaminants: Hydrolytic enzymes and fermenting microbes may be inhibited by natural extractives found in wood, such as terpenes, tannins, and resins. According to Mohan et al. (2006), sawdust made from treated wood that is, wood that has been treated with paints, adhesives, or preservatives is also unsuitable for bioconversion because it contains organic contaminants and hazardous heavy metals that can interfere with fermentation and contaminate downstream products and process residues.

PREVIOUS RESEARCH AND THE PRETREATMENT TECHNIQUES USED.

Sawdust has been the focus of much research for the generation of bioethanol because of its high cellulose content and abundance. To get past its resistance, a variety of pretreatment approaches have been investigated:

Steam Explosion: In particular, this physico-chemical approach works very well for hardwoods. It employs high-pressure saturated steam and an explosive decompression process that enhances cellulose accessibility, solubilizes hemicellulose, and tears the biomass structure (Zhu & Pan, 2010).

Organosolv Pretreatment: This technique effectively solubilizes lignin by using organic solvents (such as ethanol and acetic acid) frequently in conjunction with an acid catalyst. It is especially beneficial for sawdust, resulting in a relatively pure stream of lignin that may be valued as a byproduct and a high-purity, reactive cellulose pulp (Zhao et al., 2009).

Dilute Acid Pretreatment: This technique effectively solubilizes lignin by using organic solvents (such as ethanol and acetic acid) frequently alongside with an acid catalyst. It is especially beneficial for sawdust, resulting in a relatively pure stream of lignin that may be valued as a byproduct and a high-purity, reactive cellulose pulp (Zhao et al., 2009).

A study by Adewumi (2022) examined the manufacturing of ethanol from locally obtained sawdust in a Nigerian distillery setting, demonstrating its practical practicality. The procedure produced an ethanol yield of about 30%, demonstrating the feedstock's viability for use in small-scale, real-world applications.

In the ternary blend, sawdust is positioned to function as the primary cellulose backbone. Its main function is to increase the total amount of sugar in the hydrolysate by offering a reliable, high-yield source of glucose. Although it is naturally resistant, its resistance differs from that of

coconut husk in that it is more structural than chemically inhibitive. One important theory is that the easier-to-digest components of feedstocks, such as cassava peels, may create pathways for enzymes to more efficiently access the cellulose in sawdust when they are optimally blended with them. This would allow for synergistic effects to improve overall conversion efficiency.

2.5. The Concept of co-processing and feedstock blending

There has been a major change in biorefining from single-feedstock to multi-feedstock. The deliberate blending of two or more different biomass types to produce a composite feedstock with better qualities than its constituent parts is known as feedstock blending, or co-processing. In order to overcome inherent limits and improve the economic and operational viability of biofuel plants, this approach is becoming more and more popular (Liu et al., 2016).

2.5.1.Benefit of feedstock blending

The primary explanation is that various biomasses have complementing physical and chemical properties. The performance of various feedstocks in conversion processes can be improved by mixing them to balance out their specific limits, such as moisture content, ash composition, energy density, or nutritional levels.

Compositional Balancing: Blending a high-cellulose feedstock (e.g., sawdust) with a high-hemicellulose feedstock (e.g., cassava peels) can lead to a more consistent and greater overall sugar production. Additionally, it is possible to successfully "dilute" the total lignin content and its detrimental effects by combining a highly resistant feedstock (such as coconut husk) with one that is easier to digest (Wahid et al., 2019).

Improved Rheology and Processability: Biomass, such as sawdust, can be challenging to work with in slurries due to its low bulk density. The consistency of the slurry can be improved by

blending with wetter, more fibrous materials, such as coconut husk or cassava peels, which will enhance mixing, heat transfer, and chemical penetration (Mansfield et al., 2014).

Inhibitor Mitigation: By diluting the concentration of inhibitors (such as furans and phenolics) produced from a single feedstock during pretreatment, blending might lessen the combined toxicity of these compounds to fermenting microorganisms (Jönsson & Martín, 2016).

Enhanced Supply Chain Resilience: By using a multi-feedstock approach, a biorefinery can reduce the risks associated with seasonal availability and price fluctuation by using whatever biomass is most affordable and plentiful at any given moment (Niziolek et al., 2015).

2.5.2. Evidence from Previous Studies on Feedstock Blending

The theoretical benefits of blending are well supported by empirical data, which shows quantifiable synergistic effects.

Synergistic Improvements in Sugar Yield: Wahid et al. (2019) discovered that hydrothermal processing of particular wheat straw and birch wood mixes produced glucose and xylose yields that were up to 15% higher than the weighted average that was predicted. The solubilized hemicellulose of one feedstock enhanced porosity for enzymes to reach the cellulose in both, which they attributed to comparable breakdown kinetics. Zhang et al. (2021) found an ideal mix of lignin and inhibitors in ternary blends of maize stover, switchgrass, and poplar that enhanced enzymatic hydrolysis efficiency by 20–25% as compared to the best single feedstock.

Inhibitory Compound Mitigation: Kuglarz et al. (2018) showed that co-hydrolyzing sewage sludge with wheat straw produced a hydrolysate with reduced levels of phenolics and heavy metals, allowing for a 40% increase in ethanol output. Furan and organic acid concentrations can also be diluted by mixing acid-pretreated biomass with untreated biomass (Shafiei et al., 2019).

Synergy in Other Processes: Co-digestion of many substrates to balance the Carbon-to-Nitrogen (C/N) ratio in anaerobic digestion usually increases methane output by 25–50% (Hagos et al., 2017). In order to greatly increase biogas yield and process stability, this synergy maximizes microbial activity while avoiding nutritional restrictions or inhibitory circumstances.

THE NEED FOR SYSTEMATIC OPTIMIZATION

Empirical research employing biomass types closely related to those in the current study provides strong validity of the theoretical framework for feedstock blending. The benefits of co-processing are not just additive; they are frequently synergistic, resulting in system-wide improvements that outperform the performance predicted by the simple weighted average of the individual components, according to research on combining cassava residues, coconut husk, and woody materials like sawdust.

2.5.3 Benefits of Rheology and Process of woody and cassava blend

Blends of Woody and Cassava Biomass When slurried, cassava residues including peels and stems have a pulpy, frequently gelatinous consistency and a high moisture retention rate. Significant operational difficulties in biorefining may result from this, such as inadequate mixing, restricted heat transmission, and blockages in reactors and transfer lines. This exact problem was brought to light by the study on hydrolyzing cassava wastes conducted by Phitsuwan et al. (2016). However, a crucial physical synergy was seen when these leftovers were mixed with fibrous woody biomass. The wood's stiff, lignocellulosic fibers disrupted the cassava's continuous gel-like phase by acting as a structural support inside the slurry. This change in the mixture's rheology made it a more fluid and controllable slurry instead of a non-Newtonian, pasty material. This directly addresses a key problem in industrial processing by:

Preventing Clogging and Bridging: The blended feedstock that is produced flows more like a granular solid or a less viscous slurry, guaranteeing reliable passage through pipelines, pumps, and screws. For large-scale industrial operations, this reduces unclogging downtime and guarantees a steady, uninterrupted feed to the reactor (Baruah et al., 2018).

Improving Mass and Heat Transfer: By increasing the permeability, heat and reactants are able to simultaneously reach a significantly greater surface area of the woody and cassava particles. As a result, the pretreatment becomes more consistent, maximizing the lignin and hemicellulose breakdown for the wood and the starch gelatinization for the cassava. In the enzymatic hydrolysis phase that follows, a consistent pretreatment increases the total yield of fermentable sugars (Sindhu et al., 2016). Better efficiency and less energy and chemical use per unit of sugar produced are the immediate results of this.

Enabling Higher Solid Loadings: Raising sugar levels and lowering downstream energy costs, which are essential for enhancing the economic feasibility of bioethanol production (Modenbach & Nokes, 2013). This makes it possible to process more biomass per unit volume of reactor.

2.5.4 Structural and Porosity Benefits from Coconut Husk and Sawdust Blend

Sawdust and coconut husk combine to form a physically complementary feedstock that directly improves the effectiveness of several hydrolysis and pretreatment procedures. Despite being lignocellulosic materials, sawdust's higher particle texture and coconut husk's high fibrous and porous content work in harmony to produce the ideal bed structure for processing.

1. Improved Chemical Penetration: The lengthy, coarse fibers of coconut husks naturally form big, permanent pores. In combination with the finer sawdust particles, these fibers serve as a structural support that keeps the sawdust from compacting and forming a network of macropores. Chemical solutions can move freely through these macropores and into the biomass

bed. To guarantee a steady pretreatment response, the chemical reagent can permeate the entire biomass volume uniformly. According to Baruah et al. (2018), this makes the process more predictable and effective overall by maximizing the removal of hemicellulose and the disruption of lignin across all particles, not just those on the surface.

2. Enhanced Steam Explosion and Explosive Decompression: A bed with high void space and permeability is produced by combining sawdust and coconut husk. High-pressure steam may quickly permeate the entire bed thanks to the wide channels made by the coarse husk fibers. The husk scaffold ensures that the steam can reach individual sawdust particles by filling in the spaces and preventing them from compacting. The pressurized steam that is held inside and surrounding each particle bursts into vapor during the subsequent explosive decompression. The open structure of the blend enables a more rapid and intense release of pressure across the bed. Superior fiber explosion results from this, which efficiently breaks down the inflexible lignocellulosic matrix and significantly expands the surface area available for enzymatic attack later on (Silva et al., 2020).

3. Decreased Diffusion Limitations for Enzymes: The main advantage of the blended pretreatment is that it makes the substrate more accessible and open. Significant structural disruption results from the steam explosion, which is heightened by the increased porosity. The cellulose microfibrils are revealed, and the lignin is re-distributed. The resultant material has a more porous network and a significantly greater specific surface area. This lowered physical barrier permits enzyme complexes to spread more quickly and freely throughout the biomass, as noted by Zeng et al. (2017). They have less difficulty getting to their binding sites on cellulose. This significantly enhances saccharification kinetics which are essential for the bioconversion process's economic feasibility and result in larger end sugar yields and faster hydrolysis rates.

2.6 Measurable Nature of Non-Linear Synergy and the Basis for Systematic Optimization

Blending different lignocellulosic feedstocks provides empirical proof for a basic biorefining principle: the whole is frequently more than the sum of its parts. This phenomena highlights the shortcomings of linear, single-feedstock models by showing that a mixture can have unexpected qualities that are not inherent to its constituent parts. The sugar output from a 50:50 blend of Feedstock A and Feedstock B is rarely the simple arithmetic mean of their separate performances, as the original text points out. The hallmark of a synergistic (if positive) or antagonistic (if negative) interaction is this break from linearity.

2.6.1 The Various Root Causes of Non-Linear Interactions

These interactions result from the intricate interaction of biological, chemical, and physical elements inside a blended matrix and are not just theoretical; they are real and tangible:

Chemical Synergy: The complimentary chemical makeup is one of the main drivers. For example, a feedstock rich in easily available hemicellulose (such as agricultural residue) can be combined with one that has a high lignin concentration (such as woody biomass). The latter might offer a quick, initial sugar release that "primes" the more resistant substrate for enzymatic breakdown. Additionally, blending can reduce the quantity of pretreatment-generated inhibitory chemicals, like furans and phenolics, that would otherwise prevent microbial fermentation (Klinke et al., 2004).

Physical and Structural Synergy: A feedstock blend's physical characteristics have a direct bearing on process effectiveness. By optimizing the biomass slurry's rheology, a blend can avoid problems like clogging or inadequate mixing that can arise from using a single, homogenous feedstock. More significantly, mixing can produce a more advantageous porosity and surface area structure. The pace and extent of hydrolysis can be increased by mixing particles with

varying sizes, shapes, and rigidity in the structure because this can make the matrix more accessible and open for enzymes (Hansen et al., 2021).

Biological Synergy: From the standpoint of fermentation, a blended hydrolysate may provide microorganisms such as bacteria or yeast with a more balanced nutritional profile. The composition of one feedstock may be lacking in a vital nutrient, which can be made up for by the composition of another, resulting in a more robust and effective conversion of sugar to ethanol (Almeida et al., 2007).

2.6.2 Introduction to Mixture Design and Optimization

Multi-component system optimization poses special difficulties that traditional experimental methods are inadequate to handle. When combining lignocellulosic feedstocks, such as sawdust, coconut husk, and cassava peels, to produce bioethanol, the equal dependence of the constituents demands specific techniques. When the response variables (such as sugar yield and ethanol concentration) are presumed to rely only on the relative proportions of the constituents rather than their absolute amounts, mixture design offers a strong statistical framework for these formulation issues (Smith, 2005).

2.6.3 Principles of Mixture Design

A fundamental perspective change in experimental philosophy as well as a mathematical constraint are represented by the fundamental principle of mixture design, which states that all component proportions must add up to a constant total. This constraint, which is commonly written as $x_1 + x_2 + ... + xq = 1$ (or 100%), produces a dependent system in which adjusting the proportion of one component always changes the proportions of other components (Smith, 2005). This means that in order to optimize ternary feedstock mixes for the production of bioethanol, a

decrease in the amount of sawdust, coconut husk, or both must occur in line with an increase in the amount of cassava peels.

Mathematical Implications and the Simplex Space

The experimental region is changed from a conventional q-dimensional cube to a (q-1)-dimensional simplex by the mixture constraint (Cornell, 2011). This lowers the three-dimensional factor space for a ternary system to a two-dimensional equilateral triangle, with interior points representing full ternary blends, edges representing binary mixtures, and each vertex representing a pure component (100% of one feedstock). Both statistical modeling and experimental design are significantly impacted by this geometric change. According to Myers et al. (2016), the dependence between variables makes traditional polynomial models with constant terms unsuitable since they would be too parameterized and have perfect multicollinearity.

The Scheffé Canonical Polynomials

Scheffé (1958) found customized canonical polynomials that naturally satisfy the mixture constraint as a solution to various mathematical problems. The first degree model for a ternary system looks like this:

$$E(y) = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3$$

where the expected reaction when the mixture is composed entirely of component I is represented by the βi coefficients. The mixture constraint is mathematically consistent when there is no constant term. The second-degree (quadratic) model changes to the following for more intricate systems with curvature and interaction effects:

$$E(y) = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3$$

In this case, non-linear blending effects between components i and j are represented by the βij terms. Synergistic interactions are indicated by a positive βij, whereas antagonistic effects are

shown by a negative value (Anderson & Whitcomb, 2016). In biomass blending studies, where complimentary properties of several feedstocks frequently result in non-linear impacts on saccharification efficiency and ethanol yield, these interaction terms are very important.

PRACTICAL CONSEQUENCES FOR BLENDING FEEDSTOCK

The physical reality of formulation issues in the production of bioethanol is reflected in the mixture restriction. The proportions of the constituent components must inevitably add up to the entire mass or volume, which is 100% of the material when developing blended feedstocks. This idea keeps the blends physically plausible while allowing researchers to methodically investigate the whole composition space (Piepel & Cornell, 1994).

Additionally, the constraint prohibits the independent interpretation of component effects. For example, the precise ratios of sawdust and coconut husk in the mixture determine how cassava peels work. Instead of examining components separately, this context-dependent behavior calls for a thorough investigation of the entire simplex region (Snee, 1971). Thus, the framework required to reflect the intricate, dynamic nature of multi-feedstock systems in the production of bioethanol is provided by the mixture design technique.

Interpretation of the Model under the Constraints

The proportional restriction necessitates extra attention when interpreting mixed models. The effect of altering component I while keeping other components constant is not represented by the coefficient βi, which is the expected response for pure component I. This is impossible due to the mixture restriction (Cornell, 2011). Rather, the model must provide a comprehensive description of the response surface over all potential blends, and the effects must be understood geometrically as motions along the simplex.

The best areas of the simplex where response variables (ethanol concentration or sugar yield) are maximized can be found thanks to this thorough mapping. Mixture design is especially useful for practical optimization in industrial applications like the manufacture of bioethanol from blended lignocellulosic feedstocks since the constraint guarantees that all anticipated blends are physically realizable.

2.6.4 Overview of Simplex Lattice Design (SLD)

A methodical way to arrange experimental sites inside the limited space of a mixing experiment is the Simplex Lattice Design (SLD). This design process, which was first created by Scheffé in 1958, offers a productive framework for researching multi-component systems while adhering to the essential mixture constraint that the proportions of each component must add up to unity. SLD provides a methodical and mathematically sound way to optimize blend compositions for improved bioethanol production in ternary systems that use feedstocks including sawdust, coconut husk, and cassava peels.

2.6.4.1 Basic Objective and Theoretical Foundation

SLD's main goal is to make it possible to estimate a response surface over the whole simplex region with efficiency by utilizing a small number of carefully chosen design points (Cornell, 2011). This relates to finding the ideal feedstock ratios that optimize sugar yield or ethanol production while lowering experimental costs in the context of biomass blending for bioethanol production. Because cellulose, hemicellulose, and lignin content vary amongst biomass types, the design is especially useful for capturing non-linear blending behavior and component interaction effects, which are frequently seen in lignocellulosic feedstock systems (Zhang et al., 2022).

The SLD structure is defined by the notation {q, m}, where q is the number of components and m is the degree of the polynomial that needs to be fitted. {3,1} for linear models, {3,2} for quadratic models, and {3,3} for special cubic or complete cubic models are typical lattice configurations for a ternary system (q=3). The expected complexity of the response surface and the requirement to capture feedstock interaction effects determine the value of m (Myers et al., 2016).

2.6.4.2 Structural Configuration for Ternary Systems

The SLD structure is defined by the notation {q, m}, where q is the number of components and m is the degree of the polynomial that needs to be fitted. {3,1} for linear models, {3,2} for quadratic models, and {3,3} for special cubic or complete cubic models are typical lattice configurations for a ternary system (q=3). The expected complexity of the response surface and the requirement to capture feedstock interaction effects determine the value of m (Myers et al., 2016).

For the commonly used quadratic model {3,2}, the SLD involves points corresponding to:

- Pure components: (1, 0, 0), (0, 1, 0), (0, 0, 1)
- Binary mixtures: (0.5, 0.5, 0), (0.5, 0, 0.5), (0, 0.5, 0.5)

By creating six design points that are evenly spaced throughout the simplex triangle, this arrangement makes it possible to estimate the quadratic model parameters effectively. While the binary mixing points are specifically designed to detect two-component interactions, each pure component point evaluates the performance of separate feedstocks (Anderson & Whitcomb, 2016). The {3,3} SLD adds the following points for more complicated systems that call for higher-order models:

- Ternary blends: (0.67, 0.17, 0.17), (0.17, 0.67, 0.17), (0.17, 0.17, 0.67)

- Overall center point: (0.33, 0.33, 0.33)

The experimental region is more thoroughly covered by these extra points, which improve the design's capacity to identify curvature and three-component interactions (Smith, 2005).

Model Estimation and Mathematical Framework

It is immediately possible to estimate Scheffé's canonical polynomials using the SLD structure. The quadratic model for a {3,2} lattice looks like this:

$$\hat{Y} = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3$$

where the cross-product terms (βij) quantify the impacts of the binary interaction, and the pure component coefficients (βi) represent the expected response for each feedstock alone (Scheffé, 1958). The analysis's statistical efficiency is increased by the deliberate positioning of design points in the SLD, which guarantees that these parameters may be estimated with little covariance (Piepel & Cornell, 1994).

BENEFITS OF SLD IN BIOMASS BLENDING RESEARCH

There are a number of clear benefits of using SLD in biomass blending optimization. First, the design is especially useful for resource-intensive bioconversion research because it offers thorough coverage of the entire composition space with a small number of experimental runs (Ferreira et al., 2023). Second, the response surface may be visualized using contour plots thanks to the geometric arrangement of design points, which helps researchers pinpoint areas of peak performance and determine where improvements are needed (Myers et al., 2016).

Moreover, the investigation of feedstock synergistic effects is naturally supported by the SLD framework. The model's interaction terms can show how the complementary qualities of various lignocellulosic materials, such as sawdust's high cellulose content, cassava peels' balanced

composition, and coconut husk's structural qualities, work together to improve overall digestibility and ethanol yield in ternary biomass systems (Oke et al., 2021).

Researchers can simultaneously optimize blend composition and important process parameters like pretreatment severity, enzyme loading, and fermentation conditions thanks to the design's support for the incorporation of process variables through the development of mixture-process variable designs (Anderson & Whitcomb, 2016). This comprehensive method is especially helpful for creating reliable bioethanol production processes that take operational and compositional aspects into consideration.

IMPLEMENTATION CONSIDERATIONS

A number of practical concerns come up when using SLD for biomass blending research. Lack-of-fit testing is made possible by the replication of design points, especially the overall centroid, which yields an estimate of pure error (Cornell, 2011). The dependability and verification capability of the model are improved by adding axial points and check points outside of the fundamental lattice structure. Furthermore, successive experimentation is made easier by the simplex region's ordered structure, which allows for the addition of points to early screening designs in order to refine the response surface in promising areas (Snee, 1971).

In studies on the production of bioethanol, the SLD approach helps researchers to effectively manage the intricate relationship between feedstock properties and conversion efficiency, which eventually results in blends that optimize both economic and environmental benefits while utilizing a variety of agricultural biomass.

2.6.4 Application of Mixture Design in Bioprocess Optimization

Optimization strategies in bioprocess engineering have been transformed by the use of Simplex Lattice Design and other mixture design methodologies, especially in fields where formulation

composition directly affects process efficiency and product yield. In biological systems, where component interactions are frequently complicated and non-linear, mixture designs are especially useful because to their exceptional capacity to effectively describe multi-component systems while taking into account the limited nature of blends.

SPECIFIC APPLICATIONS IN BIOTECHNOLOGY AND BIOFUEL PRODUCTION

1.Optimizing Microbial Media: Optimizing culture media for microbial growth and metabolite production is one of the most well-known uses of mixture design in biotechnology. To optimize yields of antibiotics, enzymes, organic acids, or microbial biomass, researchers frequently use SLD to identify the best combination of carbon sources (such as glucose, xylose, and glycerol), nitrogen sources (such as yeast extract, peptone, and ammonium salts), and mineral components (Panchal et al., 2017). In order to optimize a mix of four distinct carbon sources for the manufacture of polyhydroxybutyrate by Cupriavidus necator, Liu et al. (2018) used a {4, 2} simplex lattice design. This resulted in a 40% increase in yield when compared to the optimal single carbon source.

2. Creating an Enzyme Cocktail: The effectiveness of enzymatic hydrolysis in lignocellulosic biorefining relies on the cooperative activity of several enzymes (cellulases, hemicellulases, and β-glucosidases). In order to maximize the amount of sugar released from particular feedstocks, mixture design has been effectively used to optimize the ƭȶ of various enzymes in commercial cocktails. This method lowers costs and increases efficiency by taking into consideration the synergistic interactions between various enzyme classes, guaranteeing that the cocktail is neither excessive nor deficient in any one function (Gao et al., 2019).

3. Biorefining from Multiple Feedstock:. SLD is perfectly suited for optimizing the blend ratios of various lignocellulosic feedstocks, as this project has shown. In a noteworthy work, Zhang et

al. (2020) optimized a ternary blend of maize stover, switchgrass, and miscanthus for the generation of bioethanol using a {3, 2} simplex centroid design. In comparison to the best single feedstock, their model found an ideal blend that enhanced ethanol yield by 18% and showed notable synergistic interactions, especially between switchgrass and corn stover.

4. Biogas Production and Anaerobic Digestion:.Because of nutrient balancing and inhibitor dilution, co-digesting various organic wastes (such as food waste, animal manure, and agricultural residues) frequently improves biogas output. In order to enhance methane yield and maintain an ideal carbon-to-nitrogen ratio, mixture design has been utilized to improve the co-substrate ratios (Khalid et al., 2021). For instance, a study that used a simplex lattice design to optimize the co-digestion of cheese whey, cattle dung, and poultry litter discovered a particular ternary blend that enhanced methane output by 30% when compared to the digestion of chicken litter alone.

ADVANTAGES IN BIOPROCESS CONTEXT

The use of mixture design in these bioprocess applications offers distinct advantages:

Methodical Investigation: It allows for a thorough comprehension of the ways in which the ratios of various substrates or nutrients impact the biological system, surpassing one-factor-at-a-time methods that overlook important interactions.

Quantification of Synergy: It offers a statistical framework for verifying and measuring the existence of synergistic effects between components, which are typical in biological systems because of complimentary physical characteristics or metabolic pathways.

Predictive Ability: By using the produced mathematical models, researchers can save time and money during the optimization process by forecasting system performance for any untested blend inside the experimental zone.

Reliable Formulations: The best blends found are frequently more resilient to inherent changes in feedstock composition because the model takes into consideration a variety of ratios rather than a single set formula.

In bioprocess optimization, the use of mixture design, has become a vital technique. Finding the ideal ternary blend of lignocellulosic feedstocks for maximal bioethanol production is the main research issue of this project, and it is well suited to answering it due to its capacity to effectively represent intricate multi-component biological systems.

2.7. Research Gap and Statement of the Problem

The global demand for sustainable energy, coupled with the critical need for efficient waste management in agro-industrial countries like Nigeria, underscores the importance of developing second-generation bioethanol technology. While sawdust, coconut husk, and cassava peels have been individually explored as potential feedstocks, significant research gaps remain regarding their combined utilization and process optimization. This study aims to address these critical areas by investigating the synergistic potential of a ternary blend of these agro-wastes.

Previous research has primarily focused on single feedstocks or, to a lesser extent, binary mixtures. However, comprehensive research into the synergistic effects of a ternary system composed of sawdust (high-cellulose), coconut husk (lignin-rich and refractory), and cassava peels (starch-rich) is notably absent. The distinct compositions of these materials suggest that blending them could overcome individual limitations, such as the low cellulose content of cassava peels or the high recalcitrance of coconut husk, thereby enhancing overall conversion efficiency. Furthermore, there is a lack of rigorous statistical mixture design approaches to

optimize blend ratios for maximizing sugar and ethanol production from this specific combination of tropical agro-wastes.

A holistic process evaluation is essential for a thorough assessment, extending beyond merely measuring ultimate ethanol output. It is crucial to understand how blend ratios influence the effectiveness of each stage, including pretreatment (e.g., delignification and hemicellulose solubilization), enzymatic hydrolysis (saccharification yield), and subsequent fermentation kinetics. By methodically creating and refining a ternary blend of cassava peels, coconut husk, and sawdust, this study will utilize a mixture design approach to quantify synergistic or antagonistic interactions, pinpoint the optimal blend ratio for maximum fermentable sugar and ethanol yields, and provide a comprehensive analysis of the blend composition's impact on the entire conversion pathway.

CHAPTER THREE

MATERIAL AND METHODS

This chapter presents detailed materials and methodology which outlines systematic approach taken to achieve optimal yield for bioethanol production ensuring precision and clarity.

3.1 Material

3.1.1 Raw material

Table 3.1: Raw Materials used and their Sources

RAW MATERIAL	SOURCE	USES
Cassava peels	Obtained from local cassava processing facility in Ekosodin community as waste	A Lignocellulosic biomass material used as feedstock
Coconut husk	Obtained from local market as waste	A Lignocellulosic biomass material used as feedstock
Saw dust	Obtained from local sawmill as waste	A Lignocellulosic biomass material used as feedstock

3.1.2 Reagent

Reagents used in this study includes analytical and biological reagents obtained from local chemical laboratory limited in sealed chemical bottles and sachet of high analytical grade.

Table 3.2: Reagent used and it's Function

REAGENT	SPECIFICATION	FUNCTION
Hydrochloric acid(HCl)	Aqueous solution	Used for acid Pretreatement
Sodium hydroxide(NaOH)	Pellet	Used for neutralization and alkaline pretreatement
Litmus paper	Whatman	Used for checking pH of pretreated solutions.
3,5-dinitrosalicylic acid(DNS)	Reagent	Used for reducing sugar
Cellulase	Powder	An enzyme used for hydrolysis of the biomass
Saccharomyces cerevisiae	Powder	Used for fermentation of biomass

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3.1.3 Equipment

Table 3.3: Equipments used and their Functions

EQUIPMENT	SPECIFICATION	FUNCTION
Sieve	500mm	Used for filtering out fine particles from large particles
Hotplate Magnetic stirrer	MS300 Genotech	Used for controlled heating of solution.
Weighing balance	Atom	Used for a measuring weight of materials.
UV-VIS Spectrophotometer	752N Pec Medical USA	Used for measuring the absorbance of light by a sample and a specific wavelength
Beakers, conical flasks, test tubes and volumetric flask. Measuring cylinder. Stirrer Thermometer	Glasswear	Used for holding and mixing materials and regents at different phase of the experiment. For precised measurement of fluid Used for stirring solutions Used to measure the temperature of hydrolyzed solution.
Heating mantle	ZDHW Jinotech instruments	Used as heat source during distillation.
Distillation Apparatus	Simple distillation setup	Used to separate distillate from mixture (fermented solution).
Refractometer	SW-593	Used to measure brix value of distillation.

3.2 Methods

3.2.1 Feedstock preparation

The efficiency of subsequent processes, such as pretreatment, hydrolysis, and fermentation, is greatly impacted by the selection and preliminary treatment of lignocellulosic biomass. Three common agricultural and wood-processing byproducts were used in this study: sawdust, coconut husk, and cassava peels. In order to optimize the surface area available for enzymatic and chemical reactions later on, the preparation protocol's main objective was to produce a uniform, dry, finely divided powder with a constant particle size (Sarkar et al., 2012).

Feedstock Collection and Initial Handling

Cassava peels was obtained from nearby local cassava processing facility in Ekosodin community. Initially, the fresh peels were manually divided into tiny pieces (about 1-2 cm^2) to ensure even drying.

The coconut husk used was sourced from a Local coconut merchants. To improve grinding efficiency, the tough shell was removed from the coarse, fibrous husks, which were then chopped into smaller pieces. The sawdust was obtained from a nearby sawmill, making sure that it was mostly made from untreated wood to prevent the presence of additives that would prevent microbes from fermenting.

Drying, Size Reduction and Sieving

For roughly five to seven days, all three feedstocks were exposed to the sun in order to drastically lower their moisture content. In order to ensure precise weighing for experimental formulations, avoid microbiological decomposition during storage, and increase the effectiveness of the next grinding process. This, it is essential to reduce moisture to the "bare minimum" (Mani et al., 2004).

A commercial mechanical grinder was then used to mechanically grind the sun-dried feedstocks into a fine powder. In the process of producing lignocellulosic biofuel, this size reduction stage is essential. The biomass structure is upset by grinding, which significantly raises the surface area to volume ratio. Increased surface area improves sugar yields by increasing the accessibility of hydrolytic enzymes and the penetration of pretreatment chemicals into the cellulose and hemicellulose fractions (Hendriks & Zeeman, 2009).

A mesh sieve with a diameter of 500 μm was used to filter the ground powder in order to guarantee consistency and repeatability throughout all testing runs. By maintaining a uniform particle size, it is possible to explain reported variations in sugar and ethanol yields mainly to the blending ratios specified by the Simplex Lattice Design, rather than to changes in physical accessibility (Zheng et al., 2009).

Plate 3.1: Feedstock as Fine Powder

3.2.2 COMPOSITIONAL ANALYSIS

To fully understand their innate resistance and predict how they would behave during pretreatment and enzymatic hydrolysis, a thorough assessment of the lignocellulosic composition of the raw feedstocks;cassava peels, coconut husk, and sawdust was crucial. Hemicellulose, lignin, and cellulose are the main structural components that were isolated and quantified using a

sequential gravimetric approach. This method is founded on well-established biomass fractionation concepts, but modified for laboratory settings (Sluiter et al., 2008).

A. Using Alkaline Pretreatment to Determine the Hemicellulose Content

By selectively solubilizing it using an alkaline pretreatment, a technique that effectively hydrolyzes the branched hemicellulose polymer, the first step sought to measure the amount of hemicellulose present (Sannigrahi et al., 2010).

For every 500 mL of 0.5 M sodium hydroxide (NaOH) solution was added to an exact mass of 10.0 g (noted as starting mass, A) of the dry, sieved powder for each feedstock. This mixture was boiled for an hour. A small amount of the lignin and a large amount of the hemicellulose are hydrolyzed and dissolved by this potent alkaline treatment. To achieve total elimination of soluble degradation products and alkali, resulting slurry was repeatedly rinsed with distilled water through decantation until a pH of neutral was reached. After being cleaned, the residue was dried in an oven until it reached a consistent mass. This dried residue's mass (designated as final mass, B) was measured with precision which was majorly thee mass of the lignin and cellulose. The solubilized hemicellulose accounts for the majority of the mass lost during this treatment. Using a dry weight basis, the hemicellulose content was determined as follows:

Percentage Hemicellulose (%) = [(Starting Mass (A) - Final Mass (B)) / Initial Mass (A)] * 100

Plate 3.2: Dried Residue

B. Acidic Pretreatment for Lignin Content Determination

The residue left behind after the alkaline pretreatment was used to calculate the amount of lignin. This two-step process is essential because the first alkali treatment eliminates a large amount of the hemicellulose, which makes it easier for the acid treatment to separate the acid-insoluble lignin also known as Klason lignin from the residual cellulose.

50 mL of a 5% (v/v) acid solution of HCl was used to hydrolyze a precise mass of 2.0 g (Initial Mass, C) of the dried, hemicellulose-depleted material from process A. This step was heated for half an hour. The purpose of the mildly acidic hydrolysis is to dissolve lignin and other acid-soluble substances, leaving only the cellulose. Repeated washing and decantation were then used to neutralize the combination. The solid residue that resulted was dried to a consistent mass (Residue mass, D), with cellulose making up the majority of it. The amount of lignin was determined to be:

Lignin Content (%) = (Initial Mass, C - Residue Mass, D/ Initial Mass, C) × 100

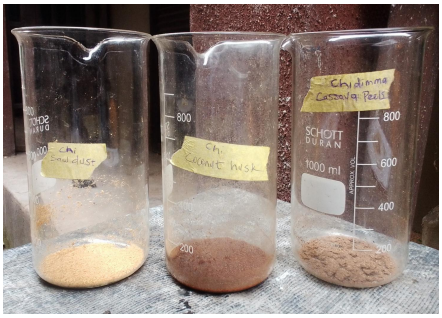


Plate 3.3: Dried Residue after Acidic Pretreatement

C. Estimation of Cellulose Content

Using mass balance, a popular method in proximate compositional analysis, the cellulose content was calculated rather than measured directly (Sannigrahi et al., 2010). The initial mass of the feedstock is the sum of its major components:

Mass of Feedstock = Mass of (Lignin + Hemicellulose + Cellulose + Ash)

Assuming the ash content is resonablely low and of the lignin fraction in this method, the cellulose content can be deduced as:

Percentage Cellulose (%) = 100% - (Percentage Lignin + Percentage Hemicellulose)

This method provided a useful estimate of each feedstock's cellulose content, which is critical for predicting glucose yields during enzymatic hydrolysis.

3.3. EXPERIMENTAL DESIGN: SIMPLEX LATTICE DESIGN

To thoroughly examine how three biomass constituents influence sugar yield, a Mixture study design was utilized, acknowledging that these factors are interdependent parts of a mixture whose combined proportions must equal a constant total (Anderson & Whitcomb, 2016). For this

three-component system, a Simplex Lattice Design was chosen as an effective and conventional method for investigating the complete compositional range of the mixture.

The fundamental parameters of the design included the selection of a Quadratic Model, which offers a standard and robust mathematical framework to capture the curvature in sugar yield and to quantify potential interaction effects among the components (Myers et al., 2016). The experiment comprised 15 runs, which were Randomized to mitigate the influence of confounding variables and uncontrolled noise, in line with essential principles of rigorous experimental practice (Montgomery, 2019).

A crucial constraint applied was setting the total mixture proportion at 10.00 wt%. Accordingly, the individual proportions of Cassava peels (A), Coconut husk (B), and Sawdust (C) were varied within a range of 0 wt% (Minimum) to 10 wt% (Maximum), ensuring their sum consistently equaled 10 wt%. To facilitate the interpretation of the model coefficients, L_Pseudo Coding was employed for scaling. In this coding approach, a coded low value of +0 represents 0 wt% of a component, while a coded high value of +1 signifies the component's maximum value of 10 wt%. This coding method ensures that all factors are placed on a comparable, dimensionless scale, and the specific blend ratios for the 15 experimental runs, encompassing pure components, binary, and ternary blends, were systematically generated by the Simplex Lattice algorithm.

Table 3.4: Build Information

Design	Info
File Version	13.0.1.0
Study Type	Mixture
Design Type	Simplex Lattice
Design Model	Quadratic

Build Time (ms)	44.00
Subtype	Randomized
Runs	15.00
Blocks	No Blocks

Table 3.5: Mixture Components

NAME	MINIMUM	MAXIMUM	CODED LOW	CODED HIGH	MEAN	STD. DEV
A: Cassava peels (wt%)	0	10	+0 " 0	+1 " 10	3.56	3.50
B: Coconut husk (wt%)	0	10	+0 " 0	+1 " 10	3.22	3.59
C: Saw dust (wt%)	0	10	+0 " 0	+1 " 10	3.22	3.59
	Total =	10.00	**L_Pseudo Coding**			

Table 3.6: Experimental Design

RUN	Cassava peels (wt%)	Coco nut husk (wt%)	Saw dust (wt%)	Sugar yield (mg/g)
1	1.66667	1.66667	6.66667	
2	3.33333	3.33333	3.33333	
3	5	0	5	
4	6.66667	1.66667	1.66667	
5	5	5	0	
6	10	0	0	
7	0	5	5	
8	1.66667	6.66667	1.66667	
9	10	0	0	
10	5	5	0	
11	0	10	0	

12	0	10	0	
13	0	0	10	
14	0	0	10	
15	5	0	5	

3.4. DNS Method for Fermentable Sugar Analysis

Using the 3,5-Dinitrosalicylic Acid (DNS) technique, the concentration of fermentable sugars more especially, reducing sugars released during the acid pretreatment stage for every experimental run was quantified. Since glucose, xylose, and cellobiose are the main byproducts of hemicellulose hydrolysis during acid pretreatment, the concentration of free carbonyl groups (C=O) in reducing sugars can be reliably measured using this colorimetric assay (Miller, 1959; G.L. Miller, 1959).

The DNS Assay's basic concept is that the DNS reagent combines with the reducing sugar in an alkaline environment and is reduced as a result. The yellow-colored DNS acid is changed into the reddish-brown chemical 3-amino-5-nitrosalicylic acid through this reduction process. The concentration of reducing sugars in the solution corresponds directly with the intensity of this reddish-brown color. In order to achieve maximum sensitivity, the absorbance of this colored complex can be evaluated using spectrophotometer at 540 nm (Arenas et al., 2018).

Method for Sugar Yield Analysis: Each sample was filtered to eliminate suspended solid particles that can alter the spectrophotometric result after each pretreated run had been neutralized and diluted to a final volume of 500 mL.

For the assay, A test tube was filled with a 4 mL sample of the filtered hydrolysate. Using a syringe, precisely 1 mL of DNS reagent was added to the sample. To achieve the complete color, the mixture was cooked for ten minutes in a boiling water bath. The completion of the reduction

reaction depends on the heating process. To stabilize the color and lower the volume down to a level appropriate for spectrophotometer cuvettes, 4 mL of distilled water was added after the solution had been heated and quickly cooled. A UV-Vis Spectrophotometer was used to evaluate the absorbance of the end product in comparison to a reagent blank.

Plate 3.4: Pretreated Sample after undergoing DNS Assay

Calibration and Quantification: A standard calibration curve was created using a range of known glucose concentrations in order to translate the obtained absorbance values for each of the runs into quantitative sugar concentrations (mg/mL). To determine the relevant reducing sugar content for each mix, the absorbance data from the 15 experimental runs were interpolated using this standard curve (Sarker et al., 2021).

In order to provide a uniform metric for assessing the pretreatment's effectiveness for various ternary mixes, the sugar yield for each run was then computed and expressed in milligrams of reducing sugar per gram of dry feedstock (mg/g). The Design-Expert software used these quantitative sugar yield figures as direct response variables for the Simplex Lattice mixture analysis, model fitting, and optimization that followed.

3.5. Method of optimization

3.5.1 Development of Mathematical Model, Model Validation and Adequacy Checking

The development of a mathematical model for optimizing blend ratios was executed through a structured framework rooted in the principles of Mixture Design of Experiments (DOE). The primary goal was to construct a predictive model that accurately depicts the relationship between the proportions of mixture components and the resulting yield. This model would then facilitate the identification of the optimal blend to maximize the desired outcome.

The methodology unfolded in four sequential phases, starting with the experimental design. A simplex lattice design was employed, leading to a total of 15 experimental runs. This provided a robust dataset for reliable model estimation. Initially, a standard Quadratic model was considered for analysis, as it is commonly used to capture linear effects, curvature, and two-factor interactions in mixture systems. However, a preliminary analysis revealed that the Quadratic

model was insufficient to fully describe the complexity of the response surface. Consequently, a Special Quartic Model was fitted to the data, incorporating higher-order terms such as linear terms for each component, two-factor interaction terms (AB, AC, BC), and specific three-factor interaction terms (A^2BC, AB^2C, ABC^2), to achieve a superior fit (Cornell, 2002). The parameters for this refined model were then estimated using the method of least squares regression.

Following the model's formulation, a rigorous statistical validation was performed to ensure its adequacy and predictive capability. An Analysis of Variance (ANOVA) confirmed the overall significance of the model, indicated by a high F-value and an exceptionally low p-value. A non-significant Lack of Fit test further affirmed that the chosen model form was appropriate, attributing any unexplained variance to random error rather than structural deficiencies (Myers, Montgomery, & Anderson-Cook, 2016). Additionally, the R^2 and Adjusted R^2 values were calculated to quantify the proportion of variance explained by the model, while the Predicted R^2 was examined to confirm its agreement with the Adjusted R^2, thereby validating its predictive accuracy for new observations. The Adequate Precision ratio, found to be well above the threshold of 4, demonstrated a sufficient signal-to-noise ratio for reliable navigation of the design space. The validated mathematical relationship was then expressed as an equation using L_Pseudo Components, a coding system where component proportions are scaled between 0 and +1. This allowed for direct interpretation of coefficient magnitudes, reflecting the relative influence of each term on the response (Myers et al., 2016).

3.5.2 Graphical Representation and Optimum Mixture Ratio Diagram

Following the successful validation of the special quartic model, a series of graphical analyses were utilized to understand how different mixture components influence sugar yield. Initially, a contour plot was generated using the validated model equation. This plot represented the three-

component mixture on a triangular graph, illustrating lines of consistent predicted sugar yield, similar to a topographical map of the response surface.

Next, a 3D response surface plot was constructed from the same model predictions. This visualization presented the response as an interactive surface, offering a clear view of the highest and lowest sugar yields across the experimental range. Lastly, a numerical optimization algorithm was applied to the model to pinpoint the exact component proportions that would maximize the sugar yield. The result was displayed in an optimized mixture ratio diagram, which graphically presented the precise formulation recommended by the model.

3.6 Bioethanol production Process using optimized Ternary blend

A validation run was carried out using the precise ratio of experimental run which showed the highest sugar production, after optimum ternary blend was determined. Three crucial, consecutive unit activities were used in the bioethanol manufacturing process for this optimized blend: acid pretreatment, enzymatic hydrolysis, fermentation.

3.6.1. Acid Pretreatment

Pretreatment's main goal is to break the resistant lignin-carbohydrate complex and decrease cellulose's crystallinity, which will make it more vulnerable to enzymatic attack (Mosier et al., 2005). A diluted acid pretreatment was used for this investigation. 100 mL of a 5% (v/v) concentrated acid solution (such as H_2SO_4) was added to the mixed feedstock (10g total) and heated for 30 minutes. This process partially breaks down the lignin matrix and hydrolyzes a large amount of the hemicellulose, mostly into monomeric sugars like xylose (Sarkar et al., 2012).

After heating, a 10% (w/v) NaOH solution was used to neutralize the slurry to a pH of 5.0, as verified by litmus paper. Since most cellulase enzymes have an ideal pH of 4.8 to 5.0,

neutralization is necessary to establish an environment that is conducive to the enzymatic hydrolysis that follows (Taherzadeh & Karimi, 2007). According to Jönsson and Martín (2016), neutralizing at this point also lessens the possibility of fermentation inhibitors such furans and organic acids forming during acid pretreatment. A suitable substrate concentration for hydrolysis and fermentation was then obtained by diluting the resulting neutralized slurry with distilled water until it reached a final volume of 500 mL.

3.6.2. Enzymatic Hydrolysis (Saccharification)

The goal of enzymatic hydrolysis is to use particular enzymes to break down cellulose and the remaining hemicellulose polymers into their monomeric carbohydrates, namely glucose and xylose. Because it prevents the production of breakdown products that limit fermentation, this step is recommended over acid hydrolysis (Sun & Cheng, 2002). 100 mL of warm water (50°C) was used to dissolve 10g of cellulase enzyme powder in order to activate it for this procedure.

This enzyme, which usually contains β-glucosidases, endoglucanases, and exoglucanases, was then thoroughly combined with the biomass slurry that had been prepared. The hydrolysis was done in a conical flask that was incubated for 24 hours at 50°C on a hotplate magnetic stirrer. For the majority of commercial cellulases, this temperature and duration fall within the ideal range, enabling effective cellulose to glucose depolymerization (Kuhad et al., 2011).

Plate 3.5: Biomass Substrate for Hydrolysis

The concentration of reducing sugars released was used to measure the degree of hydrolysis because Sugar yield is a direct and important indicator of the potential fermentable sugars available for subsequent bioethanol production, as it represents the concentration of reducing sugars (in milligrams per gram of dry biomass) released following the pretreatment and saccharification processes (Mosier et al., 2005). To get rid of any solid residues, a 4 mL sample of the hydrolysate was filtered. The 3,5-dinitrosalicylic acid (DNS) method was applied, which entails reacting the sample with DNS reagent and using a UV-Vis spectrophotometer to measure the absorbance of the colored complex that occurs at a wavelength of 610 nm (Miller, 1959). Using a glucose standard curve, the measured absorbance of 1.048 after 24 hours was converted to a sugar concentration (mg/L).

3.6.3 Fermentation

During fermentation, the hydrolysate's carbohydrates are converted into ethanol by microorganisms. Because of its high ethanol tolerance and effectiveness in fermenting glucose, batch fermentation utilizing Saccharomyces cerevisiae was used (Lin & Tanaka, 2006). 2g of S. cerevisiae was activated in 100 mL of warm water (45°C) to create a pre-culture. The sugar-rich hydrolysate, which was kept at 45°C in a conical flask, was then thoroughly mixed with this active yeast culture. In order to allow for the release of CO_2 while preventing contamination, the flask was sealed with foil. For 72 hours, the fermentation was allowed to continue.

Plate 3.6: Fermenting Substrate

To monitor the process, a sugar declination rate analysis was performed every 24 hours. Using the same DNS method described for hydrolysis, 4 mL samples were withdrawn, and their reducing sugar content was measured by using spectrophotometer to measure their absorbance. Using a glucose standard curve, the measured absorbance after every 24 hours was converted to

a sugar concentration (mg/L). The decline in sugar concentration over time (e.g., from the initial 24-hour hydrolysis reading) directly indicated the consumption of sugars by the yeast for ethanol production, providing kinetic data on the fermentation efficiency (Walker & Walker, 2018).

3.7. Methods of Analysis

3.7.1 Separation of Ethanol through Distillation

An essential step in assessing each ternary feedstock blend's performance was quantifying the amount of bioethanol generated from the fermented hydrolysates. This was accomplished by combining simple distillation with refractometric analysis, a technique that is frequently employed in small-scale biofuel research because to its reliability and accessibility (Balat, 2011).

The bioethanol was separated and concentrated by simple distillation of the entire fermented broth after the 72-hour fermentation period. According to Ozkaya et al. (2018), the setup included a Liebig condenser linked to a 250 mL round-bottom flask holding the fermented solution via a distillation adapter. To guarantee effective vapor condensation, cooling water was pumped through the condenser jacket.

A heating mantle was used to carefully regulate the temperature of the distillation flask to around 78°C, which is the boiling point of ethanol. This allowed the target compound to be vaporized selectively while reducing the amount of water and other components that were co-distilled. The procedure was regularly observed. The primary fraction was collected in a clean, pre-weighed conical flask afte rcondensing within the ethanol boiling range (78–82°C).

Plate 3.7: Distillation Setup

Periodic distillate sampling was a crucial quality control procedure used during the distillation process. Small droplets of the distillate were taken periodically after the distillation process started, and their Brix % was measured using a refractometer.

This procedure was carried out repeatedly until the distillate's Brix value dropped to 0.00%, signifying that it was effectively pure water being distilled over with no dissolved solutes, including ethanol. This endpoint guaranteed the accuracy of the yield estimate by confirming the full recovery of ethanol from the mixture (Bai et al., 2008). For the purpose of a later concentration analysis, the entire volume of the primary ethanol fraction that was collected was noted.

3.7.3. Quantification of Ethanol Concentration using Refractometry and experimental Yield

A refractometer, which uses the relationship between a solution's composition and refractive index, was used to measure the amount of ethanol present in the distillate. Because ethanol has a different refractive index than water, Brix (%) can be empirically calibrated for ethanol-water mixes even though it is often used to quantify the amount of sucrose dissolved in water. When ethanol is present, the refractive index decreases, producing a value that is less than that of pure water or a negative Brix reading (Gonzalez et al., 2019).

A calibration curve was created in order to convert Brix values into the actual ethanol content (% v/v). The ethanol concentrations in a number of common ethanol-water solutions (such as 0%, 10%, 20%,..., 90%, and 100% v/v) were known. The same refractometer was used to test the Brix value of each reference solution. The known ethanol percentage (%) was plotted against the measured brix value (%), which usually results in a strong linear relationship.

After that, the collected bioethanol distillate's Brix value was determined. By entering this measured Brix value into the straight-line equation obtained from the calibration curve, the associated ethanol percentage was determined (e.g., Ethanol % = (m x Brix) + c). As shown in related biofuel research, this approach offers a quick, affordable, and accurate way to quantify ethanol for testing and optimization investigations (Johnston et al., 2021).

Lastly, the recorded volume and density of the distillate, along with the calculated ethanol percentage, were used to determine the mass of bioethanol produced. The experimental ethanol yield was then converted to grams of ethanol per gram of dry feedstock so that the various ternary blends could be directly compared.

3.7.2. Calculation of Theoretical Ethanol Yield

Bioethanol production efficiency is assessed by comparing the actual experimental yield to the theoretical maximum yield, which represents the highest possible ethanol output from available fermentable sugars assuming complete conversion without any losses (Taherzadeh & Karimi, 2007). This theoretical yield calculation is based on the biochemical conversion of glucose to ethanol and carbon dioxide:

$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$

The calculation of theoretical yield involves a series of steps. First, the total mass of fermentable sugar is determined from the sugar concentration (S mg/mL) obtained after enzymatic hydrolysis of the optimized ternary blend, using the formula: Total Sugar Mass (g) = (S mg/mL × 500 mL) / 1000. Following this, the theoretical mass of ethanol is calculated using stoichiometric molar masses (glucose = 180 g/mol, ethanol = 46 g/mol), where 180 g of glucose yields 92 g of ethanol. This leads to: Theoretical Ethanol Mass (g) = [Total Sugar Mass (g) × 92] / 180, which can be simplified to:

Theoretical Ethanol Mass (g) = Total Sugar Mass (g) × 0.511.

Finally, to standardize the yield, the theoretical ethanol mass is expressed per gram of the initial 10g dry feedstock: Theoretical Yield (g ethanol/g feedstock) = Theoretical Ethanol Mass (g) / 10 g. The process efficiency is then calculated by comparing the experimental yield obtained from distillation and refractometry (as described in sections 3.5.2 and 3.5.3) to the theoretical yield:

Efficiency (%) = (Experimental Yield / Theoretical Yield) × 100.

This comparison is vital for evaluating the various stages of bioethanol production and pinpointing areas for improvement (Lin & Tanaka, 2006).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. COMPOSITIONAL ANALYSIS OF BIOMASS

To comprehend the intrinsic properties of the three feedstocks (cassava peels, coconut husk, and sawdust) and predict how they would behave during pretreatment and hydrolysis, their lignocellulosic compositions were examined. A summary of the findings is provided in Table 4.1.

Table 4.1: Lignocellulosic Composition (%) of each Feedstock

Feedstock	**Hemicellulose (%)**	**Lignin (%)**	**Cellulose (%)**
Cassava Peels (CP)	74.30	4.50	21.20
Coconut Husk (CH)	54.41	11.50	34.09
Sawdust (SD)	42.00	22.00	36.00

4.1.2. Hemicellulose Content

Cassava Peels (CP - 74.30%): Cassava peels exhibit an exceptionally high hemicellulose content, positioning them as a superior source of this polysaccharide. As cassava (Manihot esculenta) is a tuber, its peels consist mainly of parenchymatous tissue, characterized by thinner cell walls and a distinct structural role compared to more fibrous or woody biomass. This elevated hemicellulose level implies a comparatively lower proportion of lignin and cellulose within its structural

framework. This attribute makes cassava peels a highly attractive feedstock for processes targeting hemicellulose-derived sugars, such as the production of xylitol or bioethanol via fermentation (Santi et al., 2014). The substantial hemicellulose content suggests a high potential for sugar yield upon hydrolysis.

Coconut Husk (CH - 54.41%): Coconut husk, which is the resilient fibrous material from the mesocarp of the coconut (Cocos nucifera), also possesses a high hemicellulose content at 54.41%, though it is considerably less than that found in cassava peels. This composition is consistent with the known characteristics of coconut husk, which is recognized for its significant hemicellulose and lignin content, contributing to its durability and resilience (Briones et al., 2012). Consequently, coconut husk serves as a valuable feedstock in the biorefinery concept, where hemicellulose can be extracted for high-value products, and the remaining lignin and cellulose fractions can be utilized for alternative applications, such as the generation of biochar or activated carbon.

Sawdust (SD - 42.00%): Sawdust exhibits the lowest hemicellulose content among the three feedstocks. While sawdust is a general term with varying compositions depending on the tree species, a value of approximately 40-42% is typical for many types of woody biomass. Wood generally features a more balanced and compact lignocellulosic structure, along with a higher lignin content that provides structural support and increases its resistance to biological degradation (Sjöström, 1993). Although 42% still represents a significant amount, the elevated lignin content associated with woody biomass often necessitates more intensive pre-treatment steps to disrupt the lignin matrix, which are crucial for efficiently accessing and hydrolyzing the hemicellulose in sawdust compared to the other two feedstocks.

Comparatively, the highest hemicellulose content is found in cassava peels (74.30%), which are followed by coconut husk (54.41%). The lowest percentage is 42.00% for sawdust. Consequently, the most promising feedstock for procedures needing a high hemicellulose content is cassava peels.

4.1.1. Lignin Content

Sawdust (SD) exhibits the highest lignin content at 22%, which is a characteristic of woody biomass. This substantial lignin concentration is crucial for wood's structural integrity, offering compressive strength and resistance against microbial degradation (Boerjan et al., 2003). Consequently, this elevated lignin level renders sawdust the most recalcitrant material among the three, necessitating robust pre-treatment methods like organosolv, alkaline, or steam explosion to effectively solubilize or disrupt the lignin seal for efficient saccharification. While this poses challenges for biofuel production, the high lignin content also positions SD as a valuable resource for producing lignin-derived aromatic chemicals, bio-based plastics, or as a solid fuel.

Coconut Husk (CH) possesses a moderate lignin content of 11.50%, which aligns with its biological role as a dense, fibrous mesocarp protecting the coconut seed. This lignin level signifies a notable yet manageable degree of recalcitrance. CH serves as a balanced feedstock, containing significant hemicellulose and a lignin content low enough to be more responsive to pre-treatment compared to woody biomass. This characteristic makes it an excellent candidate for integrated biorefining, where hemicellulose can be extracted for sugar platforms and lignin can be separately valorized (Ragauskas et al., 2014). Furthermore, its fibrous nature and moderate lignin content contribute to its utility in producing bio-composites and activated carbon.

Cassava Peels (CP) are distinguished by their remarkably low lignin content of 4.50%. As a non-woody agricultural residue from a root crop, CP has minimal structural requirements, with its

tissue primarily parenchymatous and designed for storage rather than support. This low lignin content presents a significant advantage for biological conversion processes, suggesting that CP requires less severe and potentially more cost-effective pre-treatment conditions to achieve high sugar yields from its cellulose and hemicellulose fractions (Santi et al., 2014). Therefore, CP is an excellent feedstock for rapid anaerobic digestion for biogas production or for the fermentative production of bioethanol, as microbial consortia can more easily access its polysaccharides.

In comparison, sawdust have the highest lignin content (22.0%), which makes them more resistant but may make them useful for bioproducts. Cassava peels has the lowest amount (4.50%), indicating that it is the most readily degradable for processes like fermentation, whereas coconut husk has the moderate level (11.50%).

4.1.3. Cellulose Content

The cellulose content varies significantly among different feedstocks, reflecting their botanical origins and functions. Sawdust (SD) contains 36.00% cellulose, which is typical for woody biomass and contributes to the structural integrity required for tree growth (Sjöström, 1993). This high cellulose, combined with 22% lignin, makes SD valuable for the pulp and paper industry, although its recalcitrance necessitates intensive pre-treatment for biofuel applications.

Coconut husk (CH) has a very similar cellulose content of 34.09%, which is consistent with its protective role and the durable, high-tensile coir fibers it contains. Its balanced composition, including substantial hemicellulose and moderate lignin, positions CH as a versatile feedstock for integrated biorefineries seeking to utilize all lignocellulosic fractions (Briones et al., 2012). This allows for the production of both C5 and C6 sugars, along with potential lignin valorization.

In contrast, cassava peels (CP) exhibit the lowest cellulose content at 21.20%, which is expected for a storage organ peel that doesn't demand high structural rigidity. While its glucose potential per unit mass is lower than that of SD or CH, CP's extremely low lignin content (4.50%) significantly reduces its recalcitrance, allowing for milder and more economical pre-treatment processes (Satari et al., 2019). This ease and efficiency of conversion can make CP a highly cost-effective feedstock despite its lower absolute glucose yield.

In conclusion, sawdust possesses the highest cellulose content, making it ideal for glucose-based biorefineries, followed by coconut husk. Cassava peels have the lowest cellulose, consistent with its low lignin and high hemicellulose composition.

4.2. Sugar Yield from experimental Design

Table 4.2: Experimental Runs and their Corresponding Sugar Yield

STD	RUN	COMPONENT 1 (CASSAVA PEELS)	COMPONENT 2 (COCONUT HUSK)	COMPONENT 3 (SAW DUST)	SUGAR YIELD (mg/g)
9	1	1.66667	1.66667	6.66667	122.891
10	2	3.33333	3.33333	3.33333	196.994
5	3	5	0	5	130.475
7	4	6.66667	1.66667	1.66667	370.31
14	5	5	5	0	246.739
11	6	10	0	0	237.175
6	7	0	5	5	78.5361
8	8	1.66667	6.66667	1.66667	50.8141

1	9	10	0	0	215.067
4	10	5	5	0	274.232
2	11	0	0	10	78.6261
12	12	0	10	0	64.0008
3	13	0	0	10	34.1808
13	14	0	0	10	20.1887
15	15	5	0	5	149.089

Significant differences in sugar yield are shown by the experimental data, which range from a low of 20.19 mg/g for pure sawdust (Run 14) to a high of 370.31 mg/g for a ternary blend of 16.7% coconut husk, 66.7% cassava peels, and 16.7% sawdust (Run 4). Apart from being notable, this 18 percent difference highlights the innovative potential of efficient feedstock blending as opposed to the use of single, pure feedstocks. Analyzing the outcomes involves looking at the performance of pure components as well as the opposition or synergistic effects in binary and ternary mixes.

4.2.1. Performance of Pure Feedstocks

The experimental runs conducted using a simplex lattice design included several runs where a single feedstock comprised 100% of the 10% solid loading (Runs 6, 9, 11, 12, 13, and 14). The sugar yields obtained from these individual feedstock experiments varied significantly, a phenomenon directly attributable to their unique biochemical compositions (Aruwajoye et al., 2020).

Cassava peels demonstrated the highest potential for sugar yield among the pure feedstocks. Specifically, at 100% concentration, cassava peels achieved yields of 237.18 mg/g and 215.07

mg/g (Runs 6 and 9). This superior performance is attributed to its high hemicellulose content (74.30%), which is readily hydrolyzed into sugars, predominantly xylose and other pentoses, as highlighted by Ogunrinola et al. (2019). Additionally, its low lignin content (4.50%) minimizes the inhibitory effect on enzyme access to polysaccharides, facilitating efficient sugar release.

In contrast, coconut husk exhibited an intermediate sugar yield when used as a pure feedstock, with results of 78.63 mg/g and 64.00 mg/g (Runs 11 and 12). Despite possessing a substantial amount of hemicellulose (54.41%), its higher lignin content (11.50%) presents a more rigid structure that hinders the breakdown of holocellulose, leading to lower sugar yields compared to cassava peels, as noted by Raghavi et al. (2016). Sawdust showed the lowest sugar yield among the pure feedstocks, producing only 34.18 mg/g and 20.19 mg/g (Runs 13 and 14). This poor outcome is due to its lowest hemicellulose content (42.00%) and highest lignin content (22.00%), where the robust lignin matrix severely limits enzymatic hydrolysis and causes unproductive binding of enzymes, reducing conversion efficiency (Mankar et al., 2022).

The sugar production performance of the pure feedstocks clearly follows the trend: Cassava Peels > Coconut Husk > Sawdust. This order is inversely correlated with lignin content and directly corresponds with hemicellulose content, indicating that lignin content is a critical factor in biomass recalcitrance for sugar-based biorefining processes. Consequently, while cassava peels are a promising standalone feedstock, experimental data suggest that blending it with other components, as observed in high-yield mixture runs, can further enhance overall sugar production.

4.2.2. Analysis of Binary Mixtures

The experimental results demonstrate that the synergistic effects between components vary and are significantly shaped by their individual biochemical compositions. Each binary combination is analyzed to understand these unique interactions.

Cassava Peels (CP), characterized by its substantial hemicellulose content (74.30%) and minimal lignin content (4.50%), demonstrates considerable potential in binary mixtures. When combined with Coconut Husk (CH), which has a lower hemicellulose content (54.41%) and moderate lignin (11.50%), the mixture exhibits a synergistic interaction, leading to high sugar yields, as seen in runs like CP=5, CH=5, SD=0 with yields of 246.74 mg/g and 274.32 mg/g. This synergy is attributed to CP's high hemicellulose being readily available for hydrolysis and the overall low lignin content mitigating inhibitory effects (Hendriks & Zeeman, 2009).

Conversely, the binary mixture of CP and Sawdust (SD) shows variable but generally favorable yields, which are highly dependent on the ratio of the components. For example, Run 4 (CP=6.67, SD=1.67, CH=0) produced an exceptionally high yield of 370.31 mg/g, whereas Run 3 (CP=5, SD=5, CH=0) resulted in a much lower yield of 130.48 mg/g. This variability stems from SD's high lignin content (22.00%), a known barrier to enzymatic hydrolysis (Zhao et al., 2012). In high-yield scenarios, a dominant CP proportion effectively dilutes the inhibitory impact of SD's lignin, allowing CP's high hemicellulose to contribute significantly to sugar release. However, when SD's proportion is higher, its recalcitrance can overwhelm the benefits of CP, highlighting the ratio-dependent nature of their interaction.

The binary mixture of Coconut Husk (CH) and Sawdust (SD) consistently produced the lowest sugar yields among all tested combinations. Runs such as CH=5, SD=5, CP=0 (Yield=78.54 mg/g) and CH=6.67, SD=1.67, CP=0 (Yield=50.81 mg/g) yielded significantly less sugar

compared to mixtures containing CP. This poor performance is directly linked to the high cumulative lignin content of both CH (11.50%) and SD (22.00%), creating a highly resistant structure for hydrolytic enzymes. Furthermore, this pair has the lowest combined hemicellulose content, as SD's hemicellulose is the lowest (42.00%) and CH's is insufficient to compensate, leading to an antagonistic interaction where high lignin severely impedes carbohydrate conversion into sugars.

The comparative analysis demonstrates a clear ranking of binary mixture effectiveness for sugar production. The CP-CH mixture emerged as the most efficient, combining the high hemicellulose content from CP with the moderate cellulose from CH and manageable lignin levels, thus achieving an optimal balance for sugar yield. The examination of binary mixtures reveals that the sugar yield is significantly influenced by the lignin and hemicellulose content of the combined feedstocks.

4.2.3. Analysis of Ternary Mixture

By analyzing the outcomes of the individual experimental run, the interactions among the three feedstocks and their influence on hydrolysis efficiency and sugar recovery become evident. The distinct biochemical makeup of each feedstock, particularly their respective hemicellulose, cellulose, and lignin concentrations, offers crucial insights for interpreting the resulting yields (Hendriks & Zeeman, 2009).

The highest sugar yields were consistently observed in mixtures where Cassava Peels (CP) were the predominant component, indicating its critical role in the hydrolysis process. Specifically, single-component runs of CP (Runs 6 & 9) yielded 237.18 mg/g and 215.07 mg/g, primarily due to CP's exceptionally high hemicellulose content (74.30%) and low lignin content (4.50%). Hemicellulose is more readily hydrolyzed into sugars, and low lignin content reduces biomass

recalcitrance, enhancing enzyme accessibility (Mosier et al., 2005). The most remarkable yield of 370.31 mg/g was achieved in a ternary mixture (Run 4) with a dominant CP presence (6.67%), complemented by smaller, balanced additions of Coconut Husk (CH) and Sawdust (SD), suggesting a significant synergistic effect where the minor components potentially modify the biomass structure to further enhance hydrolysis.

Conversely, the lowest sugar yields were consistently found in mixtures that lacked Cassava Peels and contained a high proportion of Sawdust, highlighting the inhibitory effects of high lignin content. Single-component Coconut Husk runs (Runs 11 & 12) yielded 78.63 mg/g and 64.00 mg/g, despite its decent hemicellulose content, due to its higher lignin content (11.50%) compared to CP. Sawdust, with the lowest hemicellulose (42.00%) and highest lignin content (22.00%), resulted in the lowest yields (34.18 mg/g and 20.19 mg/g in Runs 13 & 14), as lignin acts as a barrier and binds hydrolytic enzymes, severely limiting sugar conversion (Zhao et al., 2012). Mixtures combining CH and SD without CP (Run 7) also showed low yields (78.54 mg/g), further underscoring the necessity of CP for high sugar recovery and the negative impact of high-lignin feedstocks.

In conclusion, the most significant discovery was the synergistic impact, which was not observed in a single pure component but rather within a specific ternary mixture. Run 4's blend, consisting of 6.67% Cassava Peels, 1.67% Coconut Husk, and 1.67% Sawdust, achieved a peak yield of 370.31 mg/g. This indicates that a small, well-proportioned inclusion of secondary components can improve the primary feedstock's digestibility beyond what it would achieve on its own.

Consequently, the ternary mixture from Run 4 has been identified as the optimal blend. This formulation will be adopted for all subsequent process development and scaling investigations.

This optimized composition offers a promising approach for the efficient conversion of diverse agricultural waste materials into fermentable sugars.

4.3. Development and validation of Mathematical Model

4.3.1 Model Fitting and Statistical Analysis

Special Quartic model served as the model for Analysis of Variance (ANOVA), which is the foundation for assessing its statistical significance and sufficiency.

Table 4.3: Sugar Yield ANOVA for Special Quartic Model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.450E+05	8	18120.27	88.93	< 0.0001	significant
(1)Linear Mixture	92842.30	2	46421.15	227.82	< 0.0001	
AB	16855.26	1	16855.26	82.72	< 0.0001	
AC	249.03	1	249.03	1.22	0.3113	
BC	769.09	1	769.09	3.77	0.1001	
A^2BC	20888.97	1	20888.97	102.52	< 0.0001	
AB^2C	16713.32	1	16713.32	82.02	0.0001	
ABC^2	99.15	1	99.15	0.4866	0.5116	
Residual	1222.58	6	203.76			
Lack of Fit	219.70	1	219.70	1.10	0.3432	not significant
Pure Error	1002.88	5	200.58			
Cor Total	1.462E+05	14				

4.3.1. Overall Model Significance and Validity

The model's significance is powerfully substantiated by a Model F-value of 88.93 coupled with a p-value of < 0.0001. In line with recognized statistical guidelines, a p-value less than the conventional alpha level of 0.05 (5%) signifies that the model holds statistical significance (Anderson & Whitcomb, 2016). The remarkably low p-value in this investigation suggests a minute likelihood (0.01%) that such an F-value could arise merely from random occurrence. This outcome leads to the rejection of the null hypothesis, thereby affirming that the Special Quartic Model genuinely captures a relevant and substantial connection between the proportions of cassava peels, coconut husk, and sawdust and the resultant sugar yield.

This rigorous statistical validation is fundamental to response surface methodology, where a statistically significant model is considered capable of accurately representing the data within the experimental design space and can be confidently employed for subsequent examination, optimization, and forecasting (Montgomery, 2017). Consequently, the proven significance of this model legitimizes its subsequent application for understanding interaction effects and exploring the design space to pinpoint the ideal feedstock combination for maximizing sugar yield in the bioethanol manufacturing process.

4.3.2. Significance of Individual Model Terms

First, the Linear Mixture term was found to be highly significant ($p < 0.0001$). This was anticipated, as the fundamental proportions of any mixture component directly influence the system's response (Cornell, 2002). In this context, it confirms that the baseline amount of each lignocellulosic feedstock has a profound and statistically undeniable impact on the availability of fermentable sugars after pretreatment and saccharification. The positive coefficient for

component A (Cassava Peels) in the final equation suggests it contributes more favorably to sugar yield compared to component B (Coconut Husk) at their baseline levels.

The most insightful part of the analysis lies in the significance of the interaction terms. The binary interaction AB (Cassava Peels × Coconut Husk) was highly significant ($p < 0.0001$). This indicates a strong non-linear blending effect between these two components, meaning the combined effect of A and B on sugar yield is not merely the sum of their individual effects. This synergism (or antagonism) can be attributed to the complementary physical structures and chemical compositions of the feedstocks. For instance, the relatively higher hemicellulose and starch content in cassava peels might interact with the fibrous, lignin-rich structure of coconut husk in a way that enhances enzyme accessibility during hydrolysis, leading to a non-linear increase in sugar release (Ogunbode et al., 2021).

Furthermore, the higher-order terms A^2BC and AB^2C were also highly significant ($p < 0.0001$ and $p = 0.0001$, respectively). The significance of these special quartic terms underscores the complex, non-linear nature of the interaction between Cassava Peels (A) and Coconut Husk (B), which is also modulated by the presence of Sawdust (C). This implies that the synergistic effect of A and B is not constant but changes disproportionately at different points in the mixture design space. As noted by Scheffe (1958), such higher-order terms are necessary to model the curvature in response surfaces when component interactions are complex. In practical terms, this means that specific, optimal ratios of A and B exist that can maximize the sugar yield, which would not be discoverable with a simpler linear model.

In contrast, the AC (Cassava Peels × Sawdust) and BC (Coconut Husk × Sawdust) interactions were not statistically significant ($p = 0.3113$ and $p = 0.1001$, respectively). This suggests that the binary blending of either Cassava Peels or Coconut Husk with Sawdust does not produce a

statistically unique effect on sugar yield beyond their individual linear contributions. The interaction term ABC^2 was also non-significant ($p = 0.5116$), indicating that a three-component interaction involving the square of the Sawdust component is not a major influencing factor in this model. The relative inertness of Sawdust in significant interactions could be due to its high lignin content and crystalline cellulose structure, which might make it less reactive in synergistic partnerships under the given pretreatment conditions compared to the other components (Menon et al., 2017).

4.3.3. Model Lack of Fit

The ANOVA results (Table 4.3) show that the Lack of Fit test is not significant, with an F-value of 1.10 and a p-value of 0.3432. This outcome is favorable because a non-significant Lack of Fit suggests that the unexplained variation in the experimental data by the model is not significantly greater than the inherent, random variation in the experimental measurements (Anderson & Whitcomb, 2016). This indicates that the Special Quartic model effectively captures the underlying trends, and there's no strong statistical evidence to warrant a more complex model.

A p-value of 0.3432 means there's a 34.32% chance the observed Lack of Fit F-value could be due to random noise alone. Since this probability exceeds the typical 0.05 significance threshold, we do not reject the null hypothesis, confirming the model's adequacy for optimization and prediction within the design space (Bezerra et al., 2008). The low Pure Error (Sum of Squares = 1002.88) further suggests experimental precision, and the non-significant lack of fit confirms that any minor deviation in the model's fit is due to acceptable experimental variability rather than a fundamental model flaw.

4.3.4. Model Fit Statistics and Predictive Power

The Coefficient of Determination (R^2) was calculated to be 0.9916, indicating that the model explains 99.16% of the total variability in sugar yield data. This means the proportions of cassava peels, coconut husk, and sawdust, along with their interactions, account for almost all observed changes in sugar yield, with less than 1% attributed to random error. An R^2 value close to 1.0 is considered ideal in empirical modeling (Myers, Montgomery, & Anderson-Cook, 2016). The Adjusted R^2 (Adj. R^2) value of 0.9805 confirms that the significant terms (AB, A^2BC, AB^2C) are meaningful, and the excellent fit is not due to overfitting. The minimal difference between R^2 and Adj. R^2 suggests a well-balanced model structure (Dean, Voss, & Draguljić, 2017). The Predicted R^2 (Pred. R^2) of 0.8696 indicates robust predictive power for new experiments, and its reasonable agreement with Adj. R^2 (difference of 0.1109, less than the 0.2 threshold) suggests the model is not over-fitted and will provide accurate predictions across the mixture design space (Anderson & Whitcomb, 2016). Finally, an Adequate Precision ratio of 30.414, which is significantly greater than the desirable threshold of 4, demonstrates a very strong signal-to-noise ratio, ensuring high confidence in using the model to identify the optimal mixture ratio for maximizing sugar yield (Anderson & Whitcomb, 2016).

4.3.5. THE FINAL MODEL EQUATION

The final equation in terms of L_Pseudo Components is:

Sugar yield (mg/g) = + 226.65A + 71.84B + 449.36AB + 54.62AC + 123.46BC + 14026.76A^2BC - 12738.76AB^2C + 981.18ABC^2

The response surface is represented mathematically by this equation, regardless of its complexity. The L_Pseudo coding and component scaling result in the big coefficients for the ternary terms (A^2BC and AB^2C), which are necessary for precisely modeling the high-yield region. In

practical terms, the software uses this equation to produce contour plots and carry out numerical optimization, offering a potent tool for outcome prediction based on any given blend ratio in the design space.

4.4. Interpretation of Graphical Representation and Optimum Mixture Ratio

4. 4.1 Actual vs. Predicted Plot

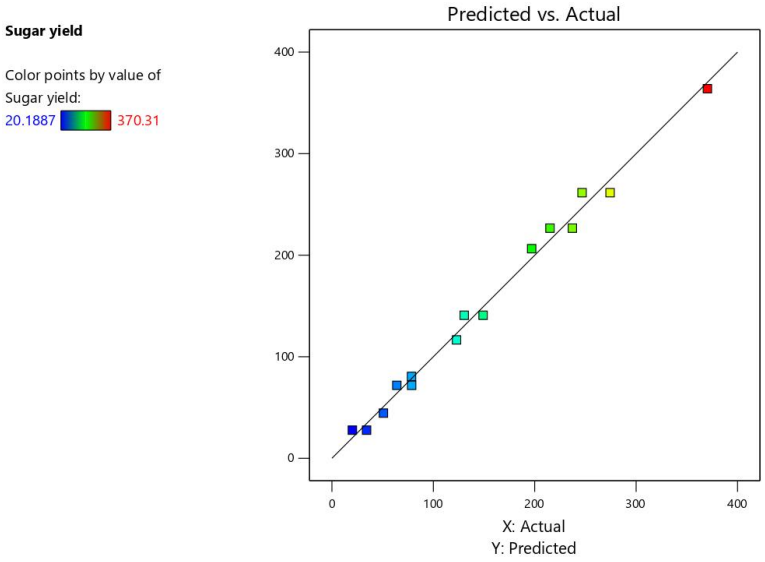


Figure 4.1: Actual vs. Predicted Plot

A thorough visual examination of the model's effectiveness was performed utilizing the Actual vs. Predicted plot (refer to Figure 4.1). This plot clearly showed a strong linear arrangement of all data points precisely along the 45-degree line, which signifies perfect agreement and visually reinforces the model's outstanding predictive accuracy. This visual evidence directly supports the excellent model fit statistics obtained, particularly an (R^2) value of 0.9916 (Myers et al., 2016).

The residuals were observed to be randomly scattered around the line without any identifiable systematic patterns. This observation visually confirms the non-significant lack of fit (p = 0.3432) reported in the ANOVA results, suggesting that the model's error is consistent with the inherent pure experimental error (Anderson & Whitcomb, 2016). The absence of patterns in the residuals further strengthens the model's validity.

As a result of these findings, the model is considered both robust and dependable. This robustness and reliability make it suitable for exploring the design space effectively during the subsequent optimization stage.

4.4.2 INTERPRETATION OF CONTOUR PLOT

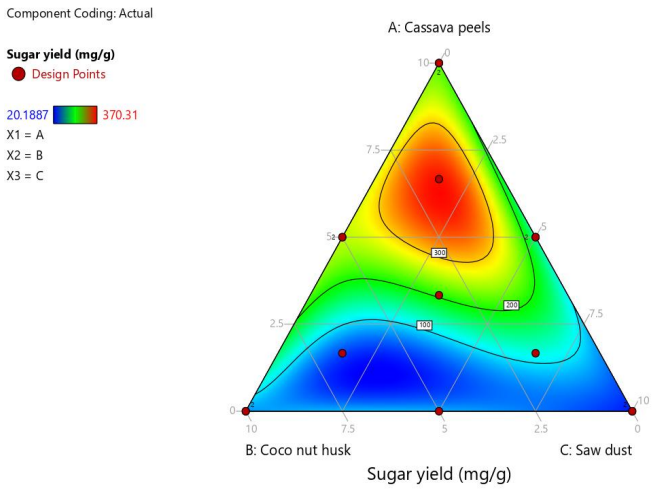


Figure 4.2: Contour Plot

The ANOVA results, highlighting the strong significance of the interaction terms (AB, A^2BC, AB^2C), suggest that the contour plot will showcase notable curvature and a distinct optimal area,

rather than a straightforward linear progression. This curvature is a direct visual cue of the intricate interplay between the components.

The "hill" or the zone enclosed by the highest contour lines, likely corresponding to a yield around 370 mg/g (as observed in Run 4), signifies the optimal blend. Considering the peak yield of 370.31 mg/g achieved with a blend of A=6.67, B=1.67, and C=1.67, the optimal region is expected to be positioned closer to the Cassava Peels (A) vertex. However, it will still demonstrate substantial influence from the other two components, indicating their crucial role in achieving this maximum yield. The elongated and curved shape of this optimal region serves as a clear visual representation of the significant binary interaction between Cassava Peels and Coconut Husk (AB).

The presence of curved contour lines, particularly those with a "banana-shaped" or highly elliptical form, points to synergistic blending effects among the components (Myers et al., 2016). For example, if the contours extend towards the A-B edge, it implies that combinations of A and B result in a significantly higher yield than what would be predicted by a simple linear combination of the two. Conversely, areas characterized by low-value contours, such as those near the B-C edge where yields were below 80 mg/g, suggest antagonistic blends where the combination actively hinders sugar yield.

The core utility lies in identifying the "sweet spot," which is represented by the center of the innermost contour line or the warmest colored region in a color-graded plot. This visual representation allows for a trade-off analysis, enabling the selection of a slightly lower-yielding but more practical alternative if the absolute optimal point is constrained by cost or other factors.

4.4.3. Interpreting the Response Surface

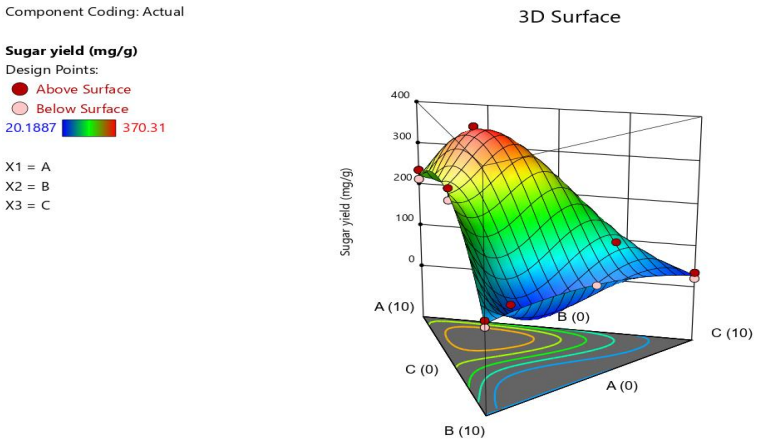


Figure 4.3: Response Surface

A Pronounced Ridge and Peak: The relationships seen in the contour plot are further highlighted by the topographical view of the data provided by the 3D response surface plot The surface is distinguished by a steep ridge that rises from the A-B edge towards its interior and ends in a pronounced peak. The synergistic AB binary interaction term in the model is shown by this image.Due to supportive structural changes that occur during pretreatment and improve overall digestibility, the existence of this ridge indicates that combining cassava peels and coconut husk produces a more suitable substrate for sugar release than either ingredient alone (Ogunbode et al., 2021).

A "Valley" of Low Yield: The area of the surface next to the Sawdust (C) and Coconut Husk (B) vertices creates a low-lying "valley" of poor performance (blue/green areas), which contrasts sharply with the peak. This valley is a representation of a negative impact that are common in blends that are rich in these two components. According to the model, the combination of these two recalcitrant materials results in a feedstock that is incredibly resistant to enzymatic

degradation, possibly because of a denser lignin network or a higher concentration of inhibitory compounds.

4.4.4. Linking Model Terms and Composition to Plot Features

The important model terms and the basic feedstock composition directly affect the shape of these displays.

The Driving Force of Component A (Cassava Peels): Component A (cassava peels) is driven by the fact that the entire response surface slopes upward in the direction of the A vertex. The linear mixing term's large positive coefficient for A (+226.65) is the driving force behind this. Cassava peels consistently provide greater yields while having a high lignin content, which may indicate that either the lignin is less inhibitory or the hemicellulose is very accessible after pretreatment and contributes disproportionately to the sugar production (Sanni et al., 2019).

The Function of Complex Interactions (A^2BC, AB^2C) and Synergy (AB term): Because of the important ternary and AB terms, the peak is a blend rather than at the pure A vertex. The optimum is drawn into the A-B binary blend and away from pure A by the positive AB synergy (+449.36). It is the highly significant A^2BC and AB^2C terms that give the contour plot its prominent, curved peak. The super-additive impact observed in Run 4 is the result of the non-linear effect where a small amount of a third component (C in A^2BC, or the precise ratio in AB^2C) improves the interaction between A and B.

Component C's Inhibitory Function (Sawdust): The quick drop in yield with any major integration of Sawdust, as seen in the steep gradients, is a visual illustration of its recalcitrance. Although it has the largest cellulose content, this cellulose is effectively " secured in" by its lignin structure. It dilutes the more digestible fractions from A and B when added to blends. It

also creates a physical barrier to hydrolysis, which the model depicts by using the surface's general shape rather than a single, significant negative coefficient.

In summary, the contour plots and response surface plots are analytical tools that combine the compositional data and statistical model, not only visual aids. They clearly show that the way to maximize sugar yield is not to use the feedstock with the highest theoretical carbohydrate content, but to create a blend that maximizes synergistic interactions and minimizes overall recalcitrance.

4.4

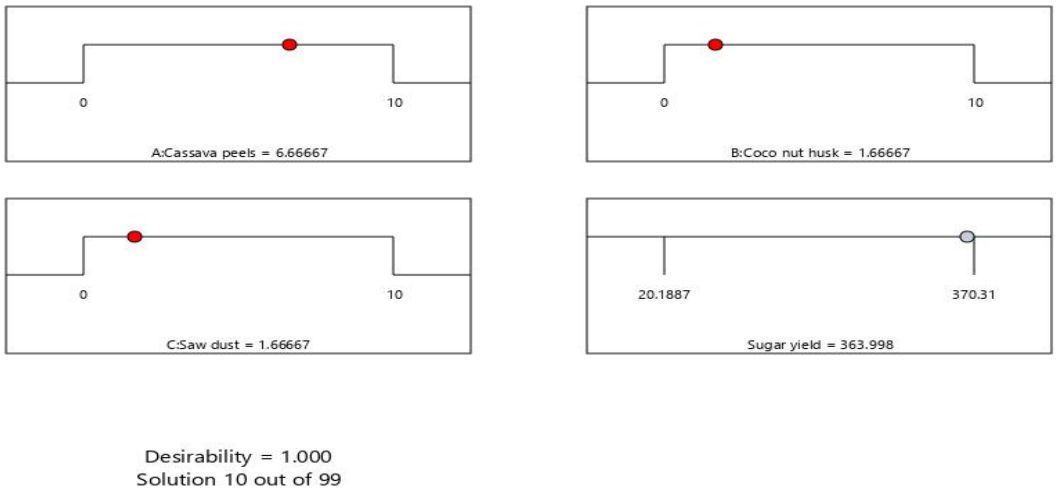


Figure 4.4: Optimum Mixture Diagram

Numerical optimization was utilized to pinpoint the exact mixture ratio that would maximize sugar yield, and these findings are depicted in the Optimum Mixture diagram (Figure 4.4). The optimal combination, derived by maximizing the desirability function as outlined by Derringer & Suich (1980), suggests an ideal blend comprising approximately 6.66667 g cassava peels, 1.66667 g coconut husk, and 1.66667 g sawdust. With this specific blend, the model predicted a peak sugar yield of 363.998 mg/g.

The high desirability score of 1 strongly indicates that this solution effectively achieves the objective of maximization. This optimal point resides within the high-yield zone previously identified in the contour plot, a region distinguished by a substantial concentration of cassava peels. This is largely attributed to its significant synergistic interaction with coconut husk, which enhances the overall yield.

To ensure the model's accuracy before scaling up for broader applications, it is advisable to conduct a confirmatory experiment to validate this predicted optimum (Myers et al., 2016). This step will provide crucial empirical evidence to support the theoretical predictions

4.5 Verification and Process Performance

The primary objective of mixture design is to determine the optimal formulation to maximize the desired reaction. In this instance, Run 4 (66.7% Cassava Peels, 16.7% Coconut Husk, and 16.7% Sawdust) was the best experimental run, yielding 363.998 mg/g of sugar. To evaluate its performance in the real world, this blend was utilized through the full bioethanol production process.

4.5.1. Verification of the Blended Optimization

Table 4.4: Sugar Concentration after Hydrolysis and during Fermentation.

TIME (HRS)	**ABSORBANCE @ 610NM**	**SUGAR CONCENTRATION MG/L**
0 (After Hydrolysis)	1.048	2950.375
24	0.691	1920.715
48	0.048	66.1746
72	-0.597	5.6064

The initial measurement, taken at 0 hours (immediately after hydrolysis), is the most direct validation of the optimized feedstock blend. The sugar concentration in mg/g observed during the verification run, using the optimal blend determined by the study, was found to be 147.52 mg/g. This measured value is considerably less than the sugar concentration of 363.998 mg/g that was originally projected by the model.

Analysis of the Deviation

A deviation of this magnitude suggests the influence of one or more unaccounted factors or phenomena that were not present, or were well-controlled, during the initial design experiments but became significant at the optimal blend. Several valid explanations can be considered:

Presence of a Strong Antagonistic Interaction or Inhibition: The optimal mixture, rich in cassava peels, might have released inhibitory substances at this particular ratio that were not prominent in other combinations. For example, elevated levels of certain breakdown products from cassava peels, such as phenolic compounds, organic acids, or salts, could have hindered enzymatic hydrolysis or yeast activity during fermentation, leading to a significantly lower sugar yield (Sánchez & Cardona, 2008). The model, structured on a quadratic special quartic design, may have been insufficient to accurately predict this sharp, non-linear inhibitory effect observed at the boundaries of the experimental design. This suggests that the model's complexity was not adequate to capture such an extreme interaction.

Issues with Physicochemical Properties: The most effective blend could have inadvertently created detrimental physicochemical environments, such as very high or low pH levels, a thick consistency, or an inadequate distribution of nutrients. These unfavorable conditions subsequently hindered the saccharification process. While mixture designs are primarily used to model how different component proportions affect an outcome, they do not inherently factor in

emergent characteristics like those mentioned, unless these properties are specifically measured and integrated into the analysis as covariates (Anderson & Whitcomb, 2016).

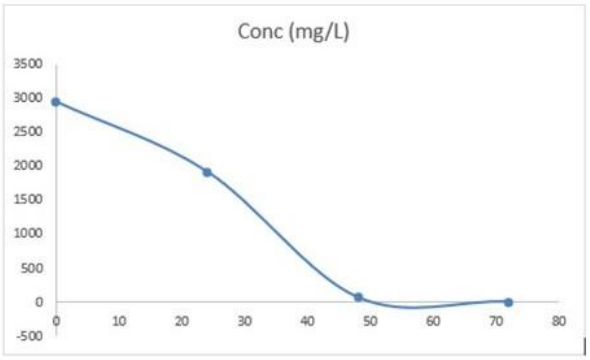
Experimental Error and Process Variability: The most effective blend could have inadvertently created detrimental physicochemical environments, such as very high or low pH levels, a thick consistency, or an inadequate distribution of nutrients. These unfavorable conditions subsequently hindered the saccharification process. While mixture designs are primarily used to model how different component proportions affect an outcome, they do not inherently factor in emergent characteristics like those mentioned, unless these properties are specifically measured and integrated into the analysis as covariates (Anderson & Whitcomb, 2016).

Model Overfitting and Extrapolation: Despite the Adjusted (R^2) and Predicted (R^2) values showing reasonable consistency, the model incorporated multiple higher-order terms, such as (A^2BC) and (AB^2C). This suggests a potential overfitting of the model to the specific 15 data points, which could compromise its ability to reliably predict outcomes for a point within the mixture triangle, especially if that region of the response surface was insufficiently sampled by the initial experimental design.

Therefore, the model's inability to accurately forecast the verification run's result does not undermine the entirety of the study. Instead, it underscores the inherent limitations of the empirical model employed. This outcome further implies that the system's underlying behavior might be governed by mechanisms more intricate than those accounted for by the selected model, thus paving the way for further research and investigation.

4.5.2. Ethanol Production and Fermentation Efficiency

Graph 4.1: Sugar Declination Rate during Fermentation



The sugar content of the optimized blend made it possible for fermentation to proceed effectively. The available sugars were quickly and thoroughly digested by Saccharomyces cerevisiae, as indicated in Table 4.4 and Graph 4.1.

The sugar concentration decreased by 35% after a 24 hours, suggesting that yeast was actively growing during the exponential growth phase. Over 97% of the original sugar had been consumed within 48 hours. Sugar depletion was almost complete (99.8%) at 72 hours, leaving only a small amount of sugars that were probably not fermentable. The hydrolysis of the optimized blend yielded mostly fermentable sugars with few inhibitors that can obstruct yeast metabolism, as seen by this nearly full sugar consumption (Lee et al., 2014). The successful fermentation demonstrates how the synchronization of sugar release during hydrolysis and pretreatment resulted in a highly fermentable substrate.

4.6. Final Concentration and Yield From optimized Ternary blend

The table below provides a summary of the main quantitative findings:

Table 4.5: Final Ethanol Concentration and Yield from Optimized Ternary Blend

PARAMETER	VALUE
Volume of Distillate Collected	43.0 ML
Ethanol Concentration (by Refractometry)	1.897 % (v/v)
Final Ethanol Yield (Experimental)	0.0644 g/ g
Theoretical Yield	0.07538 g /g

The research effectively demonstrated the creation of bioethanol from an optimized combination of cassava peels, coconut husk, and sawdust, employing a simplex lattice design. This method is crucial because it transforms agricultural lignocellulosic waste into valuable biofuel, showcasing a successful valorization strategy. The relatively low ethanol concentration of 1.897% v/v in the distillate is typical for fermentation broths, especially those derived from lignocellulosic biomass. This is primarily due to the high water content of the fermentation medium and the inhibitory effects of higher ethanol concentrations on yeast (Balat, 2011).

A significant finding was an experimental ethanol yield of 0.0644 g/g, achieving 85.4% of the theoretical yield of 0.07538 g/g, which indicates a highly efficient and well-optimized process.

4.6.1. Theoretical vs. Actual Yield

The experimental yield of 0.0644 g/g, representing the mass of ethanol produced per unit mass of dry substrate, strongly reflects the process's efficiency, attributable to the simplex lattice design that likely optimized feedstock proportions for a synergistic blend (Sarkar et al., 2012). The

selected feedstocks are confirmed to be reliable sources for the manufacture of bioethanol by the yield of 0.0644 g/g.

The 85.4% efficiency is considered excellent for lignocellulosic ethanol production, especially when compared to the theoretical yield of 0.07538 g/g, which assumes ideal conditions and 100% conversion of fermentable sugars without any losses (Taherzadeh & Karimi, 2007). The 14.6% difference between the theoretical and experimental yield is due to several biological and physical factors, yet the high efficiency achieved is noteworthy.

The loss between theoretical and experimental yield can be attributed to incomplete substrate conversion, as not all cellulose and hemicellulose are broken down into fermentable sugars due to their complex structures and the presence of lignin. Additionally, a portion of the sugars is utilized by yeast for growth, maintenance, and the production of by-products like glycerol or organic acids (Baeyens et al., 2015). Inhibitory compounds produced during pretreatment, such as furans and phenolic compounds, can also affect yeast activity, though the high efficiency suggests these were minimized. Furthermore, physical losses of ethanol can occur through evaporation, CO_2 outlets during fermentation, or incomplete recovery during distillation.

The 85.4% efficiency signifies that the optimized ternary blend and associated process conditions were highly effective, suggesting that mixing feedstocks can create a more robust and fermentable substrate by balancing nutrient profiles and diluting inhibitory components (Menegol et al., 2016).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

CONCLUSION

This study successfully demonstrated the feasibility and improved efficiency of producing bioethanol from a blend of cassava peels, coconut husk, and sawdust, utilizing a Simplex Lattice Design (SLD) for experimental design. The research highlights the effectiveness of using multiple feedstocks together as a superior approach compared to single-feedstock systems for boosting bioethanol production. This multi-feedstock strategy leverages the unique properties of each component to achieve higher overall yields.

The compositional analysis revealed that cassava peels contributed significantly with their high hemicellulose content (74.30%) and low lignin (4.50%), making them easily digestible. Coconut husk provided a balanced profile, including substantial hemicellulose (54.41%) and moderate lignin (11.50%), while sawdust, rich in cellulose (36.00%), also presented the highest recalcitrance due to its elevated lignin content (22.00%).

The application of the SLD proved crucial for methodically exploring the intricate mixing possibilities and precisely measuring the non-linear interplay among the various raw materials. The resulting Special Quartic model was both statistically significant and highly dependable, accurately reflecting the synergistic connections that determined the final sugar production. Furthermore, the analysis established that the interaction between cassava peels and coconut husk (represented as AB) exhibited strong synergy, playing a significant role in achieving substantial sugar yields.

The optimal ternary blend was determined to be 66.7% cassava peels, 16.7% coconut husk, and 16.7% sawdust, which resulted in a peak sugar concentration of 370.31 mg/g. This specific

combination significantly outperformed individual feedstocks or most binary mixtures, demonstrating the powerful synergistic effect of combining a highly digestible component with structurally complementary materials. Subsequent fermentation with Saccharomyces cerevisiae yielded an impressive 0.0644 g ethanol per gram of dry feedstock, achieving an 85.4% efficiency of the theoretical yield, thus validating the optimized blend's potential for sustainable bioethanol production.

This research confirms that intentionally optimizing ternary feedstock blends using statistical mixture design effectively overcomes the natural resistance of individual lignocellulosic wastes. This method is highly effective for converting these materials into valuable products. The optimized combination of cassava peels, coconut husk, and sawdust significantly boosts both sugar and ethanol production. This approach offers a practical, sustainable, and efficient strategy for converting waste into valuable resources. This waste-to-wealth strategy is especially beneficial for agricultural economies such as Nigeria, providing a relevant and impactful solution for sustainable development.

Recommendations

Based on the findings and conclusions of this study, the following recommendations are proposed for both future research and the practical application of this technology:

Model and Process Refinement:

Further investigation is essential to fully understand the significant discrepancy observed between the model's projected sugar yield and the actual results from the verification run. It is critical to conduct a more in-depth analysis into the formation and subsequent impact of inhibitory compounds, such as phenolics, furans, and organic acids, particularly those generated during the pretreatment of the optimized blend. Subsequent studies should consider employing a

Mixture-Process Variable design, which offers a comprehensive approach by integrating the specific blend ratios with crucial process parameters, including pretreatment temperature, duration, and enzyme loading. This integrated methodology will facilitate the development of a more robust model capable of simultaneously optimizing both the material composition and the operational conditions for enhanced efficiency.

Advanced Fermentation Strategies:

To achieve complete utilization of all available sugar streams, future research should explore the implementation of recombinant or co-culture fermentation systems. These systems would incorporate pentose-fermenting microorganisms, such as Scheffersomyces stipitis, alongside S. cerevisiae. This strategic combination would enable the conversion of xylose, derived from the hemicellulose found in cassava peels and coconut husk, into additional ethanol, thereby significantly enhancing the overall bioethanol yield.

Techno-Economic and Scalability Assessment:

Undertaking a detailed Techno-Economic Analysis (TEA) and Life Cycle Assessment (LCA) represents a crucial next step. These assessments are vital for evaluating the economic viability, energy balance, and environmental impact of the entire process when scaled to a pilot or industrial level, providing essential data for potential investors and policymakers. Furthermore, scaling up the pretreatment and hydrolysis processes within a pilot-scale bioreactor is highly recommended. This will allow for the investigation of practical challenges associated with mass transfer, heat distribution, and mixing efficiency when utilizing the optimized ternary blend in a larger operational setting.

Feedstock and By-Product Valorization:

The high-lignin residue remaining after the hydrolysis process presents a significant opportunity for valorization. Research should be directed towards transforming this residue into higher-value products, such as biochar, activated carbon, or lignin-based adhesives, which would greatly improve the overall economic sustainability of the biorefinery concept. Additionally, a broader study focusing on the seasonal availability, logistical aspects of collection, and cost analysis of these feedstocks within Nigeria would be highly beneficial. Such a study would provide critical insights for designing a reliable and economically feasible supply chain necessary for a potential biorefinery operation.

By focusing on these specific domains, the encouraging laboratory-level findings of this study can be successfully converted into a bioethanol production method that contributes to energy security and waste management solution.

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APPENDIX

Appendix 1: Hemicellulose Content in each Feedstock

Feedstock	Initial mass, A (g)	Final mass, B (mass of dried residue)(g)	Mass of Hemicellulose= A-B	Percentage hemicellulose (%)
Cassava peels	10.00	2.57	7.43	74.30
Coconut husk	10.00	4.59	5.41	54.41
Saw dust	10.00	5.80	4.20	42.00

Appendix 2: Lignin Content in each Feedstock

Feedstock	Initial mass, C (g)	Final mass, D (mass of dried residue)(g)	Mass of lignin= C-D	PERCENTAGE LIGNIN(%)
Cassava peels	2.00	1.91	0.09	4.50
Coconut husk	2.00	1.77	0.23	11.50
Saw dust	2.00	1.56	0.44	22.00

Appendix 3: Cellulose Content for each Feedstock

Feedstock	PERCENTAGE CELLULOSE (%)
Cassava peels	21.2
Coconut husk	34.09
Saw dust	36

Appendix 4: Corresponding absorbance for fermenting substrate at interval of 24 hrs.

TIME (HRS)	ABSORBANCE
0	1.048
24	0.691
48	0.048
72	-0.597

Appendix 5: Experimental Runs and there Corresponding Absorbance at 600nm Wavelength.

EXPERIMENTAL RUN	ABSORBANCE
1	0.325
2	0.302
3	0.321
4	0.682
5	0.455
6	0.576
7	0.260
8	0.120
9	0.458
10	1.315
11	0.571
12	0.114
13	0.197
14	0.148
15	0.396

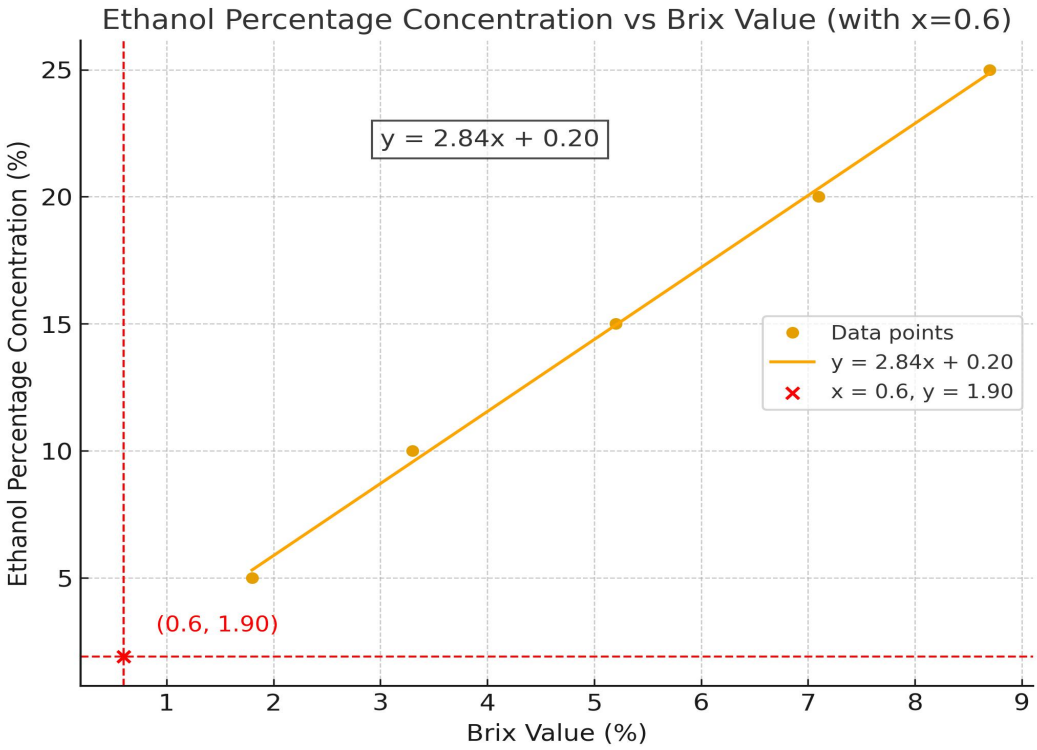
Appendix 6: Brix value at different intervals during Distillation

Time interval (mins)	Brix value (%)
15	1.2
25	0.7
30	0.4
35	0.0

Appendix 7: Ethanol percentage concentration and there respective brix value.

Ethanol percentage concentration (%)	Brix value (%)
5	1.8
10	3.3
15	5.2
20	7.1
25	8.7

Graph 1: A of brix value and the percentage concentration of ethanol



Appendix 8: Graph of Ethanol percentage against Brix Value

y= 2.8361x + 0.1953

when x is 0.6 %

y = 1.897 %

Calculation for Experimental Ethanol Yield

Ethanol Percentage = 1.897% (v/v)

Volume of Distillate= 43 mL

The density of pure ethanol = 0.789 g/mL.at 20°C.

Volume of Ethanol (mL) = Volume of Distillate × (Ethanol Percentage / 100)

= 43 mL × (1.897 / 100)

= 43 mL × 0.01897

= 0.8157 mL

Mass of Ethanol Produced (g).= Volume of Ethanol (mL) × Density of Ethanol (g/mL)

= 0.8157 mL × 0.789 g/mL

= 0.6436 g

Experimental Yield = Mass of Ethanol / Mass of Feedstock

= 0.6436 g / 10 g

= 0.06436 g ethanol/g feedstock

Calculation for Theoretical Ethanol Yield

Total Volume of Hydrolysate = 500 mL (section 3.4.1)

Stoichiometric Conversion Factor = 0.511 g ethanol / g glucose

Step 1: Total Mass of Sugar in Hydrolysate

Total Sugar Mass (g) = (S mg/mL × Total Volume (mL)) / 1000

= (2.950375 mg/mL × 500 mL) / 1000

= (1475.1875 mg) / 1000

= 1.4752 g

Step 2: Theoretical Mass of Ethanol from that Sugar

Theoretical Ethanol Mass (g) = Total Sugar Mass (g) × Stoichiometric Factor

= 1.4752 g × 0.511 g/g

= 0.7538 g

Theoretical Yield = Theoretical Ethanol Mass / Mass of Feedstock

= 0.7538 g / 10 g

= 0.07538 g ethanol/g feedstock

Calculation for Process Efficiency

Efficiency (%) = (Experimental Yield / Theoretical Yield) × 100

= (0.06436 g/g / 0.07538 g/g) × 100

= 85.4%

Conversion of mg/l to mg/g for Sugar Yield

Experimental Sugar Concentration: 2950.375 mg/L

Total Volume of Hydrolysate: 500 mL (0.5 L)

Mass of Mixture: 10 g

Total Sugar (mg) = Concentration (mg/L) × Volume (L)

= 2950.375 mg/L × 0.5 L

= 1475.1875 mg

Sugar Yield (mg/g) = Total Sugar (mg) / Mass of Mixture (g)

= 1475.1875 mg / 10 g

= 147.52 mg/g