SUSTAINABLE BIOETHANOL PRODUCTION FROM ZAMBIAN CORN STOVER

BY

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DECLARATION

Declaration by the Candidate

I do hereby declare that the contents of this thesis being submitted herein are my original work and they have not been previously submitted to any University for award of a degree or any other qualification.

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DEDICATION

To

My wife, Milimo Choongo Mwanakaba

My sons

My daughters

&

My siblings

Whose support, love & guidance gave me the strength to endure loneliness and possible discouragement on this academic journey.

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ABSTRACT

Commercialization of second-generation bioethanol production is hindered by the lack of sustainable, cost-effective, and environmentally friendly pretreatment technology. The use of Deep Eutectic Solvents (DES) is a promising alternative. This study aimed to optimize DES pretreatment of Zambian corn stover to maximize bioethanol production. The specific objectives were to determine engine performance and emissions of bioethanol/gasoline blends; ascertain the ideal conditions for cellulose yield, enzymatic hydrolysis, and bioethanol generation; and conduct a techno-economic feasibility study of major scale DES-based bioethanol production. The factors studied during pretreatment included time (6–15 hours), temperature (60°C–150°C), choline chloride to lactic acid ratio (1:2, 1:6, and 1:10), and substrate-to-solvent ratio (SLR) (1:08–1:32). Hydrolysis was conducted at temperatures between 45°C and 50°C for 60–72 hours. Optimization of pretreatment and hydrolysis was performed using Central Composite Design (CCD), Response Surface Methodology (RSM), Artificial Neural Networks (ANN), and Gradient Boosted Regression Trees (GBRT). Mathematical models were developed to estimate cellulose and fermentable sugar yields. The optimal pretreatment conditions:105°C, 10.5-hour reaction time, and a 1:6 ChCl:LA ratio yielded a 46.1% cellulose recovery, with model predictions achieving 43% (quadratic) and 46.1% (GBRT) at R^2 values of 91% and 80%, respectively. Optimal enzymatic hydrolysis conditions enzyme loading of 10 mg per gram of biomass, 50°C, and 72-hour reaction time resulted in a fermentable sugar yield of 78%, validated through High-Performance Liquid Chromatography (HPLC). Fermentation using Saccharomyces cerevisiae produced bioethanol with an 80% yield, confirmed via Gas Chromatography-Mass Spectrometry (GC-MS). Distillation was conducted at 78.5°C using a computer-controlled bioethanol process unit. Through laboratory-level distillation, 2.82 g of bioethanol was obtained, leading to a final production volume of 3.57 L. Bioethanol/gasoline blends (G100, E10, E20, E30, and E40) were tested on an Atico computer-controlled hybrid test bench engine. Brake power and brake specific fuel consumption (BSFC) results were 31.42, 32.72, 34.03, 30.11, and 28.8 kW and 0.2706, 0.2516, 0.2333, 0.2765, and 0.3194 kg/kWh for G100, E10, E20, E30, and E40 blends, respectively. E20 provided the best balance between performance and emissions, increasing brake thermal efficiency (BTE) by 7.4% while reducing carbon monoxide (CO) and hydrocarbon (HC) emissions by 21% and 26%, respectively. Higher ethanol blends (E30 and E40) further reduced emissions but required modifications in ignition timing and fuel injection for optimal engine performance. A techno-economic analysis (TEA) assessed the feasibility of scaling up DES-based bioethanol production for a 50,000-liter capacity plant. The DES process was found to be 27% more cost-effective than conventional methods due to the recyclability and biodegradability of lactic acid and choline chloride, reducing overall fuel costs. A life cycle assessment (LCA) showed a 32% reduction in greenhouse gas emissions compared to fossil fuel-based gasoline. The results confirm the potential of DES-based pretreatment to enhance bioethanol production and improve economic viability.

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ACRONYMS AND SYMBOLS

A	Temperature
AB	Interaction Between Temperature and Time
A^2 and B^2	Nonlinear Squared Term Effect
ABV	Alcohol by Volume
ACO	Ant Colony Optimization
AFEX	Ammonia Fibre Explosion
AI	Artificial Intelligence
ANN	Artificial Neutral Network
ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
B	Time
BBD	Boxed Behnken Design
BP	Brake Power
BRT	Gradient Boosted Regression Tree
BSFC	Brake Specific Fuel Consumption
BTE	Brake Thermal Efficiency
β	Intercept
CBP	Consolidated Bio-Processing
Capex	Capital Expenditure
CC	Construction Cost
CCD	Central Composite Design
C5	Pentose Sugars (Xylose and Arabinose)
CF_t	Cash Flow at a Given Time

ChCl	Choline Chloride
CO	Carbon Monoxide
CO_2	Carbon Dioxide
C_p	Cost of Fermentation Tank
CV	Coefficient of Variation
D	Number of operating days
DCF	Discount Cash Flow
DE	Differential Evolution
DES	Deep Eutectic Solvents
DOE	Design of Experiment
ERB	Energy Regulation Board
E10	10% Ethanol Blend
FCCD	Face Centred Central Composite Design
FFD	Full Factorial Design
G100	100% Gasoline
GA	Genetic Algorithms
GBRT	Gradient Boosted Regression Trees
GC-MS	Gas Chromatography Mass Spectrometry
GHG	Greenhouse Gas
GH_z	Gigahertz
HC	Hydrocarbon
HDI	Human Development Index
HFO	Heavy Fuel oil
HMF	Hydroxymethylfurfural

HOV	Heat of Vaporization
HPLC	High Performance Liquid Chromatography
HVED	High Voltage Electrical Discharge
IL	Ionic Liquid
IP	Indicated Power
IPCC	Intergovernmental Panel on Climate Change
IRR	Internal Rate of Return
ISO	Internation Organization for Standardization
KOH	Potassium Hydroxide
LA	Lactic Acid
LCA	Life Cycle Assessment
LCB	Lignocellulosic Biomass
LFS	Laminar Flow Speed
LHV	Fuel Lower Heating Value
LHW	Liquid Hot Water
LPG	Liquefied Petroleum Gas
LPMO	Lytic Polysaccharide Monooxygenases
$\dot{m}_f$	Fuel mass flow rate
MH_z	Megahertz
ML	Machine Learning
MPa	Megapascal
MSE	Mean Square Error
n	Project Life Time
N	Engine Speed

N_aCS	Sodium Cumene Sulphonate
N_aOH	Sodium Hydroxide
NH_3	Ammonia
NO_x	Nitrogen Oxide
NPV	Net Present Value
Opex	Capital Expenditure
PB	Payback Period
PEE	Pulse Electric Energy
PEF	Pulse Electric Field
pH	Acidity
PSO	Particle Swarm Optimization
Q	Volume of Bioethanol Production
r	Discount Rate
RF	Random Forests
RID	Retractive Index Detector
RMSE	Roof mean Square Error
RPM	Revs/Minute
RSM	Response Surface Methodology
SA	Simulated Annealing
SAE	Society of Automotive Engineers
SEM-EDX	Scanning Electron Microscopy Energy-Dispersive -X-Ray
SHF	Separate Hydrolysis and Fermentation
SPORl	Sulphuric Acid Pretreatment with Overlining
SSCF	Simultaneous Saccharification and Co-Fermentation

SSF	Simultaneous Saccharification and Fermentation
SVM	Support Vector Machine
T	Torque
TEA	Techno-Economic Analysis
T_w	Plant Working Hours
μ	Microns
V_c	Volume of Fermentation Tank
X	Independent Variables
Y	Model Equation Response
ZABS	Zambia Bureau of Standards
$\sum$	Summation

CHAPTER ONE: INTRODUCTION

1.1 Background

The search for renewable and sustainable energy sources has accelerated due to the world's increasing energy demand, the depletion of fossil fuel reserves, and growing environmental concerns. Bioethanol, a renewable fuel derived from biomass, has emerged as a viable alternative to fossil fuels, particularly in the transportation sector. Corn stover, a lignocellulosic agricultural waste product, is very common in Zambia. This makes it possible to produce second-generation bioethanol without compromising food availability.

Figure 1. 1: Corn stover raw material for ethanol production (Satnum farms, Zambia)

However, because corn stover is hard and challenging to break down, it requires thorough pretreatment to convert cellulose and hemicellulose into sugars that can be fermented. This research investigates the application of Deep Eutectic Solvents (DES) as an eco-friendly pretreatment technique to augment biofuel production, enhance energy security, and promote rural development.

Historically, the transportation industry worldwide has relied on fossil fuels, such as petrol and diesel, to operate (Umar et al., 2021). As the global population increases—estimated at 8,045,311,447 in 2023 (C.K. Sun, 2024)—the demand for fossil fuels continues to rise correspondingly. However, fossil fuels are limited resources that take millions of years to form and can't be replenished within a human lifetime (Bond, 2022). Their depletion is inevitable, as each well typically lasts only about 200 years on average (Schmieder & Kring, 2020). This unsustainable way of using energy highlights the importance of finding cleaner, renewable, and alternative sources of energy that can meet the needs of both the world and the United States.

Biofuels have thus become increasingly popular as alternative fuels for transportation, especially in developing countries where access to clean and affordable energy is crucial for economic growth and societal well-being (Talukdar, 2021). Zambia is a good example of this problem because it is consistently concerned about energy security, as it relies heavily on imported oil products. Many changes in the global fuel market, as well as fluctuations in the value of foreign currencies, have a significant impact on the local fuel market, making energy prices unstable and unpredictable (Zhang et al., 2024). Climate-related issues, such as prolonged droughts and unpredictable rainfall patterns, have exacerbated these energy problems by hindering hydroelectric generation and highlighting the need for sustainable and adaptable energy solutions (Zenda, 2024).

For social, economic, and environmental progress, it is essential to have access to cheap and reliable energy. For developing nations such as Zambia, access to energy directly facilitates poverty alleviation, industrial advancement, and employment generation, which are essential elements of the Human Development Index (HDI) (Hariram et al., 2023; Downs et al., 2020). Energy availability supports critical aspects of society, such as education, healthcare, sanitation, and access to clean water, particularly in rural areas. Promoting renewable energy sources, such as bioethanol, not only helps address environmental and economic challenges but also enables Zambia to achieve its long-term objectives of inclusive growth, rural industrialization, and sustainable development.

The blending policy is currently being developed under the biofuels sector, which means that the production of biofuels is being promoted at this time. In Zambia, all petroleum products are imported (Tembo et al., 2020). Because there aren't enough domestic petroleum sources, the import bill is extremely burdensome for the nation. However, because of the country's large amount of arable land and the highly productive agricultural sector, appropriate waste management would significantly aid in the production of biofuels based on lignocellulosic materials.

The petroleum subsector, the electricity subsector, and the biomass subsector comprise Zambia's energy sector. In this instance, the petroleum industry and the nation's cooking technologies are the main subjects. Firewood, charcoal, electricity, and gas are the four primary cooking technologies. It has been noted that 54% of Zambians cook with firewood, 27.99% with charcoal, 0.1% with LPG, and 17% with electricity (Baltruszewicz et al., 2021). But Zambia's rural and urban areas differ greatly from one another. 81% of rural households cook and stay warm using firewood, 16% use charcoal, and 3% use electricity (Scott & Archer, 2021). In contrast, 51% of people in urban settings cook with charcoal, 43% with electricity, 6% with firewood, and a very small percentage with liquefied petroleum gas (LPG).

The Energy Regulation Board of Zambia [ERB] (2023) as illustrated in Tables 1.1 and 1.2 display Zambia's energy mixes for electricity supplies and petroleum respectively,

Table 1. 1 : Electricity Sector- Energy Mix in Zambia (Source: ERB, 2023)

No.	Technology	Installed Capacity	Percentage (%)
1	Hydro	2702.50	81.5
2	Thermal (Coal)	330	9.95
3	Thermal (Diesel)	84.80	2.55
4	Heavy Fuel Oil	110	3.3
5	Solar	89.13	2.7
	Total	**3316.43**	**100**

At 81.5%, hydropower is Zambia's primary source of electricity supply. Additional sources include solar at 2.7%, heavy fuel oil at 3.3%, diesel at 2.55%, and coal at 9.95%. The data makes it abundantly evident that insufficient funds have been allocated to renewable energy sources (Kaoma & Gheewala, 2020). Although hydropower is a clean energy source, its generation capacity is impacted during drought years and is completely dependent on weather patterns.

With the exception of kerosene and heavy fuel oil (HFO), the average daily consumption of fossil fuels rose nationally as illustrated in Table 1.2. Diesel consumption rose from 2.98 million liters in 2020 to an average of 3.33 million liters in 2021. In 2021, the amount of gasoline consumed per day rose from 1.24 million liters to 1.45 million liters. Similarly, the average national consumption of Jet-1 and LPG increased from 57,115.02 liters and 21,767.23 kg to 76,012.50 liters and 21,866.03 kg, respectively.

Table 1. 2: Daily average consumption, 2020-2021-Fossil. (Source: ERB, 2023)

PRODUCT	2020	2021	%Change
Diesel (L)	2,977,214.48	3,327,465.70	11.8
HFO (kgs)	123,350.82	42,150.53	-65.8
Jet A-1 (L)	57,115.02	76,012.50	38.1
Kerosene (L)	25,699.21	8,687.82	-66.2
LPG (kgs)	21,767.23	21,866.03	0.5
Unleaded Petrol (L)	1,239,930.92	1,448,991.30	16.9

Source: 2021 Energy sector report

However, the national daily consumption of kerosene and HFO decreased from 25,767.23 liters and 123,350.82 kg to 42,150.53 kg and 8,687.23 liters, respectively (Stritzke & Jain, 2021).

1.1.1 Use Of Fossil Fuels

Zambia's daily fossil fuel consumption is shown in table 1.2 above. According to a 2018 study by the Intergovernmental Panel on Climate Change (IPCC), emissions from fossil fuels are the main contributors to global warming. According to research, the industry and fossil fuels are responsible for 89% of CO_2 emissions (Rashedi et al., 2020). More than 90% of the fuel used in the transportation sector comes from petroleum, which also generates significant amounts of greenhouse gases. There is no longer any doubt about the necessity of alternative fuels, such as biodiesel, bioethanol, propane, hydrogen, and electricity derived from renewable resources like solar, wind, biomass, and geothermal. Emissions of carbon dioxide and other greenhouse gases may be decreased by switching to alternative fuels (Litvak & Litvak, 2020). Greenhouse gas emissions could have a detrimental effect on infrastructure development, energy crop farming, and the frequency of droughts and floods if they are not cut quickly. Currently, fossil fuels account for about one-third of global carbon emissions. The current known deposits are predicted to last the world until 2052, which is just 27 years from now, if no new reserves are discovered(Kalair et al., 2021).

1.1.2 Alternatives To Gasoline And Diesel

To lessen the negative effects of fossil fuels, particularly gasoline and diesel, researchers are creating a range of technologies. Electrically powered vehicles, biodiesel, and bioethanol are currently at the top of the list. The emphasis is on bioethanol as a gasoline substitute. A variety of biomass types could be used as feedstocks to produce bioethanol (Ayodele et al., 2020). These biomass types can be broadly classified into three groups based on their chemical makeup, including materials that contain sugar, such as sugarcane, sugar beet, molasses, whey, and sweet sorghum. Last but not least is lignocellulosic biomass derived from straw, agricultural waste, crop, and wood residues. The second category includes feedstocks that contain starch, such as grains (corn, wheat), and root crops like cassava and sweet potatoes (Mujtaba et al., 2023).

The first-generation feedstocks of sugar and starch compete with food needs and may generally cause resistance. Due to its affordability, accessibility, and lack of competition with food and animal feed crops, lignocellulosic biomass (2nd generation) is an obvious substitute source for producing bioethanol (Mignogna et al., 2024). Because algae can be directly converted into energy, they are regarded as a potential feedstock for the production of third-generation bioethanol. Generally speaking, the marine environment and technological advancements determine how this feedstock is used to produce bioethanol. According to (Larkum et al., 2020), algae belong to a broad category of photosynthetic organisms. There is ongoing debate regarding the classification of algae, especially with regard to the status of cyanobacteria. Algae can be either multicellular (macroalgae) or unicellular (microalgae). Because of their lipid content, microalgae (phytoplankton) frequently float on the water's surface, whereas macroalgae (seaweeds) are typically found affixed to rocks or other structures (Saraf & Dutt, 2022). Researchers studying biofuels from around the globe have already taken notice of microalgae. The potential of macroalgae as a third-generation feedstock has recently been recognized by "Biofuel World," which has prompted more research on the subject. Bioethanol can be produced from algae by fermenting their algal polysaccharides, which include cellulose, starch, and other special polysaccharides. Furthermore, biodiesel can be produced from triacylglycerols (Jakubowski et al., 2021).

1.1.3 Use Of Corn Stover For Bioethanol Production

The corn stover in Africa is the largest agricultural waste biomass with challenges in disposal. It is important to understand that value addition is possible to corn stover because they contain lignin and cellulose, which can be processed into bioethanol (Adeleke et al., 2023). In Zambia, corn is a staple food and is mainly grown by peasant farmers. The Food and Agriculture Organization (FAO) research in 2020 in Zambia reported that the corn stover yield stood at 2,777,713 tons and corn cobs at 552,595 tons. These are huge tonnages of biomass that are usually burnt as a way of disposal (Kojakovic et al., 2022). The corn stover is a complex natural biopolymer composed of three main components, which include lignin, hemicellulose, and cellulose. The most abundant component in lignocellulosic structure is cellulose, with a linear polymer of β-1.4 linked glucose units It is associated with hemicellulose, a branched polymer composed of sugars such as pentoses and hexoses. The plant cell contains lignin, primarily concentrated in the middle lamella and the primary cell wall. Bioethanol, C_2H_5OH, structural formula CH_3CH_2OH, is an organic molecule with hydroxyl functional (-OH). It is an alcohol homologous group. Particularly, bioethanol is primarily an alcohol with two carbon atoms with one hydroxyl group (Pandey et al., 2023).

There are three components associated with each other in a complex bond of a recalcitrant structure that limit enzymatic hydrolysis of cellulose. These include lignin, which is an aromatic polymer macromolecule with three different phenolic monomers that cannot be fermented for bioethanol production (Vaidya et al., 2022). The lignin is the main obstacle to enzymatic hydrolysis of cellulose since it hinders cellulase access to cellulosic substrate as a steric hindrance, during which it nonproductively absorbs cellulase, causing loss of enzyme activity (Huang et al., 2022). Therefore, pretreatment is necessary to overcome the above obstacles before enzymatic hydrolysis and fermentation to enhance enzymatic digestibility of lignocellulosic biomass and bioethanol yield (Sun et al., 2022). The identified feedstock in this case is the corn stover, which in most cases is left to dry in the fields and to house termites and is burnt when bush fires start in summer. Being agricultural waste, these feedstocks do not in any way compete with the food requirements of the people (Koul et al., 2022).

1.1.4 Need For Treatment

Cellulose and hemicellulose are made up of sugars just like starch-related feedstocks. Nevertheless, cellulose is naturally in the form of lignocellulose. Lignocellulose is a complex structure kind of material, naturally found in plants (Mujtaba et al., 2023). he first step, therefore, is to pretreat lignocellulosic biomass to remove the lignin and enhance the penetration of hydrolysis agents without destruction of cellulose and hemicellulose chemically (Kumar et al., 2020). Pretreatment is a crucial tool for cellulose conversion processes, as it alters the structure of cellulosic biomass to make cellulose more accessible to enzymes that convert the carbohydrate polymers into fermentable sugars (Mustafa et al., 2022).

1.2 Problem Statement

In Zambia, a significant amount of corn stover, a significant agricultural waste product from maize harvesting, is either burned in the open or left to rot in the fields. These practices significantly harm the environment by polluting the air, releasing greenhouse gases like carbon dioxide and methane, and wasting a potentially valuable renewable biomass resource. Although there is a lot of this lignocellulosic residue, it is not very useful for producing bioethanol because the plant material is naturally very complex.

Maize stover consists of a tightly bound lignocellulosic matrix made up of cellulose, hemicellulose, and lignin. This rigid structure makes it difficult for enzymes to access the cellulose and prevents its release during hydrolysis, resulting in low conversion rates and reduced bioethanol yields. The challenge of breaking down this stubborn structure effectively and sustainably has been a significant obstacle to producing second-generation bioethanol.

Using strong acids, alkalis, and other chemical reagents as part of traditional pretreatment methods has been shown to work for delignification and making cellulose more accessible. However, these methods often involve high operational costs, damage equipment, produce harmful by-products, and pollute the environment. These issues make it difficult for developing countries like Zambia to produce bioethanol in a way that is both affordable and environmentally friendly. This is because affordable and sustainable technologies are essential for rural industrialization and the growth of green energy.

To address these challenges, this research explores the use of Deep Eutectic Solvents (DES) as an alternative, environmentally friendly pretreatment method for corn stover. DESs are affordable, biodegradable, and recyclable solvents known for breaking down lignocellulosic biomass structures with minimal environmental impact. This study aims to effectively reduce lignin content, increase cellulose accessibility, and enhance enzymatic hydrolysis efficiency through optimized DES pretreatment conditions. The study intends to demonstrate the feasibility of DES-based pretreatment as a sustainable approach to boost bioethanol production, reduce dependence on fossil fuels, and promote the adoption of renewable energy Zambia.

1.3 Justification Of The Study

The study was warranted by the pressing necessity to advocate for sustainable and eco-friendly energy alternatives to diminish reliance on fossil fuels and alleviate the repercussions of environmental pollution. In Zambia, a lot of corn stover are made each year as agricultural waste. Most of it was burned or left to rot, which released greenhouse gases and wasted a valuable renewable resource. Changing this biomass into bioethanol was a useful way to turn waste into energy and make renewable energy. But traditional ways of pretreating, like acid and alkaline processes, were too expensive, harmful to the environment, and not long-lasting. It was therefore reasonable to use Deep Eutectic Solvents (DES) as pretreatment agents because DES is a green, biodegradable, low-cost, and recyclable option that can make cellulose more accessible, speed up enzymatic hydrolysis, and boost ethanol yield.

It was also important to test how well bioethanol-gasoline blends worked in an internal combustion engine to see if the biofuel could be used in real life. The examination of engine efficiency, combustion properties, and exhaust emissions yielded valuable information regarding the viability of incorporating bioethanol into current fuel systems. The addition of a techno-economic analysis made the study even more valid by showing how economically feasible, energy-efficient, and scalable the process was. The study was justified, as it addressed significant environmental, technical, and economic challenges related to bioethanol production, thereby contributing to Zambia's initiatives in renewable energy development, rural industrialization, and sustainable green growth.

1,4 Significance Of Study

This study was important because it helped develop sustainable energy by using corn stover, an abundant but underused agricultural waste that was often burned or left to rot, which caused pollution and greenhouse gas emissions. Transforming this biomass into bioethanol offered an alternative renewable energy source, encouraged waste valorization, and supported initiatives to decrease environmental harm and reliance on fossil fuels.

From a scientific standpoint, the research enhanced comprehension regarding the utilization of Deep Eutectic Solvents (DES) as environmentally friendly pretreatment agents for lignocellulosic biomass. Using DES instead of traditional chemical pretreatments was a low-cost, biodegradable, and recyclable option that made cellulose more accessible and sped up enzymatic hydrolysis. The optimized pretreatment conditions resulted in an increased ethanol yield and validated the viability of DES-based methodologies for sustainable bioethanol production.

The study demonstrated the feasibility of utilizing bioethanol-gasoline blends as cleaner transportation fuels through the assessment of engine performance and emissions. The techno-economic analysis also showed how the process could be made more cost-effective and scalable, which would help both the environment and the economy. The findings of this study collectively endorsed Zambia's transition towards the adoption of renewable energy, rural industrialization, and the sustainable development of biofuels.

1.5 The Objectives Of The Research

1.5.1 Main Objective

The main objective of this research was to optimize the use of Deep Eutectic Solvents (DES) in pretreatment of Zambian corn stover to maximize bioethanol production

1.5.2 Specific Objectives

The specific objectives are to:

1. Characterise and pretreat corn stover using DES and determine conditions for optimal cellulose yield.
2. Identify the optimal conditions for enzymatic hydrolysis that maximize bioethanol yield.
3. Assess engine performance and emission characteristics of produced bioethanol /gasoline blends
4. Assess the techno-economic feasibility of the integrated DES-based bioethanol production process at large-scale operations.

1.6 Research Questions

1. What are the optimal conditions for DES pretreatment and enzymatic hydrolysis to maximize cellulose and fermentable sugar yields from corn stover?

2. How much has the optimization of the pretreatment and enzymatic hydrolysis process of corn stover using DES improved fermentable sugar and bioethanol yields?
3. What is the benefit of techno-economic feasibility and scaling up DES pretreatment during bioethanol production?
4. How do bioethanol-gasoline blends perform in terms of engine efficiency and emissions compared to conventional gasoline?

1.7 Conceptual Framework

The conceptual framework of this study (Figure 1.2) illustrates the logical progression of the research design, highlighting the interconnections among inputs, processes, and outputs that collectively facilitate the optimization of bioethanol production from Zambian corn stover using Deep Eutectic Solvents (DES).

Inputs:

The primary materials used were Zambian corn stover and the DES reagents, specifically choline chloride (a hydrogen-bond acceptor) and lactic acid (a hydrogen-bond donor). Temperature, residence time, the molar ratio of choline chloride to lactic acid, and the biomass-to-solvent ratio were all independent factors in the process. These variables directly affect the physicochemical disruption of lignocellulosic bonds (Mustafa et al., 2022; Vaidya et al., 2022).

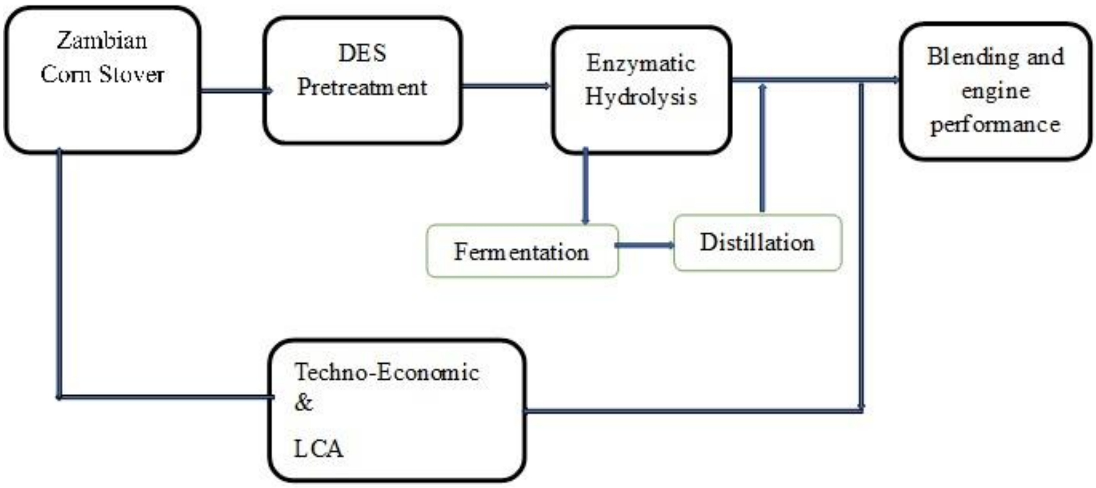

Figure 1. 2: A framework for ideas

Processes:

The study's process pathway consisted of five main steps: pretreatment, enzymatic hydrolysis, fermentation, engine testing, and techno-economic and environmental evaluation.

1) Pretreatment stage: The DES pretreatment breaks up the lignin–cellulose–hemicellulose network, making cellulose easier to get to (Kumar et al., 2020). This stage was modelled and optimised using Response Surface Methodology (RSM) and Central Composite Design (CCD), and further validated with machine-learning algorithms—Artificial Neural Networks (ANN) and Gradient Boosted Regression Trees (GBRT)—to identify nonlinear relationships between process parameters and cellulose yield

2) Enzymatic hydrolysis: Under the right conditions, cellulase enzymes broke down pretreated biomass into fermentable sugars. High-Performance Liquid Chromatography (HPLC) was used to measure the amount of sugars produced.

3) Fermentation: Saccharomyces cerevisiae turned sugars that could be fermented into bioethanol, which was confirmed by Gas Chromatography–Mass Spectrometry (GC–MS).

4) Engine testing: A hybrid single-cylinder engine was used to test different blends of bioethanol and petrol (E10–E40) for brake thermal efficiency, torque, fuel consumption, and exhaust emissions.

5) Techno-economic and environmental assessment: A Techno-Economic Analysis (TEA) looked at how profitable the process was, and a Life Cycle Assessment (LCA) measured how much less greenhouse gas (GHG) emissions it could produce than traditional methods (Kaoma & Gheewala, 2020).

Output:

The anticipated outcomes of the study comprised: • Optimised DES pretreatment parameters maximising cellulose recovery; • Improved fermentable sugar and ethanol yields, substantiated through experimental and statistical validation; • Identification of the most effective ethanol–gasoline blend (E20) for engine performance and emissions; • Assessment of the economic viability and environmental advantages of DES-based bioethanol production. Connections and Feedback: The conceptual framework incorporates feedback between process outputs and optimisation models. Laboratory experiment data (e.g., cellulose yield and sugar concentrations) guided the refinement of models in RSM, ANN, and GBRT to guarantee predictive accuracy (Mujtaba et al., 2023). The feedback from TEA and LCA confirmed that the process could be scaled up, closing the loop between optimising it in the lab and using it in industry.

This comprehensive framework illustrates how DES pretreatment is the primary process that yields both technical and sustainable results. It aligns with Zambia's goals for renewable energy policy by linking the use of agricultural waste to clean energy production, rural industrialization, and reduced carbon emissions (ERB, 2023; Tembo et al., 2020).

1.8 Outline Of The Thesis

This thesis comprises five chapters: Chapter One introduces the study and its context; Chapter Two reviews related literature and identifies research gaps; Chapter Three details materials and methods; Chapter Four presents and discusses results; and Chapter Five outlines conclusions, recommendations, and future work:

Chapter one has an overview of the study, which include background information at international scope and Zambian energy consumption trends, bioethanol production, and relevant policies. It as well defines the research problem, outlines the study objectives, and explains the importance of exploring bioethanol production from Zambian corn stover.

Chapter two reviews existing literature related to the study, including hydrolysis processes, kinetic studies of lignocellulosic biomass, large-scale bioethanol production viability, biomass classification, various pretreatment technologies, fermentation techniques, bioethanol purification, process modeling, and economic assessments and gaps/challenges.

Chapter three details the materials, equipment, and experimental procedures used in the research. It outlines the methods for biomass collection, pretreatment using Deep Eutectic Solvents (DES), enzymatic hydrolysis, fermentation, and distillation. Also, it covers computational modeling, statistical analysis, and performance evaluation of bioethanol/gasoline blends.

Chapter four presents the research findings, including the impact of different process variables on bioethanol yield, engine performance assessments, and economic feasibility. The results are analyzed and interpreted in relation to existing studies.

Chapter five summarizes key conclusions drawn from the study and provides recommendations for future research, policy considerations, and industrial implementation of DES-based bioethanol production

CHAPTER TWO: LITERATURE REVIEW

2.0 Chapter Introduction

There is a lot of promise for the sustainable production of bioethanol and other biochemicals from lignocellulosic biomass (LCB), which is obtained from sources such as forestry residues, industrial byproducts, and agricultural wastes like corn stover. In particular, corn stover is a readily available agricultural residue that serves as an environmentally friendly feedstock because it does not interfere with food production or contribute to deforestation. LCB, which consists of cellulose, hemicellulose, and lignin, is difficult to break down because of its complex structure, so it needs special treatment to help enzymes work better and to break down the lignin barrier. While traditional methods like acid or alkaline treatments are commonly used, newer and better options such as deep eutectic solvents (DES), ionic liquids, and microwave-assisted techniques offer more effective and sustainable solutions.

The process of turning LCB into bioethanol relies on effective enzymatic hydrolysis and fermentation, along with proper pretreatment. Even though there are still major issues with how tough the material is and the price of enzymes, advancements in enzyme engineering, such as heat-resistant cellulases and mixes of different enzymes, have greatly improved the efficiency of breaking down the material. Genetically modified yeasts and bacteria that can survive the harmful substances created during pretreatment can ferment both hexose and pentose sugars. Besides ethanol, valuable chemicals like lactic acid, succinic acid, and butanol, which are important for different industries, can be produced from sugars derived from lignocellulosic biomass (LCB). However, technical and financial obstacles continue to limit commercial-scale implementation.

By lowering greenhouse gas emissions and encouraging rural development without causing deforestation, the use of agricultural residues such as corn stover helps achieve environmental goals. Corn stover is especially well-suited for sustainable biofuel projects because of these advantages. Emerging tactics to increase productivity and cut expenses include leveraging computational tools to optimise processing conditions and integrating biomass conversion with complementary processes, like biogas production. To fully realise the potential of LCB as a renewable energy and biochemical resource, it will take more research, policy support, and cooperative innovation throughout the biomass value chain to overcome current obstacles.

2.1 Bioethanol From Corn Stover

The most plentiful agricultural biomass in most African nations is corn stover, a lignocellulosic biomass. With 45 million cubic meters of bioethanol produced from the accessible corn stover worldwide, 60% of all production is represented(Khan, ur Rehman, et al., 2022). Usually, burning corn stover biomass helps to clear the fields for the next farming season. Being lignocellulosic biomass, corn stover falls into the second-generation feedstock for bioethanol manufacturing (Aghaei et al., 2022).

A globally recognized substitute for gasoline in the transportation industry is bioethanol (C2H5OH). First-generation feedstocks from food crops and a suitable replacement for gasoline make up the first trials in the manufacturing of bioethanol. Being food crops, there has always been conflict between trade and mass food consumption(Amornraksa et al., 2020). Food needs for the people come before issues of commerce and trade under normal conditions; thus, it is necessary to substitute second-generation lignocellulosic biomass instead of first-generation feedstocks. Available in enormous quantities worldwide, lignocellulosic biomass is affordable and sustainable. Many nations produce huge amounts of corn stover. For large-scale bioethanol generation, corn stover is thus a valuable lignocellulosic source (Aghaei et al., 2022).

Traditionally, one of the well-known and extensively used biofuels, bioethanol, has been added to gasoline. But its pure form (anhydrous bioethanol) is being used straight in gasoline engines more and more (Kulanthaivel et al., 2021). Typically, microbial fermentation of sugars derived from biomass produces bioethanol, which then undergoes distillation to yield the final biofuel output. Effective biofuel generation depends on the pretreatment of carbohydrate polymers, turning them into fermentable sugars; thus, this process usually begins with that step (Chisti & Karimi, 2022).

Lignocellulosic biomass has a complex structure made up of different chemical and physical features. This covers chemical composition, fiber characterization, and cell proposition, whose influence on further saccharification is noteworthy (Okolie, Nanda, et al., 2021). Consequently, it is established that the feedstock with more cellulose, hemicellulose, reduced lignin, and silica content is fit for the process of extracting bioethanol. Appropriate feedstock for bioethanol generation are the estimations of compositional analysis of cellulose (32–47%), hemicellulose (19–27%), and lignin (5–24%). (Shukla et al., 2023) According to Shukla et al., 2023, feedstock that contains more cellulose, hemicellulose, reduced lignin, and silica is suitable for the bioethanol extraction process. The right feedstock for making bioethanol is based on the analysis showing cellulose (32–47%), hemicellulose (19–27%), and lignin (5–24%).

Corn stover, which is a common plant material, cannot be easily turned into bioethanol because it has lignin, cellulose, and hemicellulose mixed together in a complicated structure that prevents enzymes from reaching the cellulose; therefore, it is not directly converted into bioethanol. Before hydrolysis, fermentation, and purification can happen, pretreatment is an important first step that aims to break apart the tough structures of the material. The study did not go into particular pretreatment technologies.(Zabed et al., 2023). Pretreatment therefore is used with the aim to breakdown the crystalline and polymeric structures which is a crucial step prior to hydrolysis, fermentation and final purification (Sołowski et al., 2020).

Historically, first-generation resources, more especially, food crops have been the main basis of bioethanol generation. This trend has sparked interest in second-generation feedstocks, which use non-food biomass, as it raises questions about the competition between biofuel production and food supply. Corn stover, a form of lignocellulosic biomass, serves as a sustainable second-generation feedstock, despite its use as animal feed. It is plentiful all around and presents a beneficial substitute for first-generation resources. To get the cellulose needed to make fermentable sugars, corn stover has to be treated first, which means breaking down the lignin that protects the plant's inner structure (Amornraksa et al., 2020).

Under the 2nd generation category, lignocellulosic biomass, corn stover, is the most alternative resource accessible for bioethanol generation, unlike first-generation feedstocks. The advantage is corn stover (cob, stalk, and leaves) does not compete with the food demand of the people (Shakelly et al., 2023). Furthermore, lessening environmental issues is the use of corn stover as a feedstock for bioethanol manufacturing since disposal often results in environmental damage (Hundie, 2021). Still, production of bioethanol is by pretreatment, much as with any other lignocellulosic biomass. The paper listed all the pretreatment techniques; thus, its main goal was an academic one.

2.2 Bioethanol Production Process From Corn Stover

2.2.1 Grinding/Milling

The first and one of the most essential steps in the preparatory process of a corn stove for bioethanol production was grinding or milling. This step made sure the biomass was chopped into smaller pieces, which helped make the next steps like pretreatment, enzymatic hydrolysis, and fermentation work better. Typically, the biomass was reduced to sizes that passed through a 3.2 mm screen (Jewiarz et al., 2020). The grinding process had a direct impact on how efficiently bioethanol was produced because it changed important physical and chemical features of the biomass, like moisture content, average size of the ground particles, and how the particle sizes were spread out. These parameters played a critical role in determining the efficiency of enzymatic hydrolysis and fermentation processes (Wróbel, 2020).

Mechanical cutters and hammer mills were the most popular tools for breaking down lignocellulosic biomass, with hammer mills being especially preferred because they create consistent particle sizes that are good for enzymatic digestion (Krátký, 2022). The effectiveness of the milling process largely depended on the biomass type, initial moisture content, and the specific energy input (Sitotaw et al., 2023). A properly adjusted milling process made the biomass particles smaller, increased their density, and helped break them apart, which disturbed the tightly packed structure of the lignocellulosic material. This decrease in crystallinity and fiber tangling made it easier for enzymes to reach the material, which in turn improved the efficiency of breaking it down and fermenting it (Balcerek, 2022).

For bioethanol production using corn stover, particle size reduction typically ranged between 0.5 mm and 3.0 mm. This size range is considered the best for breaking down the material with enzymes, balancing the energy used and the amount of sugar produced (Pengilly et al., 2022). Fine grinding led to increased surface area, allowing for better enzyme penetration and enhanced cellulose conversion. However, excessive milling led to unnecessary energy consumption and generated fine dust particles that posed operational challenges, such as handling difficulties and increased risk of combustion (Kanageswari et al., 2022). Studies showed that optimizing the milling process improved bioethanol yield and reduced energy requirements. For example studies by Yang et al., (2023) demonstrated that reducing the size of corn stover particles from 5 mm to 1 mm resulted in a 25% increase in enzymatic hydrolysis efficiency. Also, when biomass is ground correctly, it mixes better in slurries, leading to more even mixing and fewer problems with mass transfer during fermentation.

Therefore, grinding and milling were critical steps in the bioethanol production process, influencing the overall conversion efficiency of corn stover into fermentable sugars. Making the pieces smaller helped increase bioethanol production, but it was important to find the right balance between energy use, how well the process worked, and the costs of producing bioethanol. New energy-saving milling methods, like vibratory milling and selective grinding, are still being studied to make large-scale bioethanol production from lignocellulosic biomass more cost-effective.(Hodaifa et al., 2022). Advances in energy-efficient milling techniques, such as vibratory milling and selective grinding, continued to be explored to further enhance the economic viability of large-scale bioethanol production from lignocellulosic biomass (Pérez-Merchán et al., 2022).

2.3 Pretreatment

Pretreatment is an important stage in the preparation of lignocellulosic biomass after sizing it to small particles by grinding. The purpose of pretreatment is to break down the complex structure and separate the lignin from the cellulose and the hemicellulose (Mankar et al., 2021). Common pretreatment methods are physical, physicochemical, chemical, biological, and pulsed electrical methods. Figure 2.1 below shows the original status of biomass and the process of pretreatment. The pretreatment breaks down the lignin shell that surrounds the cellulose and hemicellulose, allowing the cellulose and hemicellulose to be collected for enzymatic hydrolysis and turned into fermentable sugars (Pereira & Arantes, 2020).

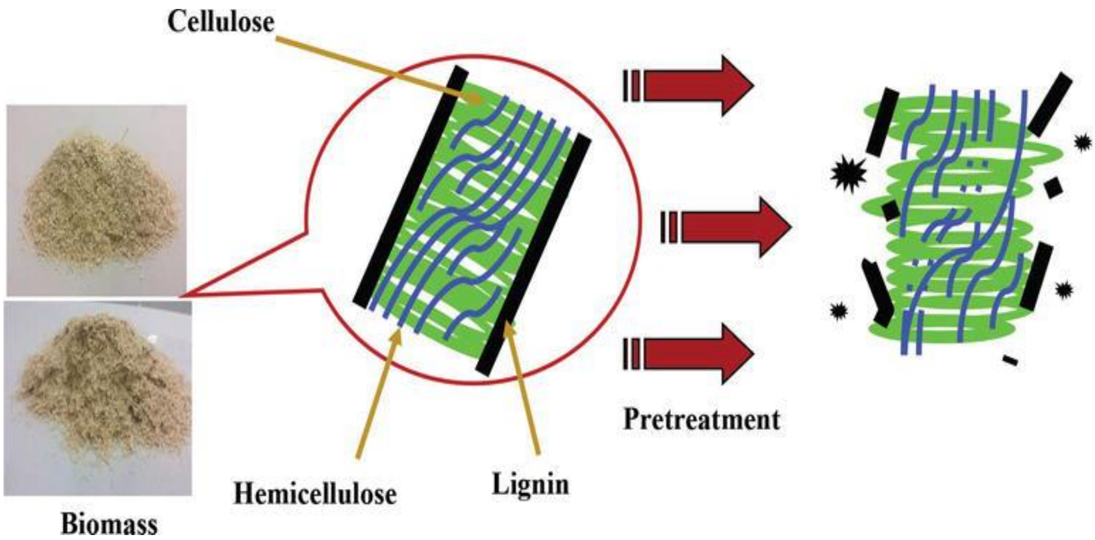

Figure 2. 1: Breakdown structure of pretreated biomass, (Source: Renewable Resources and Bio References)

The pretreatment process is crucial in the efficient conversion of lignocellulose into valuable biofuel products. Key factors in this process include residence time, reaction temperature, and the ratio of biomass to solvent, which significantly impact the yield of cellulose that can be fermented into bioethanol. Xu et al., (2020) In regions like Zambia, where there is an abundance of agricultural residues and dedicated energy crops, the sustainable management and availability of these feedstocks are critical components of a successful biofuel strategy (Atelge et al., 2020).

2.3.1 Chemical Pretreatment Methods

Lignocellulosic biomass, which is derived from plant materials such as wood, agricultural residues, and grasses, is a promising and abundant source of renewable energy. With concerns over the environmental impact of fossil fuels and the growing demand for sustainable energy sources, lignocellulosic biomass has gained significant attention as a potential feedstock for biofuels and bioproducts (N. Singh et al., 2022). The biomass is primarily composed of three key components: cellulose, hemicellulose, and lignin. Cellulose and hemicellulose have sugars that can be turned into biofuels, but lignin is difficult to break down because of its tough and complicated structure, making it hard for enzymes to work on it (Banu et al., 2021). So, being able to effectively break down the lignocellulosic matrix is key to using lignocellulosic biomass for making biofuels.

For many years now, the conversion of lignocellulosic biomass to fermentable sugars has been limited by its natural resistance to degradation. The complicated structure of lignocellulose, along with strong hydrogen bonds, makes it hard for enzymes to break down the biomass. (Thapa et al., 2020). To make cellulose and hemicellulose more accessible to enzymes, pretreatment processes are necessary to shatter the biomass structure and break down lignin and hemicellulose (Vu et al., 2020). Among the various pretreatment methods, chemical pretreatment has emerged as one of the most widely used and effective strategies due to its ability to improve the biodegradability of complex biomass materials. Historically, chemical pretreatment methods have been essential in overcoming the barriers to lignocellulosic biomass conversion, and numerous advancements have been made over the past few decades(Khan et al., 2022).

Early chemical pretreatment methods were based on the use of acids or alkalis, with sulfuric acid and sodium hydroxide being the most commonly utilized chemicals (Woiciechowski et al., 2020). After these early methods, more advanced techniques were created, such as exploring ionic liquids as potential solvents for lignocellulose and using organosolv pretreatment, which mixes organic solvents with an acid or base(Ascencio et al., 2020) Furthermore, ozonolysis—a method that oxidizes lignin using ozone (O_3)—has been recognized as a promising new pretreatment strategy(Rasid et al., 2021). Researchers have focused on making pretreatment methods better to reduce costs, boost how well biomass breaks down, and decrease the creation of harmful by-products that could slow down later fermentation and bioprocessing steps as the area of lignocellulosic biomass conversion has advanced. (Zhu et al., 2021). Therefore, the following sections will cover the mechanisms, benefits, drawbacks, and most recent advancements in the field of chemical pretreatment. These methods include sulfuric acid, sodium hydroxide, organosolv, ionic liquids, and ozonolysis (Hasanov et al., 2020). This review aims to provide an overview of these techniques, their efficacy, and the current research focused on improving pretreatment procedures for effectively converting lignocellulosic biomass into biofuels.

2.3.1.1 Dilute Sulphuric Acid

Diluted sulfuric acid (H_2SO_4) is one of the most popular and extensively researched pretreatment techniques for decomposing lignocellulosic biomass. Sulfuric acid treatment of biomass, particularly at high temperatures, is showing promise in overcoming lignocellulose's resistance (Zhang et al., 2021). Because of its outstanding effectiveness in dissolving complex biomass structures like lignin, hemicellulose, and cellulose, sulfuric acid pretreatment has remained one of the most studied techniques since its introduction in the 1950s (Woiciechowski et al., 2020). The method has evolved, with more attention on improving the pretreatment conditions to make it more efficient, cheaper, and to reduce harmful by-products that could interfere with later steps like enzymatic hydrolysis or fermentation. (Panakkal et al., 2022).

The main ways that sulfuric acid pretreatment functions are by partially breaking down lignin and hydrolyzing hemicellulose. Sulfuric acid helps break apart the hemicellulose structure into simpler sugars like xylose and arabinose when heated to moderate temperatures (150–180°C) and used in low amounts (1–5%) (Wolfaardt et al., 2021). As a result of this breakdown, the biomass becomes less structurally rigid, and cellulose is more amenable to enzymatic hydrolysis. Sulfuric acid helps dissolve lignin and break down hemicellulose, but not as effectively as stronger acids. Because lignin functions as a physical barrier that inhibits the efficient breakdown of cellulose, its removal is essential (Xia et al., 2020). However, in very harsh conditions, cellulose breaks down into smaller sugars or can create harmful substances like furfural and hydroxymethylfurfural (HMF), which is a downside of using sulfuric acid pretreatment (Kumar et al., 2020). These byproducts lower the total amount of biofuels produced because they make processes like fermentation and enzymatic hydrolysis less effective. Nevertheless, closely regulating important variables like temperature, acid concentration, and treatment duration can minimize these undesirable reactions and optimize the pretreatment procedure (Panakkal et al., 2022).

To maximize efficiency and reduce the production of unwanted byproducts, recent developments in sulfuric acid pretreatment have focused on improving reaction conditions. For instance, Wang et al., (2023) found that adding a gentle alkaline neutralization step after a two-step sulfuric acid pretreatment greatly improved the amount of sugars produced by enzymes and reduced harmful by-products. A gentle acid treatment (2% H_2SO_4) was applied in this study to dissolve hemicellulose, followed by a stronger treatment with a higher concentration (4% H_2SO_4) to break down lignin (Martins et al., 2022). By using a base to cancel out the leftover acid, this process lowered the creation of harmful byproducts and made it easier to access cellulose for hydrolysis (Olatunji et al., 2021).

Another recent study by Nordin et al., (2022) looked into the use of steam explosion in conjunction with low-concentration sulfuric acid pretreatment (1–2%). This combination made it easier for enzymes to break down cellulose by effectively getting rid of a large portion of hemicellulose and changing the structure of lignin. (Sun et al., 2020). According to the study, regulating the pH of the biomass slurry after treatment could improve sugar recovery overall and lessen cellulose loss or degradation (Talaiekhozani & Rezania, 2020). Researchers have further investigated the use of more concentrated sulfuric acid in sub- or supercritical conditions, providing a more efficient method of breaking down biomass. In their research on using sulfuric acid at very high temperatures and pressures, (Leng et al., 2021) discovered that sulfuric acid worked better as a catalyst, leading to more effective removal of lignin and greater amounts of fermentable sugars. Although the process is energy-intensive, it has the potential to increase biomass conversion efficiency on a larger scale; however, the cost of energy and specialized equipment is still a significant obstacle (Lee & Yu, 2020).

The main advantage of using sulfuric acid for pretreatment is that it can partially remove lignin from biomass and dissolve hemicellulose, making it much easier for enzymes to break down cellulose (Baksi et al., 2023). When compared to other chemicals, sulfuric acid's reasonable pricing makes it a desirable choice for large-scale biomass pretreatment. Sulfuric acid's easy recovery and reuse eliminates the need for large amounts of fresh acid (Sun et al., 2021).

Sulfuric acid use, however, comes with several difficulties. Furfural and HMF are harmful by-products that cause big issues because they stop fermentation and enzymatic hydrolysis (Tan et al., 2021). It is still challenging to strike the ideal balance between effective biomass deconstruction and low by-product formation, even though mild acid pretreatment can reduce the production of these by-products. Furthermore, sulfuric acid pretreatment raises operating costs due to the high energy inputs needed, particularly when using high temperatures (Hoang et al., 2023).

To tackle these challenges, scientists have explored mixing sulfuric acid pretreatment with other methods, such as enzymatic treatments or different solvents, to make it work better and create fewer byproducts. For example, (Alawad & Ibrahim, 2024) combined microwave irradiation with sulfuric acid pretreatment, greatly lowering the process's time and energy needs while preserving high sugar yields. This combination made it possible to deconstruct biomass more effectively while having less of an adverse effect on the environment.

By addressing energy consumption, reducing by-products, and combining it with other pretreatment techniques, sulfuric acid pretreatment is expected to become more sustainable in the future (Islam et al., 2020). Additionally, scientists are exploring how acid-loving bacteria can help break down unwanted leftover materials, reducing their negative impact on later fermentation and hydrolysis processes. Additionally, there is growing interest in developing special pretreatment methods that adjust sulfuric acid conditions for specific materials, such as algae, forestry waste, and agricultural leftovers. These customized methods could maximize the conversion of different biomass types into biofuels and biochemicals and increase overall process efficiency (Kasinath et al., 2021).

2.3.1.2 Alkali Heat Pretreatment

One of the most studied chemical processes for converting lignocellulosic biomass is alkaline pretreatment. To break down the structure of lignocellulose and improve the cellulose's subsequent enzymatic digestibility, alkaline chemicals like sodium hydroxide (NaOH), potassium hydroxide (KOH), or ammonia (NH_3) are primarily used (Mankar et al., 2021). The main goals of alkaline pretreatment are to reduce the lignin content and change the structure of hemicellulose, making it easier for enzymes to break down cellulose. (Yuan et al., 2021). The process has drawn a lot of attention because it works well, has less of an impact on the environment than other approaches, and uses widely accessible and reasonably priced chemicals (Rasid et al., 2021). Alkaline pretreatment is also easily combined with other techniques to increase the efficiency of biomass conversion.

Alkaline pretreatment works by partially removing lignin and deacetylating and depolymerizing hemicellulose. Hemicellulose dissolves, and the biomass loses some lignin because the hydroxide ions in alkaline solutions break the bonds that connect lignin and hemicellulose (Azelee et al., 2023). Because lignin serves as a physical barrier that shields cellulose from enzymatic attack, its removal is especially crucial. Alkaline pretreatment makes it easier for enzymes to break down cellulose by removing or changing the lignin structure. Additionally, the alkaline conditions decrease cellulose's degree of crystallinity, making enzymatic hydrolysis even easier (Yuan et al., 2021). The goal of recent alkaline pretreatment research has been to optimize the procedure to increase productivity while lowering operating expenses. Scientists have looked into pretreating different kinds of lignocellulosic biomass, such as agricultural residues, with sodium hydroxide (NaOH). Studies have shown that using NaOH to pretreat lignocellulosic biomass effectively breaks down hemicellulose and reduces lignin content (Jung et al., 2020). To get the best results, it has been essential to optimize variables like temperature, treatment duration, and NaOH concentration. Research shows that the best breakdown of biomass with the least sugar loss happened at temperatures between 120 and 160°C using an NaOH concentration of 3-5 (Sharma et al., 2023).

Combining alkaline pretreatment with other techniques to increase its efficacy is one intriguing research topic. When combined with alkaline chemicals, ultrasonic or microwave treatments greatly shorten treatment times and increase biomass deconstruction's overall effectiveness (Yan et al., 2021). Researchers discovered that using microwaves or ultrasonic waves during alkaline pretreatment helped remove lignin and made cellulose less organized, which made it easier to access the sugars for fermentation (Shabbirahmed et al., 2023). Researchers have also been looking into using ammonia (NH_3) for alkaline pretreatment instead of sodium hydroxide. In terms of lignin removal and cellulose accessibility, ammonia pretreatment has demonstrated comparable or superior outcomes to NaOH (Zhao et al., 2020). Ammonia is less corrosive than sodium hydroxide, which makes it easier to handle and less taxing on equipment. This property is the main benefit of ammonia-based pretreatment. Also, ammonia pretreatment lowers the creation of harmful byproducts by creating ammonium lignin complexes, which are easier to remove in the next processing steps (Novia et al., 2022). Additionally, recent research has demonstrated that low-concentration alkaline solutions (less than 2% NaOH) can be used to accomplish efficient pretreatment while using fewer chemicals. In comparison to conventional alkaline pretreatment, researchers were able to achieve excellent enzymatic digestibility with significantly lower chemical costs by optimizing the treatment time and temperature (Olatunji et al., 2021). This method maintains high biomass conversion efficiency while reducing the process's environmental impact by using fewer chemicals.

Alkaline pretreatment has several benefits, such as being environmentally friendly, reasonably priced, and utilizing readily available chemicals like sodium hydroxide. In addition, it produces fewer harmful byproducts than more forceful pretreatment techniques like acid hydrolysis (Olatunji et al., 2021). The process's capacity to recover and repurpose alkaline solutions, such as sodium hydroxide, further enhances its sustainability. Alkaline pretreatment is also a more energy-efficient choice than high-temperature procedures like steam explosion or supercritical treatments because it requires mild operating conditions (Nunes & Borges, 2021).

However, alkaline pretreatment poses certain challenges that require resolution. One of the primary problems is that, in comparison to acid pretreatment techniques, longer pretreatment times are required, particularly when using lower alkaline solution concentrations. Energy use and operating expenses may rise as a result (Atelge et al., 2020). The overall quantity of biofuels produced can go down because a lot of valuable sugars are lost when hemicellulose and lignin dissolve too easily in alkaline solutions. Also, while alkaline pretreatment is good at removing lignin, it might not work as well as other methods like organosolv or oxidative pretreatment in completely breaking down the lignin structure.. (Meng et al., 2020). Sodium hydroxide can be recycled, but the presence of dissolved organic compounds and the formation of lignin degradation products can make the recycling process more difficult. Recovery of the alkaline chemicals following pretreatment is another challenge (Rashid et al., 2021). To increase the effectiveness of chemical recovery and reduce environmental impact, researchers are investigating several tactics, including solvent recovery and purification techniques (Ait-Touchente et al., 2024).

Alkaline pretreatment's future depends on increasing its effectiveness and lessening its negative effects on the environment. Combining alkaline pretreatment with physical techniques like microwave or ultrasonication offers significant potential for process improvement. Combining these techniques could improve biomass conversion rates while drastically cutting down on treatment time and energy use (Rashid et al., 2021). Researchers are also looking into biomass-specific pretreatment methods that change the alkaline conditions to fit different types of biomass, like algae, forestry waste, and agricultural waste (Ezealigo, 2022). For instance, research has shown that different biomass types have distinct ideal ammonia pretreatment conditions. Tailoring the procedure to particular feedstocks may result in increased yields and reduced chemical usage (Zanuso Jiménez, 2022).

The creation of ecologically friendly alkaline pretreatment methods is another area of emphasis. To make breaking down biomass easier and lessen the harm caused by chemicals, researchers are looking into using eco-friendly solvents like ionic liquids or deep eutectic solvents along with alkaline pretreatment (da Costa Lopes, 2021). Furthermore, alkaline pretreatment may become more economical and sustainable over time due to developments in energy recovery systems and process integration (Khoshnevisan et al., 2022).

2.3.1.3 Organosolv Pretreatment

Organosolv pretreatment is an important method for making biofuels and biochemicals because it has shown to be very effective at turning lignocellulosic biomass into sugars that can be fermented (Wei Kit Chin et al., 2020). Even though organosolv pretreatment has existed since the early 1900s, more people are becoming interested in it as a better option than traditional acid and alkali methods because it now understood how it works and how to improve the process. (Aggarwal et al., 2021). The process mainly uses organic solvents like methanol, acetone, bioethanol, and other similar solvents, along with an acid or base catalyst, to break down the lignocellulosic structure, especially the lignin part, while causing minimal damage to cellulose and hemicellulose (Rabelo et al., 2023).

The organosolv pretreatment's ability to dissolve lignin which is a complicated substance that helps plants stay strong and crucial for it to work well. Since it keeps enzymes from reaching the cellulose fibers, lignin is recognized as the first obstacle to the enzymatic hydrolysis of cellulose (Shu et al., 2021). The organosolv pretreatment helps produce more fermentable sugars by breaking down lignin, making it easier for enzymes to access and digest cellulose. By breaking down the lignin-carbohydrate complex, organic solvents help solubilize lignin and facilitate its extraction from biomass (Malinská et al., 2021).

To guarantee complete solvent penetration and lignin solubilization, the solubilization usually takes place at temperatures between 160°C and 220°C and moderate pressure. Pretreatment maintains the sugar content of the cellulose and hemicellulose fractions, facilitating their eventual enzymatic hydrolysis. So, organosolv pretreatment makes the surface of cellulose more accessible, which helps enzymes break down polysaccharides into sugars that can be fermented (Islam, 2021).

Organosolv pretreatment can specifically dissolve lignin, making it easier to recover both cellulose and hemicellulose, which is one of its biggest advantages. Organosolv pretreatment helps keep cellulose and hemicellulose from breaking down too quickly, unlike traditional acidic or alkaline methods that usually cause these sugars to break down (Ibrahim & Kruse, 2020). Also, organosolv processes are cheaper and better for the environment than other chemical pretreatment methods that use harmful acids or bases, because the organic solvents they use can often be recovered and reused. Additionally, this process has a safer environmental impact because organic solvents, like bioethanol, are comparatively less toxic than other solvents (Broda et al., 2022).

You can change the pretreatment process for different materials by adjusting factors like the type of solvent, temperature, pressure, and catalyst concentration, which is another benefit of organosolv pretreatment. For instance, research has found that using a mix of bioethanol and acetic acid as a solvent makes it easier to break down corn stover and results in high sugar levels, which are important for making biofuel effectively (Khan et al., 2021). Because of its versatility, organosolv pretreatment can be used on various lignocellulosic materials, including forestry biomass and agricultural residues. Despite its benefits, organosolv pretreatment has a number of drawbacks that prevent widespread use. The high price of organic solvents, which can account for a sizeable amount of total operating expenses, is one of the main obstacles (Sridevi et al., 2022). These solvents require a significant amount of energy to produce and recover, especially at high temperatures and pressures. Additionally, improper handling of solvent disposal and recycling typically results in further environmental issues (Rabelo et al., 2023).

To reduce these difficulties, researchers have focused on improving the solvent system and the process conditions to reduce expenses and raise the pretreatment's overall effectiveness. One strategy to lower solvent costs and energy consumption is to combine mild acids or bases with less expensive solvents, like bioethanol (Wei Kit Chin et al., 2020). Additionally, researchers are striving to develop more efficient solvent recovery systems that can significantly reduce the need for new solvents and enhance the sustainability of the process. Some studies suggest that developing new catalysts or co-solvents can make the lignin solubilization process work better and more precisely, while also reducing the overall energy and chemical use (Chin et al., 2021).

Recent studies have further investigated the optimization of organosolv pretreatment parameters to increase biomass conversion rates and lower related costs. For example, research indicated that ionic liquids combined with ethanol solvent could improve the delignification process and make cellulose more accessible for hydrolysis (Taokaew & Kriangkrai, 2022). Also, it has been shown that using a two-step pretreatment process, where the biomass is first gently treated with organosolv and then goes through a second step with enzymes, can increase the amount of sugar produced.(Amini et al., 2021).

Non-toxic, eco-friendly solvents are also employed to increase the sustainability of the organosolv process. Particularly, ionic liquids have drawn a lot of interest because they are recyclable, less volatile than conventional organic solvents, and effectively dissolve lignocellulosic biomass (Khoo et al., 2021). Organosolv pretreatment allows lignocellulosic biomass to be effectively turned into useful biofuels and biochemicals.

Organosolv pretreatment helps break down biomass effectively because it can dissolve lignin while keeping the cellulose intact (Duval et al., 2021). Solvent recovery, energy consumption, and cost issues continue to occur, though. The goals of ongoing research are to improve the sustainability of organosolv pretreatment, lower operating expenses, and optimize process conditions. Organosolv pretreatment may play a crucial role in the bioeconomy of the future if these issues are resolved (Sidiras et al., 2022).

2.3.1.4 Ozonolysis Pretreatment

A newer method for preparing lignocellulosic biomass is ozonolysis, which uses ozone (O_3) to help break it down (Ibrahim, 2022). The approach is based on the idea that ozone can specifically break down lignin, which is the toughest part of lignocellulose, making it easier for enzymes to break down the biomass. Because of its relatively mild reaction conditions and low production of inhibitory by-products, ozonolysis has gained a lot of attention recently as an environmentally friendly and energy-efficient method for pretreatment of biomass (Rasid et al., 2021). The method breaks apart the aromatic ring structure of lignin using a specific type of oxidation, creating small, more soluble lignin pieces that are easier to wash away later (Basak et al., 2023).

One of the most important benefits of ozonolysis is its ability to specifically target lignin without significantly harming the biomass's cellulose and hemicellulose components (Muazzam et al., 2021). To improve biomass conversion, lignin must be selectively broken down to increase cellulose's accessibility to enzymes. Additionally, compared to high-temperature chemical pretreatment techniques like acid or alkali treatments, ozone uses less energy because it is a powerful oxidizing agent that works in mild conditions (low temperature and atmospheric pressure). Because of this, ozonolysis may be a sustainable and affordable option for pretreatment of biomass, especially in the production of biofuel on a large scale (Figueirêdo, 2020). When compared to other pretreatment techniques, ozonolysis produces fewer harmful byproducts, which is another significant advantage. The process does not produce compounds like furfural and HMF, known to inhibit fermentation processes. Because it eliminates the need for expensive detoxification procedures, ozonolysis is especially appealing for downstream applications (Derco et al., 2021). Furthermore, ozone is a sustainable and environmentally friendly oxidizing agent because it can be produced from oxygen.

Ozonolysis has drawbacks despite these benefits. The comparatively high cost of ozone acquisition and generation is one of the process's main drawbacks. Electrical energy is used to produce ozone, which can be costly, especially if it is upgraded (Priyadarshini et al., 2022). Additionally, it can be difficult to manage how well ozone is used in the pretreatment process because it breaks down quickly and reacts easily (Premjit et al., 2022). By adjusting reaction parameters like reaction time, ozone concentration, and biomass particle size, researchers have come up with strategies to increase the effectiveness of ozone utilization (Zhang et al., 2022). Additionally, the procedure usually calls for an extra washing step to get rid of dissolved lignin fragments, which can raise labor and water expenses overall (Rahmati et al., 2020). To increase the overall biomass conversion efficiency, recent developments have concentrated on refining the ozonolysis process in conjunction with other pretreatment methods such as steam explosion or enzymatic hydrolysis. For example, Ibrahim, (2022) demonstrated that ozonolysis successfully got rid of lignin while keeping the cellulose structure intact, which significantly boosted glucose production from wheat straw when used with a gentle alkali treatment. This combined approach can reduce some of the technical and cost challenges of the process while still benefiting from ozonolysis's ability to break down lignin selectively (Montet, 2021).

Additionally, there have been initiatives to create more effective ozone generation systems, like using ozone generators with higher energy efficiency, as well as developments in ozone application technologies. It is believed that these advancements will lower ozonolysis's total cost, making it a more cost-effective choice for extensive industrial uses (Rekhate & Srivastava, 2020).

2.3.1.5 Ionic Liquids Pretreatment

As a promising alternative for pretreating lignocellulosic biomass, ionic liquids (ILs) have garnered a lot of interest lately as a novel and sustainable way to increase biomass conversion (Haykir et al., 2023). Since the early 2000s, using ionic liquids for preparing biomass has become more popular, with researchers looking at different ILs to see how well they can dissolve lignocellulose parts, especially lignin, while keeping the cellulose structure intact. ILs are very good at breaking down plant materials because they have special features like low vapor pressure, high thermal stability, and the ability to both dissolve lignin and free cellulose (Hasanov et al., 2020). The rare properties of ILs, for instance low vapor pressure, high thermal stability, and the ability to dissolve both lignin and cellulose, make them significantly effective for breaking down lignocellulosic materials (Eqbalpour et al., 2023). According to Usmani et al. (2020), ionic liquids are salts that are liquid at low temperatures and frequently have unusual chemical characteristics based on their anion and cation composition. These salts can be specifically tailored to fit various biomass types (Usmani et al., 2020)

To enable the solvation of lignin, hemicellulose, and other constituents, ILs work by breaking the strong hydrogen bonds and van der Waals interactions that exist between the cellulose fibers. This disruption breaks down the complex lignocellulosic structure, which otherwise prevents enzymatic hydrolysis (Annamraju, 2021). To guarantee that the cellulose component is accessible for enzymatic digestion, ILs discretely dissolve lignin, leaving the cellulose component largely intact. One of ILs' main advantages is their ability to preserve cellulose stability, unlike other pretreatment techniques that typically cause cellulose to degrade (Haron et al., 2021).

One of the main advantages of using ILs for biomass pretreatment is their recyclable nature, which allows for repeated use. Because ILs are dense and do not evaporate, additional solvent recovery techniques like distillation are not necessary for their reuse (Nguyen, 2020). High-end applications prefer ILs due to their recyclable nature, which lowers the overall cost and environmental impact of the process. Additionally, to make biomass conversion more effective, ILs are usually combined with other methods like steam explosion or enzymatic hydrolysis. Because of their adaptability, ILs can be tailored to suit various biomass feedstocks and processing circumstances (Mankar et al., 2021). Nevertheless, there are still obstacles to the commercial use of ILs in spite of these benefits. Their high price in comparison to other common solvents like sodium hydroxide or sulfuric acid is one of the main issues (Zhou et al., 2023). Ionic liquids' economic viability is currently limited by the expensive and energy-intensive processes required for their production, particularly in large-scale industrial settings (Haider et al., 2022). Additionally, while ionic liquids dissolve lignin very well, they can create byproducts that make it harder for fermentation or enzymatic hydrolysis to happen. To reduce these inhibitory effects, it is crucial to optimize the process conditions

(Radhakrishnan et al., 2021). Current research has focused on finding more economical and ecologically friendly ways to enhance the qualities of ILs. For example, researchers are looking into new ionic liquids that have low environmental toxicity or are derived from bio-based sources (Cho et al., 2021).

To make it easier to break down biomass and turn it into biofuels, researchers have looked into "task-specific" ionic liquids that mix solvents with chemical or catalytic properties (Ong et al., 2021). A promising path toward expanding the widespread use of ILs in biomass pretreatment is the creation of these novel, more reasonably priced ionic liquids. Studies have also shown that modifying important input parameters like temperature, time, and concentration can improve the effectiveness of ILs in pretreatment. For example, Usmani et al., (2020) found that mixing 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) with water greatly boosted the amount of glucose made from hardwood because the IL helped break down lignocellulose and reduced the breakdown of cellulose. Furthermore, the lignin's selective solubilization increased the enzymatic efficiency of the following hydrolysis stages. As a result, ILs are becoming more and more regarded as a viable pretreatment option, provided that the financial obstacles are removed (Han et al., 2020).

2.3.1.6 Challenges Associated with Ionic Liquids Pretreatment

Ionic liquids are expensive, toxic, and cannot be recovered once they are used. Due to this difficulty, researchers are turning to more affordable and sustainable alternatives. Because of these difficulties, alternative solutions are being looked for to guarantee that the procedures can be advanced to commercial levels (Flieger & Flieger, 2020). Since enzymatic hydrolysis and pretreatment take a long time to produce bioethanol from lignocellulosic biomass, it's preferable for the pretreatment chemicals to be cheaper and reusable (Huang et al., 2021). Recyclability issues and reagent acquisition costs have been major roadblocks to scaling up the use of ionic liquids in the pretreatment of lignocellulosic biomass for bioethanol production (Ethaib et al., 2020). These methods, which address the economic and environmental issues caused by fossil fuels, are primarily laboratory-based. Furthermore, the relatively high density of ionic liquids is a persistent disadvantage that restricts the processes meant to increase the production of bioethanol. The use of ionic liquids is often increased to overcome all of these obstacles (Rodríguez, 2021).

Ionic liquids currently have a density of 1.6 g/cm^{-3}, which is greater than that of water. Only when the alkyl chain lengthens does this density systematically decrease. Ionic liquids typically have viscosities comparable to those of oil (Prietzel et al., 2020). Because it takes more energy to mix the reagent with the biomass, this type of viscosity has a detrimental effect on power transfer and the reagent's overall penetration into the biomass. Additionally, halides, water, and volatiles are impurities found in ionic liquids, particularly during synthesis (Doblinger et al., 2020).

2.3.1.6 Deep Eutectic Solvents

A big step forward in making biomass conversion processes more sustainable and efficient is the use of Deep Eutectic Solvents (DES) as a pretreatment technology (Malolan et al., 2021). DES, which consists of natural and renewable materials, has several advantages over traditional solvents, like being less toxic, biodegradable, and able to dissolve lignocellulosic biomass under milder conditions (da Costa Lopes, 2021). Because of these features, DES is particularly suitable for countries looking to develop their bioenergy industries in an environmentally friendly way (Sekharan et al., 2022). Deep eutectic solvents (DES), a new type of reagent, have lower melting points than regular components. They are affordable, specially designed materials that are good for the environment and useful for pretreatment applications (Hansen et al., 2020).

Deep eutectic solvents must be non-toxic, recyclable, biodegradable, flammable, and reasonably priced, among other requirements. There are currently only a few of these solvents available. Deep eutectic solvents are typically inexpensive mixtures of two or three components. These constituents form a eutectic mixture with a lower melting point through hydrogen bond interactions and self-association (Cotroneo-Figueroa et al., 2022).

To improve biomass conversion processes, it's important to understand how different pretreatment conditions and DES characteristics work together. This knowledge is particularly vital in developing countries like Zambia, where creating a sustainable biofuel industry could significantly enhance economic growth and energy security (Atilhan & Aparicio, 2021). Key factors that influence how quickly and effectively cellulose breaks down include residence time, which is how long the biomass is treated, and reaction temperature, which affects how fast the reactions happen (Wong et al., 2023). Choline chloride and glycerol have been used to prepare biomass by breaking down the tough lignin layer, allowing the cellulose to be accessed for enzyme hydrolysis (Quraishi et al., 2024). The synthesis of DES and the pretreatment operating conditions are crucial. The solid-to-solvent ratio, temperature range, and DES reaction time are the factors that contribute to a successful pretreatment (Kovács et al., 2022).

2.3.2 Physical Pretreatment Methods

To improve the digestibility of lignocellulosic biomass, physical pretreatment methods employ mechanical, thermal, or pressure-based techniques to alter its structure. These methods are important for breaking apart the complex structure of cellulose, hemicellulose, and lignin, making it easier for enzymes to work on cellulose (Mankar et al., 2021). The type of biomass, the amount of moisture present, and the intensity of the mechanical or thermal forces used all effect how effective physical pretreatment is. Although these techniques can greatly increase the effectiveness of ensuing biochemical reactions, their large-scale application is difficult due to their frequent high energy requirements (Areepak et al., 2022). One of the main goals of physical pretreatment is to break down the tough lignin layer that blocks enzymes from reaching cellulose. Additionally, lignin adsorbs enzymes, decreasing their capacity to hydrolyze cellulose into sugars that can be fermented(Mohammad et al., 2020). Physical pretreatment makes it easier to break down biomass and boosts bioethanol production by reducing the tightness of cellulose and increasing the area that enzymes can work on. But prolonged exposure to high temperatures or excessive mechanical force can cause unwanted changes like sugar degradation and the formation of inhibitory compounds, which can harm the efficiency of bioethanol production (Pérez-Merchán et al., 2022).

Mechanical extrusion, pyrolysis, ball, hammer, and disk milling, as well as microwave radiation, are the main physical pretreatment techniques. These methods' modes of operation, energy needs, and impacts on biomass structure vary (Duque García et al., 2023). For example, milling efficiently breaks down cellulose crystallinity and reduces particle size, but it requires a lot of energy, which makes it less practical for large-scale industrial applications (Sitotaw et al., 2023). However, the high upfront costs of microwave-assisted pretreatment make it hard to quickly break down structures and improve access for enzymes (Cheah et al., 2020).

Physical pretreatment techniques are still essential to the production of bioethanol in spite of these drawbacks, especially when paired with chemical or biological pretreatments to increase productivity and lower energy usage. To ensure sustainable and economical bioethanol production, emerging hybrid pretreatment strategies seek to maximize the advantages of physical methods while minimizing their disadvantages (Periyasamy et al., 2022). Improving the effectiveness of physical pretreatment methods is important for encouraging large-scale production of lignocellulosic bioethanol, especially with the world's growing interest in renewable energy.

2.3.2.1 Microwave Radiation Pretreatment

The microwave radiation pretreatment is highly acceptable because it extracts fermentable sugars from the lignin shell more quickly and efficiently. Volumetric and dielectric heating produce high yields of fermentable sugars (Fernandes et al., 2023). Because of the shorter duration of contact, the process is environmentally benign and causes minimal material degradation on all sides. This process produces high yields due to minimal energy loss in the material. Nevertheless, the microwave pretreatment creates standing waves known as resonance that cause specific overheating spots because it is not uniformly applied across the material's surface. Penetration of bulk products is another difficulty (Hazeena et al., 2024).

Microwave pretreatment uses an electromagnetic wave with electric and magnetic fields. The 300 MHz and 300 GHz frequency bands are where the waves operate. The dielectric constant, shape, size, and the microwave equipment used for heating determine the 2450 MHz domestic microwave range (Romero-Zúñiga et al., 2022). A microwave's efficiency in heat transfer and shorter retention time are its advantages. Power distribution issues, uneven feedstock heating, and inefficiency with bulk materials continue to be problems (Ramos et al., 2022). Effective biomass pretreatment is a key factor that affects sufficient bioethanol yields. In this instance, sodium cumene sulfonate (NaCS) hydrotrope was used to pretreat wheat stillage to produce second-generation bioethanol. Using NaCS and microwave-assisted pretreatment, the cellulose structure was mainly preserved, while the amounts of lignin and hemicellulose were reduced. The procedure produced higher yields by converting the material completely into bioethanol in 48 hours (Kłosowski et al., 2022).

2.3.2.2 Mechanical Extrusion Pretreatment.

This approach has continued being used to pretreat lignocellulosic biomass. The method is highly adaptable and customized to fit specific setups, optimizing conditions for distinct extruder designs, biomass types, and enhancing additives. Extruders are designed according to parameters required for the identified biomass. They are adjustable in line with the size and type of biomass. The focus in mechanical extrusion methods is the energy consumption. The energy consumption in this type of pretreatment is on the higher side, resulting in increased energy costs (Konan et al., 2022).

There are a number of extruders used in the pretreatment of lignocellulosic biomass, but generally an extruder is a thermomechanical machine with two components, namely the barrel and a die. Figure 2.2 does exhibit the actual assembly of the extrusion machine. During operation, one or more liquids are injection paints. James Bramah designed and obtained the first patent in 1797. Since then, manufacturers have developed a variety of extruders for lignocellulosic biomass extrusion as a pretreatment process. The challenge remains that the design and manufacture of the equipment are costly. Below is a typical assembly of a clearly labeled extruder that is clearly self-explanatory in terms of cost inputs to the design (Konan et al., 2022).

It is one of the methods that is continuously being used in the pretreatment of lignocellulosic biomass. The method is highly adaptable and customized to fit specific setups, optimizing conditions for distinct extruder designs, biomass types, and enhancing additives. Extruders are designed according to parameters required for the identified biomass. They are adjustable in line with the size and type of biomass. The focus in mechanical extrusion methods is the energy consumption. The energy consumption in this type of pretreatment is on the higher side, resulting in increased energy costs

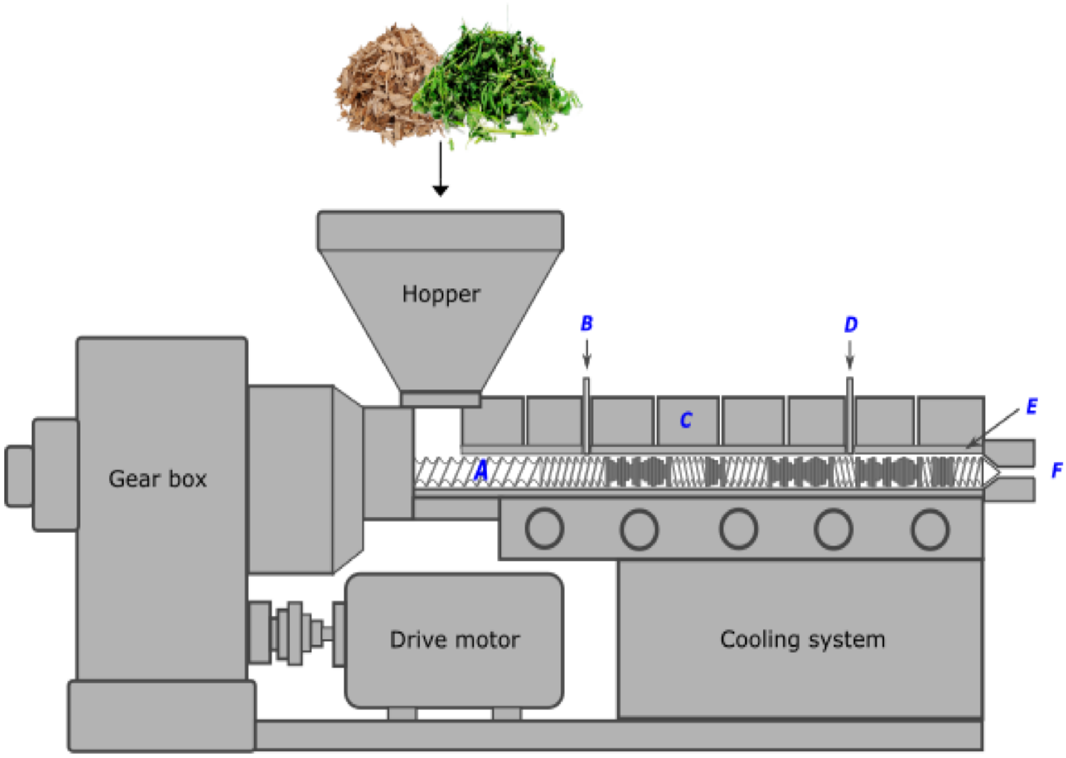

Figure 2. 2: Extruder pretreatment design (Source: Energies 2022)

Pretreatment is a crucial step in making bioethanol from lignocellulosic biomass because it helps break down the tough structure and allows for enzymatic hydrolysis, which turns carbohydrates into simpler sugars. Over the years, mechanical extrusion has become a common pretreatment technique. The capacity to crush and work with high solids is a benefit of mechanical extrusion. Additionally, it has excellent mixing, heat transfer, and versatility. Energy consumption and economic relevance continue to be the problem (Duque et al., 2017).

2.3.2.3 Pyrolysis Pretreatment

One technique for producing bio-oil is pyrolysis. Without the use of oxygen or any other agent, the procedure is carried out at temperatures between 500 and 800 degrees Celsius. There are two steps in the process: low and fast. Char and pyrolysis oil are produced as a result of the cellulose's quick breakdown during the pretreatment. The final product is influenced by various factors, such as biomass characterization and reaction parameters. Direct combustion and gasification are two examples of thermochemical processes that include pyrolysis (Aftab et al., 2019). Pyrolysis is primarily used to pretreat lignocellulose biomass to produce biochar and bio-oil. Most people use pyrolysis to produce biochar and bio-oils, with fewer reports of its use for reducing sugars. It should be clear to you, though, that 85% cellulose recovery from the lignin shell is a favorable yield whenever pyrolysis is utilized to produce bioethanol. Between 500 and 800 degrees Celsius, the temperature range stays high. The type of pyrolysis, reaction parameters, and biomass properties all affect the yields (Kumar & Sharma, 2017).

Pyrolysis is a thermal breakdown of biomass that is carried out without oxygen and is primarily used to produce biochar, bio-oil, and other gaseous products like syngas. It is considered a promising technology for biomass valorization, with yields of up to 78 weight percent and a brief retention period. The process is categorized as either slow pyrolysis or fast pyrolysis based on the heating rate. Slow pyrolysis yields solid biochar, whereas fast pyrolysis yields bio-oils in a few hours (Zadeh et al., 2020).

2.3.2.4 Pulse Electric Field

The process of turning lignocellulosic biomass into biofuel is frequently discussed in contemporary literature. New methods for preparing lignocellulosic biomass for biofuel involve using high voltage electrical discharges (HVED), such as pulse electric fields (PEF) or pulsed electric energy (PEE) (Vorobiev & Lebovka, 2017). By forming pores in the cell membrane, PEF pretreatment exposes the cellulose in biomass. Agents are able to break down cellulose into certain sugar constituents through this process. This technology exposes biomass to a random bursting high voltage at a tiny frequency (100 µs) pulse of time ranging from 5.0 to 20 KV/cm for a brief period of time (nanoseconds to milliseconds (Kumar & Sharma, 2017).

Pulsed electric field pretreatment was used to examine the structure of the corncob. The gravimetric method was used to characterize the feedstock structure, and SEM-EDX was used to control the pretreated samples. With a 60-second retention period, the field strength was 9 kV/cm. While lignin and hemicellulose were 2.02% and 12.9%, respectively, the recovered cellulose was 40.59%. The PEF-specific input energy and energy/pulse during the tests were 8.72 KJ/L and 0.0205 J, respectively (Putranto et al., 2021).

2.3.3 Physiochemical Pretreatment Method

To increase the digestibility of lignocellulosic biomass—a prerequisite for the effective production of biofuel—physicochemical pretreatment techniques are essential. These techniques make it easier for enzymes to break down cellulose by breaking apart the tough structure of lignocellulose using both physical and chemical methods. Many research methods have been developed and improved over the years, including wet oxidation, liquid hot water (LHW), steam explosion, ammonia fiber explosion (AFEX), and SPORL (sulfuric acid pre-treatment with overlining). These pretreatment procedures and techniques aim to reduce biomass recalcitrance by removing or altering hemicellulose and lignin, the primary obstacles to effective biomass conversion. Understanding the mechanisms and improvements of these methods has been greatly aided by recent research, which has provided better routes for turning lignocellulosic biomass into useful chemicals and biofuels.

AFEX is a common method that prepares lignocellulosic biomass by using high-pressure ammonia and then quickly releasing the pressure. By successfully upsetting the structure of the biomass, this technique reduces the amount of lignin and increases the accessibility of cellulose. AFEX has demonstrated exceptional efficacy in enhancing the enzymatic digestibility of various feedstocks, such as wheat straw. To increase sugar yields and decrease the production of inhibitory byproducts, recent research has concentrated on optimizing the ammonia concentration and treatment duration. Zhang et al., (2021) studied how varying ammonia levels in the AFEX pretreatment could improve the release of fermentable sugars from biomass, particularly wheat straw, while minimizing the creation of harmful byproducts, which would make the biofuel production process more efficient (Yankov, 2022). One well-known physicochemical pretreatment is steam explosion, which entails exposing biomass to high-pressure steam and then quickly decompressing it. This process breaks down hemicellulose and reduces lignin in the material, causing the biomass to explode and making cellulose easier to access for enzymatic hydrolysis. Steam explosion processes a variety of feedstocks, including softwood and agricultural waste. In their review of recent advancements in steam explosion. (Liu et al., 2022) highlighted that changing factors like temperature and pressure can improve the effectiveness of enzymatic hydrolysis and reduce the creation of substances that hinder fermentation. Their research made clear how crucial it is to regulate these variables to maximize the process for various biomass types (Zhang et al., 2022).

One efficient pretreatment method that eliminates hemicellulose and partially depolymerizes lignin without the use of chemicals is LHW, which uses water heated to high temperatures (160°C to 240°C) under high pressure. Since LHW pretreatment doesn't involve any chemical applications, it is particularly appealing due to its environmental friendliness. (Zhang et al., 2022) looked into how to improve LHW pretreatment for corn stover and found that changing factors like temperature and reaction time significantly increased how well enzymes could break it down. The study found that LHW treatment is a good method for making biofuel because it can effectively convert a large amount of hemicellulose into sugars that can be fermented, while keeping the cellulose intact (Zhang et al., 2022). The process of wet oxidation involves treating biomass with oxygen or air at high temperatures and pressures, which partially oxidizes the lignin and hemicellulose. This pretreatment method makes it easier to break down lignocellulosic biomass by reducing its resistance, allowing enzymes to access the cellulose more easily. Recently, (Sun et al., 2022) looked at using wet oxidation on rice straw and found that it significantly reduced the lignin content and made the cellulose easier to break down for enzymatic hydrolysis. Compared to other techniques like steam explosion, they discovered that wet oxidation increases sugar yield and decreases the formation of fermentation inhibitors (Wu et al., 2020).

In the process called SPORL, lignocellulosic biomass is first treated with a weak solution of sulfuric acid to break down hemicellulose. Then, lime is added to balance the acidity and get rid of lignin. Lime is then added as an overlining step to neutralize the acidity and remove lignin. By making various lignocellulosic feedstocks, including hardwoods, more digestible, this method has demonstrated encouraging outcomes. The effectiveness of SPORL on eucalyptus wood was studied by Islam et al., (2021), who found that the process made fermentation and enzyme breakdown work better. Their research showed that by improving sugar production and lowering negative effects, SPORL could be very useful for enhancing the overall efficiency of turning biomass into energy (Yankov, 2022).

2.4 Filtration

In the biorefinery process, filtration is a crucial step, particularly when converting lignocellulosic biomass to produce biofuel. Numerous pretreatment procedures, like acid hydrolysis or SPORL, produce various byproducts, including inorganic substances like calcium sulfate dihydrate (gypsum). Lime or other alkaline agents normally neutralize sulfuric acid to produce gypsum, as is often the case in certain pretreatment methods. Although useful in other applications (like building materials), this byproduct must be eliminated from the biomass slurry to prevent it from interfering with enzymatic hydrolysis and other downstream processes. These insoluble contaminants must be eliminated by filtration in order for only the biomass—free of any solid particles—to move on to the following phases of the conversion process. This step guarantees that enzymes can efficiently access the cellulose for hydrolysis and helps to avoid the possible clogging of enzymatic reactors. Additionally, eliminating gypsum can lessen the possibility of enzyme inhibition, which is essential for enhancing the conversion process's overall effectiveness.

In the SPORL process, filtration is crucial for separating gypsum from the biomass slurry, according to a study by Martău et al.,(2024). By guaranteeing that the biomass slurry is free of gypsum and other undesirable particles, the study indicated that appropriate filtration techniques do considerably increase the efficiency of the enzymatic hydrolysis process. Gypsum needs to be removed because it can harm how well the enzymes work and lower the amount of sugar produced during hydrolysis (Jiang et al., 2023).

Filtration is also crucial for preserving the pretreatment liquor's consistency. Filtration helps recover valuable chemicals like sulfuric acid and lime, which can be recycled in later processing stages, in addition to removing byproducts like gypsum (Wang & Lee, 2021). Reagent costs are decreased by this recycling, which also increases the process's sustainability and economic viability. The study (Zhao, Li, et al., 2020) suggests that better filtration methods could lessen the environmental harm of the pretreatment process and boost the amount of fermentable sugars produced

Additionally, filtration is essential for raising the general operational effectiveness of biorefinery processes. A 2023 study by Lobato-Rodríguez et al., investigated how biomass processing in biorefineries could be enhanced by sophisticated filtration systems like membrane filtration or pressure filtration. Their study highlighted how crucial it is to choose the right filtration techniques based on the particular pretreatment procedure and the biomass feedstock By effectively getting rid of small gypsum particles from the biomass mixture, they were able to lessen the chances of problems during fermentation and offer cleaner material for hydrolysis.(Lobato-Rodríguez et al., 2023). Furthermore, the development of innovative filtration materials has enabled more effective filtration procedures. For instance, a study in 2024 by Janakiraman and others examined how well nano-porous filtration membranes could remove tiny particles from biomass slurry after it was pretreated. High throughput is essential for the large-scale production of biofuel, and these sophisticated filtration technologies have proven to be very successful in eliminating gypsum and other fine particles. The study showed that by using less energy in the filtration process, these new technologies could significantly reduce costs and make the production of lignocellulosic biofuel more sustainable (Janakiraman et al., 2024).

Filtration is therefore an essential step that guarantees the effectiveness of subsequent procedures in the conversion of biomass into biofuels. Filtration helps the processes of breaking down biomass and making biofuels by removing gypsum and other solid waste, which increases the overall amount of biofuel produced. Additionally, making lignocellulosic biorefineries more sustainable and cost-effective needs advanced filtration technologies and proper recycling of chemicals.

2.5 Enzymatic Hydrolysis Lignocellulosic Biomass

One of the most important processes in turning lignocellulosic biomass into biofuels and other biochemicals is enzymatic hydrolysis. Using specific enzymes, it is essential for converting cellulose and hemicellulose into fermentable sugars(Saini et al., 2022). Enzymatic hydrolysis has several benefits over chemical hydrolysis techniques, such as reduced energy needs, fewer undesirable byproducts, and increased specificity. However, obstacles like enzyme inhibition, biomass recalcitrance, and high enzyme costs continue to prevent its widespread commercialization (da Silva et al., 2020).

Lignocellulosic biomass consists of complicated parts of plant cell walls, such as cellulose, hemicellulose, and lignin, while first-generation biomass comes from food crops like corn and sugarcane, which have simple starches and sugars that are easy to ferment (Bilal & Iqbal, 2020). Because of its structural complexity, lignocellulosic biomass is extremely resistant to degradation and needs specific enzymatic hydrolysis in order to be broken down into sugars that can be fermented. Specifically, lignin forms a barrier that makes it hard for enzymes to reach and work effectively, which reduces how well the breakdown happens (Cai et al., 2023). Furthermore, the high crystallinity of cellulose in lignocellulosic biomass makes enzymatic breakdown even more difficult. Also complicated, the hemicellulose part requires specific enzymes because it is made up of different types of sugars, both pentoses and hexoses (Arzami et al., 2022). Enzymatic hydrolysis is crucial for turning lignocellulosic biomass into biofuels because it effectively breaks down plant fibers that would otherwise be unavailable for microbial fermentation (Lynd et al., 2022).

The increasing focus on second-generation biofuels derived from lignocellulosic feedstocks stems from the urgent need for sustainable alternatives to fossil fuels that do not compete with food production. Utilizing forestry waste, agricultural residues, and energy crops, lignocellulosic biofuels are a more economical and environmentally friendly alternative to first-generation biofuels, which have sparked worries about land use and food security (Ayodele et al., 2020). To effectively release sugar and produce biofuel, advanced hydrolysis methods are needed because lignocellulosic biomass is tough to break down. (Okolie, Mukherjee, et al., 2021). Furthermore, more resilient and effective enzyme systems that can tolerate industrial processing conditions have been developed as a result of recent developments in enzyme engineering. Techniques like protein engineering and directed evolution have created enzymes that work better, last longer, and can handle inhibitors more effectively (Mesbah, 2022). These advancements are important for making enzymatic hydrolysis more practical and competitive with traditional chemical methods. Additionally, there are promising opportunities to get more energy and create less waste by using enzymatic hydrolysis together with other biorefinery methods like anaerobic digestion and microbial fuel cell technology (Costa et al., 2022). The potential of enzymatic hydrolysis as a method for producing biofuel is boosted by new strategies for recycling enzymes, using fixed enzyme systems, and combining different microbes to make the process more efficient. (Federsel et al., 2021).

2.5.1 Types Of Enzymatic Hydrolysis

Numerous hydrolysis methods have been created to increase productivity and economy. These include cellulase-based hydrolysis, multi-enzyme methods, simultaneous enzymatic hydrolysis, and diluted acid hydrolysis (Sinitsyn & Sinitsyna, 2021). Cellulase-based hydrolysis stands out among these due to its increased industrial viability, enhanced specificity, and efficiency, which are results of improvements in enzyme engineering and optimization techniques (Dey et al., 2022). A detailed analysis of these techniques is given in the sections that follow.

2.5.2 Dilute Acid Hydrolysis With Enzymatic Hydrolysis

A popular pretreatment technique for lignocellulosic biomass is diluted acid hydrolysis, which aims to increase cellulose's accessibility for enzymatic action. This method effectively dissolves hemicellulose, breaks apart lignin, and reduces the crystallinity of cellulose by using mild acids like sulfuric or hydrochloric acid at controlled levels. By subjecting cellulose fibers to enzymatic attack, the procedure increases the overall effectiveness of enzymatic hydrolysis.(Manzanares et al., 2020). One of the biggest issues with dilute acid hydrolysis is that it creates harmful substances like furfural and hydroxymethylfurfural (HMF), which can interfere with fermentation and enzyme function (Tan et al., 2021). These inhibitors, which are byproducts of the breakdown of sugar, can disrupt microbial fermentation as well as enzyme activity. To lessen these inhibitory effects, techniques like biological detoxification, overlining, and activated carbon filtration have been developed (Ciampi et al., 2022). Alternative acid catalysts, like organic acids, have also been investigated recently to lower the production of inhibitory compounds while preserving high pretreatment efficiency.

Methods to make processes better, such as high-pressure hydrothermal treatments and acid recycling systems, are new ways to improve diluted acid hydrolysis, aiming to get more sugar and lower costs. To make large-scale processing better and more cost-effective, researchers are currently looking into using diluted acid hydrolysis along with continuous hydrolysis systems (Dutta et al., 2022).

2.5.3 Concurrent Enzymatic Hydrolysis

Alternative acid catalysts, like organic acids, have also been investigated recently to lower the production of inhibitory compounds while preserving high pretreatment efficiency.

Methods to make processes better, such as high-pressure hydrothermal treatments and acid recycling systems, are new ways to improve diluted acid hydrolysis, aiming to get more sugar and lower costs. To make large-scale processing better and more cost-effective, researchers are currently looking into using diluted acid hydrolysis along with continuous hydrolysis systems (Al-Mardeai et al., 2021). One of the main advantages of concurrent enzymatic hydrolysis is that it reduces the stopping effect caused by end products like glucose and cellobiose. Higher enzyme activity leads to better sugar conversion rates because the fermenting microbes rapidly use the sugars produced during hydrolysis(Chukwuma et al., 2020). Additionally, by reducing the number of reactors needed for biomass processing, SSF lowers operating costs and contamination risks.

Notwithstanding these benefits, SSF poses process optimization difficulties. Overall efficiency may be limited because enzymes and microbial fermenting agents frequently require different ideal conditions, such as pH and temperature. To solve this problem, scientists are working to create bacteria and yeast strains that are resistant to high temperatures and acidic conditions, which will allow for more effective SSF procedures (Chilakamarry et al., 2022). Recent research has shown promising results using altered strains of Zymomonas mobilis and Saccharomyces cerevisiae that can ferment both hexose and pentose sugars together, which boosts ethanol production. (Todhanakasem et al., 2020). Hybrid processing methods like consolidated bioprocessing (CBP) and simultaneous saccharification and co-fermentation (SSCF) are also examples of SSF advancements. By combining several processes, using less energy, and producing more bioethanol, these integrated approaches seek to increase efficiency (Joshi et al., 2021). The aim of future research is to improve SSF conditions by using methods that adapt microbes, design better enzymes, and create advanced bioreactors. (Londoño-Hernandez et al., 2020).

2.5.4 Multi-Enzyme Cocktails

To accomplish more effective biomass degradation, multi-enzyme cocktails combine different enzymes, including cellulases, hemicellulases, and accessory enzymes. This method can release more sugar because it allows for the breakdown of different lignocellulosic parts at the same time (Teixeira et al., 2021). The creation of customized enzyme formulations intended to target particular feedstocks has been the main focus of recent developments. By improving the way enzyme mixtures work together, less enzyme can be used and reduce production costs (Adsul et al., 2020). To increase long-term process stability and lower operating costs, immobilized enzyme systems in which enzymes are fixed onto solid supports for reuse are also being investigated (Intasian et al., 2021).

Enzyme cocktails offer notable benefits in hydrolysis efficiency by combining β-glucosidases, which facilitate rapid sugar release by converting cellobiose into glucose; endoglucanases, which break down amorphous cellulose regions; and exoglucanases, which extract individual glucose molecules from cellulose chains. Also, hemicellulases like xylanases and arabinosidases help break down hemicellulose, which makes it easier for cellulases to access cellulose (Monterrey et al., 2022). Oxidative enzymes like lytic polysaccharide monooxygenases (LPMOs), which increase cellulose accessibility by cleaving glycosidic bonds via oxidative mechanisms, are now included in advanced enzyme formulations. LPMOs are a valuable new tool for industrial hydrolysis processes because research indicates that using them with regular cellulase mixtures can boost hydrolysis efficiency by 30 to 50% (Karnaouri et al., 2022). Adding non-catalytic proteins like expansins helps speed up enzymatic hydrolysis by loosening cellulose fibers (Sánchez-Muñoz et al., 2022). To maximize synergy and minimize process costs, future research will concentrate on optimizing the enzyme ratios in cocktails (Pinheiro et al., 2021).

2.5.5 Cellulase-Based Hydrolysis

The most sophisticated and extensively researched enzymatic hydrolysis technique is cellulase-based hydrolysis. This method is the most effective and efficient enzymatic hydrolysis technique available because it only uses cellulases (Gan et al., 2024). Cellulase-based hydrolysis guarantees a clean, high-yield process that optimizes sugar recovery while lowering energy consumption and operating expenses, in contrast to other hydrolysis techniques that might produce inhibitors, call for extra detoxification procedures, or entail harsh pretreatments (Ranjan et al., 2023).

The process of breaking down substances has become much more effective because of new types of cellulases that can work well in different conditions, like high heat and changing acidity, while still remaining stable (Akram et al., 2024). The hydrolysis process is more efficient overall, and large-scale industrial applications are more feasible thanks to these thermostable cellulases' sustained catalytic activity (Ajeje et al., 2021). Putting cellulase onto solid supports has made the enzymes more stable and reusable, which extends their working life and greatly reduces production costs. (Sulman et al., 2022). Genetically engineered microbial strains, including recombinant fungi and bacteria, have been developed to overproduce cellulases at lower costs. Engineered strains of Aspergillus niger and Trichoderma reesei release more cellulase, making it easier to scale up the process (Agrawal et al., 2023). Simultaneously, synthetic biology techniques have made it possible to create bacteria that can produce entire cellulase systems, doing away with the requirement for additional enzymes. These advancements in microbes have changed industrial enzymatic hydrolysis, ensuring a reliable and cost-effective source of powerful cellulases (Potočnik et al., 2023).

New types of cellulase with exceptional ability to break down materials and better resistance to common blockers found in plant biomass have been created using advanced methods like directed evolution and rational design (Contreras et al., 2020). Nowadays, cellulase mixtures are tailored for particular biomass types, guaranteeing highly efficient and targeted hydrolysis. Cellulase-based hydrolysis is the most reliable method for breaking down materials using enzymes, and researchers are working on creating new cellulase versions that are more stable and efficient by using artificial intelligence (AI) and machine learning (Popović et al., 2024).

Cellulase-based hydrolysis's mild reaction conditions make it especially well-suited for producing industrial biofuel since it does not require a lot of downstream processing. Since there are no harsh chemical reagents involved, cellulase-based hydrolysis is the most environmentally friendly and financially feasible method of converting biomass (Lu et al., 2020). Additionally, new advancements in reducing costs and improving efficiency come from hybrid processing methods like consolidated bioprocessing (CBP), which mixes microbial fermentation and cellulase hydrolysis in one bioreactor (Carvalheiro et al., 2024). Cellulase hydrolysis will continue to be the mainstay of the bioeconomy of the future thanks to the continuous development of integrated biorefineries that use it to produce various product streams, such as bioethanol, bioplastics, and high-value chemicals (Kavitha Shree et al., 2024).

The process is expected to be made efficiency and even better through continued research on improving cellulase mixtures, enhancing how microbial hosts secrete enzymes, and using high-throughput screening to improve enzyme engineering (Davison et al., 2020). Cellulase-based hydrolysis will undoubtedly lead the way in producing sustainable biofuels in the future thanks to its smooth integration of biotechnology advancements and industrial scalability (Usman et al., 2024).

2.6 Fermentation

A vital step in the bioconversion of lignocellulosic biomass into bioethanol, a sustainable and renewable fuel source, is fermentation. The process includes using microbes to turn simple sugars, which come from breaking down lignocellulosic biomass, into ethanol and other useful products (Rivero-Pino et al., 2023). Because it affects the process's overall yield and efficiency, this step is essential to the production of bioethanol. Improving fermentation conditions and the types of microbes used is important for making bioethanol a practical option as a commercial biofuel, especially with the increasing need for sustainable energy sources around the world (Meng et al., 2022). To maximise the production of bioethanol, it is still difficult to successfully ferment hydrolysates that contain both hexose and pentose sugars. The situation calls for sophisticated fermentation techniques (Jayakumar et al., 2022).

Many things, like choosing the right microbial strains, removing harmful substances, and improving the process, can affect how well fermentation works (Sosa-Martínez et al., 2023). Improved sugar conversion rates and increased microbial resistance to inhibitors are the results of advances in genetic engineering and bioprocessing. These advancements increase the viability of producing bioethanol on a large scale. Researchers hope to improve process efficiency and economic viability by investigating different fermentation strategies (Adegboye et al., 2021).

2.6.1 Types Of Fermentation In Hydrolysate Processing

Fermentation of lignocellulosic hydrolysate can be divided into different types depending on how microbes work, how sugars are used, and the conditions of the process. The main types, which will be explained in detail later, include co-fermentation techniques, consolidated bioprocessing (CBP), simultaneous saccharification and fermentation (SSF), and separate hydrolysis and fermentation (SHF) (Sharma et al., 2020).

The type of feedstock, the presence of inhibitors, and the intended ethanol yield all influence the choice of fermentation strategy. While some fermentation techniques optimise the efficiency of ethanol conversion, others are more economical (Karimi et al., 2021). Since scalability and process stability are essential for commercial viability, industrial applications also impact the fermentation method selection (Kosamia et al., 2022). The following sections cover the various fermentation techniques in detail, emphasising their benefits and drawbacks.

2.6.1.1 Simultaneous Saccharification And Fermentation (SSF)

SSF cuts down on process time and expenses by combining fermentation and enzymatic hydrolysis into a single step. By minimising sugar accumulation, this technique lowers microbial inhibition. However, SSF requires conditions that support both microbial fermentation and enzyme activity, which can be challenging (Pratto et al., 2020). Advances in thermotolerant and genetically engineered microorganisms have significantly improved SSF efficiency, making it a promising approach for large-scale bioethanol production. Research suggests that SSF can reduce enzyme loading, thereby making bioethanol production more cost-effective (Singhania, Patel, Raj, et al., 2022). Additionally, SSF helps to lower contamination risks by limiting the accumulation of free sugars, which can attract unwanted microbial growth. Despite these advantages, the challenge of optimising process conditions for both hydrolysis and fermentation remains a limiting factor.

Studies have indicated that SSF can achieve high ethanol yields when coupled with effective pretreatment strategies that break down lignocellulose into fermentable sugars efficiently (Jahangeer et al., 2024). The development of thermophilic microorganisms that can withstand the optimal hydrolysis conditions has significantly improved SSF efficiency. However, a major problem with SSF is that it's hard to keep the best conditions for both breaking down materials with enzymes and for the growth of microorganisms, since they often need different temperatures and pH levels (Chilakamarry et al., 2022). Also, the continuous monitoring and adjustment of process conditions are required to prevent microbial inhibition and enzyme deactivation, which can impact ethanol production efficiency (Londoño-Hernandez et al., 2020).

2.6.1.2 Consolidated Bioprocessing (CBP)

CBP aims to make bioethanol production easier by using one type of microbe that can break down lignocellulose and turn the sugars into alcohol. Although this method uses highly engineered microorganisms, it lowers operational complexity and enzyme costs (Singhania, Patel, Singh, et al., 2022). Future bioethanol production could be revolutionized by the development of robust CBP strains with improved lignocellulose-degrading capabilities, which has been the focus of recent research (Periyasamy et al., 2023). The benefit of CBP is its capacity to combine every stage of processing into a single organism, which could reduce expenses and streamline manufacturing. The development of microorganisms that can effectively perform both fermentation and hydrolysis without the need for external enzyme supplementation is still the main obstacle, though (Montaño López et al., 2022).

Recent developments in synthetic biology and metabolic engineering have produced genetically modified microorganisms with improved fermentative and cellulolytic properties. In CBP, engineered yeast strains and bacterial consortia have shown encouraging results, increasing ethanol yields while simplifying the process (Adebami & Adebayo-Tayo, 2020). However, the intricate genetic alterations needed to improve microbial performance make CBP's scalability difficult. Enhancing tolerance to inhibitors found in lignocellulosic hydrolysates and boosting microbial robustness are the main goals of future research. The ability to generate affordable microbial strains that can effectively break down lignocellulose and withstand industrial conditions will be crucial to CBP's success in industrial applications (Andhalkar et al., 2023).

2.6.1.3 Co-Fermentation Strategies

Hexose (glucose) and pentose (xylose) sugars in hydrolysates are effectively converted by co-fermentation, which uses several microbial strains. Metabolic engineering efforts have concentrated on improving pentose utilisation in yeasts like Saccharomyces cerevisiae and Escherichia coli because the majority of native fermenting microorganisms prefer glucose over xylose (Saxena et al., 2023). Sequential and simultaneous fermentation are two co-fermentation techniques that have demonstrated encouraging outcomes in increasing the yield of ethanol from mixed sugar substrates. The efficiency of the process can be greatly increased by fermenting both types of sugar at the same time. But there are still obstacles to overcome, like catabolite repression, which occurs when glucose inhibits the metabolism of xylose (Nielsen et al., 2020).

By incorporating xylose metabolic pathways into conventional ethanol-producing microorganisms, recent advances in strain engineering have enhanced co-fermentation techniques. Furthermore, strains with improved pentose metabolism have been chosen as a result of developments in adaptive laboratory evolution (Dolpatcha et al., 2023). To improve ethanol production, future research attempts to further optimise co-fermentation conditions by balancing sugar utilisation and resolving glucose repression problems. To ascertain the efficacy of co-fermentation techniques in large-scale bioethanol production, additional validation is necessary before they can be used in industry (Melendez et al., 2022).

2.6.1.4 Separate Hydrolysis and Fermentation (SHF)

SHF has emerged as one of the most effective fermentation techniques for producing bioethanol, especially when working with lignocellulosic hydrolysates. In SHF, enzymes hydrolyse lignocellulose in two distinct steps, followed by fermentation of the resulting sugars (Hawrot-Paw & Stańczuk, 2022). Higher sugar yields can be achieved by using this method, which enables ideal enzymatic hydrolysis conditions prior to fermentation. SHF's primary benefit is its adaptability; both fermentation and hydrolysis can be optimised separately. Moreover, inhibitory compounds can be removed before fermentation, enhancing microbial efficiency (Soares et al., 2020). Despite taking longer to process, SHF has been widely used in industrial bioethanol production because of these benefits.

The ability to independently optimise fermentation and hydrolysis improves microbial activity and sugar availability. SHF also allows for detoxification steps to remove inhibitors such as furfural and acetic acid, which can otherwise hinder fermentation efficiency (Kordala et al., 2021). Additionally, this method enables the use of engineered yeast strains specifically tailored for ethanol production under controlled fermentation conditions. Ethanol yields have further increased due to recent developments in SHF, such as enhanced enzymatic hydrolysis methods and adaptive laboratory evolution of microbial strains (Osman et al., 2023). SHF is a favoured option for industrial applications since studies show that it produces higher bioethanol concentrations than alternative techniques.

2.7 Bioethanol Distillation

Globally, fermentation-based bioethanol production continues to be a key component of the production of renewable energy. The distillation process is the last and most crucial step in separating bioethanol from water and other byproducts after fermentation. However, the effectiveness of distillation depends not only on its capacity to concentrate bioethanol but also on maximising energy efficiency and reducing its environmental impact. In order to increase bioethanol recovery, lower energy consumption, and improve environmental sustainability, researchers worldwide are concentrating on refining distillation methods and investigating new technologies. Given the growing need for biofuels and the part bioethanol plays in halting climate change, this is especially important. Numerous aspects of distillation are being studied in various countries, ranging from energy efficiency and process optimisation to minimising the release of hazardous byproducts.

This study examined new developments in bioethanol distillation, emphasising creative methods and technologies put forth by researchers from around the world. Reducing production costs and guaranteeing the viability of producing bioethanol economically depend on effective distillation techniques. The impact of implementing heat-integrated distillation columns to lower energy consumption was investigated in the study carried out in Pakistan by Karimi et al., (2021). This study showed that the overall energy requirement for distillation could be lowered by 15% to 20% by implementing heat integration techniques, such as using surplus heat from other phases of the bioethanol production process. When Karimi and colleagues compared a number of heat-integrated distillation setups, they discovered that multi-effect distillation and systems that used heat exchangers were especially successful in striking a balance between energy savings and the purity of bioethanol. For large-scale bioethanol plants, where energy savings directly translate into cost reductions, this novel approach has important ramifications.

The impact of fermentation by-products, such as organic acids and fusel oils, which can impede the process of bioethanol separation, is another crucial component of distillation optimisation. Brazilian researchers, under the direction of Lopes et al., (2020), looked into how these impurities affected distillation. Their study demonstrated that the presence of volatile substances in the fermentation medium, such as acetic acid, acetaldehyde, and other aldehydes, reduces the effectiveness of ethanol separation. Lopez's group suggested a two-step distillation procedure where a second, more energy-efficient distillation was performed after the fermentation broth had been treated to eliminate the majority of these contaminants. This method decreased the production of undesirable by-products, which would otherwise degrade the quality of the finished product, and increased the purity of bioethanol. When producing bioethanol on an industrial scale, these pre-treatment methods are essential for improving distillation efficiency.

The separation of bioethanol from water and other components can be improved with the use of advanced distillation techniques like membrane and hybrid systems. In China

,Li et al., (2021), investigated the application of a hybrid distillation-membrane system that combines membrane filtration and conventional distillation methods. This technique makes use of membranes' unique separation capabilities to recover ethanol more effectively. In comparison to traditional distillation techniques, this study demonstrated that membrane-assisted distillation not only improved bioethanol recovery but also used less energy. The bioethanol concentration was raised, and a more effective separation process was achieved by using an initial membrane to extract water from the bioethanol-water mixture. According to Li and associates, incorporating membrane technology into distillation systems may usher in a new era of bioethanol production that uses less energy.

Vacuum distillation has drawn interest in India as a bioethanol production alternative to conventional distillation techniques. The benefits of employing vacuum distillation to extract ethanol from fermentation broths were examined by (Kumar et al., 2022). Because vacuum distillation lowers the boiling points of the mixture's constituents, bioethanol can be separated at lower temperatures, minimizing the thermal degradation of delicate compounds and lowering the energy requirement overall. In contrast to the typical 78°C needed for atmospheric distillation, this study demonstrated that bioethanol can be extracted at temperatures as low as 60°C by operating at a lower pressure. In addition to using less energy, the lower temperature gives you leverage for upscaling and keeps volatile compounds from deteriorating during high-temperature distillation.

One fascinating area in the search for sustainable production techniques is the incorporation of renewable energy into the distillation of bioethanol. Indian researchers. Indian researchers, (Singh et al., 2024), investigated how solar thermal energy can be combined with conventional distillation columns to significantly lower the carbon footprint of bioethanol production. The team reduced their dependency on fossil fuels for the distillation process by using solar energy to warm the fermentation broth. Solar thermal collectors combined with multi-effect distillation units reduced energy consumption and greenhouse gas emissions, according to this pilot study. According to this study, distillation and solar energy can work together to produce bioethanol in a more sustainable and profitable manner, particularly in Africa, where sunlight is plentiful.

Recent studies have placed a strong emphasis on the environmental effects of bioethanol distillation, with numerous investigators comparing the energy consumption and carbon footprint of various distillation techniques. In South Africa, Sekoai et al., (2023) compared the environmental effects of membrane distillation and conventional distillation in South Africa using a life cycle assessment (LCA). Their research revealed that although membrane distillation was more expensive initially, it provided substantial environmental advantages, such as a decrease in carbon emissions and shorter energy consumption over time, which ultimately reduced the total cost. They ultimately came to the conclusion that membrane distillation might be essential to increasing the sustainability of bioethanol production, particularly in nations aiming to raise their environmental standards and lessen their dependency on fossil fuels.

In order to scale up bioethanol distillation to commercial levels, process optimization and simulation are essential. In order to optimize the design of the distillation column for the production of bioethanol, López Núñez et al., (2022) from Spain created a process simulation model using Aspen Plus. In this study, various distillation configurations were simulated and compared according to capital costs, energy consumption, and bioethanol yield. The findings demonstrated that improved reflux ratios and optimized column designs could greatly boost bioethanol recovery while lowering energy costs. López and his colleagues were able to provide useful recommendations for distillation column design through process simulation, which may aid bioethanol producers in effectively scaling up their operations.

The distillation process in bioethanol production plays a critical role in ensuring the purity and efficiency of the final product. As shown by a number of researchers from various countries, there are various approaches to improve distillation, starting from energy-saving techniques to novel separation technologies (Intan Shafinas Muhammad & A. Rosentrater, 2020). These improvements in hybrid systems, vacuum distillation, and using renewable energy are promising changes that could lower the environmental impact and energy use of bioethanol production while still being cost-effective. These studies underscore the global efforts toward optimising bioethanol distillation and ensuring that bioethanol continues to be a sustainable and renewable alternative to fossil fuels (Cherwoo et al., 2023).

2.8 Effect Of Operations Parameters

Producing bioethanol from lignocellulosic biomass, such as corn stover, requires several complex procedures and careful control of numerous operational parameters. Temperature, pH, the amount of solid material, and the choice of pretreatment chemicals are some of the factors that impact the entire bioethanol production process, which involves getting the raw materials, pretreating them, breaking them down with enzymes, fermenting, and distilling (Bhatia et al., 2020). To attain high yields and increase overall process efficiency, it is crucial to optimise the influence standards at each stage of the process. In particular, the pretreatment phase is important for breaking down the complex lignocellulosic structure into sugars that can be fermented, which can also impact the amount of bioethanol produced. The cost-effectiveness and environmental impact of making bioethanol mainly rely on the conditions used before and during pretreatment, as well as during enzymatic hydrolysis and fermentation. In this section of the study, various operational parameters that influence each stage of the bioethanol production process were reviewed from corn stover. Pretreatment, enzymatic hydrolysis, fermentation, and distillation will be emphasised as key factors that impact how much bioethanol is produced and how efficiently it is made (Xu et al., 2020).

Selecting and preparing the biomass feedstock before the pretreatment process even starts is essential to guaranteeing effective conversion in subsequent steps. Harvesting, cleaning, and particle size reduction are some of the first steps in feedstock processing that can have a big impact on the total amount of bioethanol produced. The effectiveness of the pretreatment step in the case of corn stover is influenced by several factors, including the feedstock's particle size distribution, moisture content, and the proportions of cellulose, hemicellulose, and lignin (Colla et al., 2022). Research by Akter et al., (2020), shows that controlling the moisture content and adjusting the particle size of the biomass before pretreatment makes it easier to access the cellulose, which helps it work better for enzymatic hydrolysis. Smaller particle sizes create a larger surface area, which helps chemicals or enzymes work better with the biomass, leading to higher amounts of fermentable sugars later on.

To break down the complex lignocellulosic structure and make cellulose more accessible for enzymatic hydrolysis, the pretreatment phase is one of the most important steps in the production of lignocellulosic bioethanol (Kumar et al., 2020). Pretreatment techniques come in a wide range, including combinations of physical, chemical, and biological treatments. Deep Eutectic Solvents (DESs), like lactic acid and choline chloride, have been highlighted in recent research as an efficient and eco-friendly pretreatment technique. DESs have been shown to degrade lignin and hemicellulose more effectively than conventional acidic or alkaline pretreatments Condor et al., (2022). This procedure increases the hydrolysate's concentration of fermentable sugars, which is essential for optimising the fermentation's bioethanol production. Furthermore, using DESs is less detrimental to the environment, which makes it a desirable substitute for producing bioethanol sustainably. Additionally, using DESs improves sugar recovery while lowering the need for harsh chemicals, which lessens the environmental impact of pretreatment (Pratama et al., 2023).

Prior to pretreatment, enzymatic hydrolysis is essential for turning the biomass's cellulose into fermentable sugars, primarily glucose. The enzymatic hydrolysis process uses cellulase and hemicellulase enzymes to convert cellulose and hemicellulose into simpler sugars (Houfani et al., 2020). Enzyme loading, reaction time, temperature, pH, and solid loading are some of the variables that affect the effectiveness of enzymatic hydrolysis. A study by López-Fernández-Sobrino et al., (2021) showed that the amount of enzyme used is very important for getting more sugar from hydrolysis, as using more enzyme helps break down cellulose into glucose more effectively. Striking an ideal balance between reducing expenses and optimising sugar yield is crucial, as excessive enzyme loading may lead to increased expenses. Furthermore, the concentration of biomass in the hydrolysis reactor, or solid loading, has a major impact on the rate of sugar recovery (Kasperski, 2023). It has been demonstrated that high solid loading (greater than 15% w/w) raises the hydrolysate's concentration of fermentable sugars, which can have an immediate effect on fermentation efficiency. It is crucial to remember that high solid loads may make the hydrolysis slurry more viscous, which could lower enzyme accessibility and efficiency and necessitate careful optimization (Atiku et al., 2024).

Following enzymatic hydrolysis, fermentation is the process by which yeast or other microbes turn the fermentable sugars into bioethanol. Numerous factors, such as temperature, pH, sugar concentration, and the kind of microorganism employed, have a significant impact on the fermentation process (Maleke et al., 2022). Saccharomyces cerevisiae, one of the most common yeast strains used in bioethanol fermentation, is most active at temperatures between 25°C and 30°C. According to Edeh, (2021) yeast activity is extremely sensitive to temperature, and any departure from this ideal range may result in lower yields of ethanol. In addition to temperature, pH has a big impact on fermentation. With an ideal pH range of 4.5 to 5.5, yeast thrives in slightly acidic environments. Deviations from this pH range can inhibit yeast growth and fermentation rates, thereby reducing ethanol production. Additionally, one important factor affecting fermentation efficiency is the concentration of sugars in the fermentation medium (Tse et al., 2021). Although very high sugar concentrations can put the yeast under osmotic stress, which could reduce fermentation efficiency, higher sugar concentrations result in higher ethanol yields. Thus, maximising ethanol production during fermentation requires maintaining an ideal balance of temperature, pH, and sugar concentrations (García-Depraect et al., 2021).

The process of producing bioethanol ends with the distillation stage, which comes after fermentation. Based on variations in boiling points, distillation is used to separate ethanol from water and other fermentation byproducts. Because distillation uses a lot of energy, it must be carefully optimised to guarantee energy efficiency and lower expenses (Li & Li, 2020). The amount of ethanol in the fermentation broth is one important factor affecting distillation efficiency. Because less energy is needed to extract the ethanol from the mixture, higher ethanol concentrations enable more effective distillation. Water and other fermentation byproducts, however, can make distillation more difficult and lower the purity of ethanol. Numerous methods, including membrane-assisted distillation and vacuum distillation, have been investigated to increase distillation efficiency (Popescu et al., 2021). Specifically, vacuum distillation uses less energy and maintains the quality of the ethanol by operating at lower pressures and temperatures. By incorporating heat recovery systems into the distillation process, the environmental impact of producing bioethanol is reduced and energy efficiency is further improved (Janković et al., 2024).

Therefore, the production of bioethanol from corn stover involves several interrelated steps, each influenced by specific operational factors. To increase the concentration of fermentable sugars, improve the efficiency of enzymatic hydrolysis, and break down the lignocellulosic structure, the pre-treatment procedure is essential (Morales et al., 2021). To get the best sugar output and minimise problems in the process, careful management of factors like enzyme amount and solid material is needed during the enzymatic hydrolysis stage. To guarantee high ethanol yields during fermentation, variables like temperature, pH, and sugar concentrations must be optimized (Afedzi et al., 2025). Lastly, ethanol concentration, energy efficiency, and the application of sophisticated distillation techniques all affect distillation, which extracts ethanol from the fermentation broth. By fine-tuning the parameters at every stage, the production of bioethanol from corn stover can be made more sustainable and efficient (Broda et al., 2022).

2.9 Engine Performance on Gasoline/Bioethanol Blends

The research used a Toyota TERCEL-3A four-stroke, four-cylinder SI engine, which ran on a blend of petrol and bioethanol. The tests focused on equivalence, air-fuel ratio, fuel consumption, volumetric efficiency, brake thermal efficiency, brake power, engine torque, and brake-specific fuel consumption. Sarabi & Abdi Aghdam, (2020) also looked at exhaust emissions for unburned hydrocarbons (HC), carbon monoxide (CO), and carbon dioxide (CO_2) when using bioethanol and unleaded petrol in different fuel mixtures at engine speeds between 1000 rpm and 4000 rpm. The findings showed that while brake-specific fuel consumption and the equivalent air-fuel ratio decreased, brake power, torque, volumetric and brake thermal efficiencies, and fuel consumption all increased. As CO_2 rose, CO and HC fell. E20 produced the best results in every measured engine parameter.

In the transportation industry, bioethanol is commonly used as a petrol additive. Engine-out emissions like CO, HC, and particulate matter are decreased when blended. Because bioethanol has a higher octane rating than petrol, it lessens the tendency for knocking when engines are operating Zhao & Wang, (2020). The heat of vaporisation (HOV) of bioethanol is three times that of petrol. Ethanol has a higher laminar flow speed (LFS) during the engine's expansion stroke, which helps determine useful work during the power stroke.

Tests were conducted in Colombia using an SI engine and petrol mixed with anhydrous bioethanol in different amounts: E10, E15, E20, E40, E60, E85, and E100, while the engine ran at 3600 rpm under different loads. At variable engine loads, the engine was operated at 3600 rpm. According to the results, E40 produced the best results, using 2.5% less brake-specific fuel than the E10 blend (Mariaca & Castaño, 2018). Nitrogen oxide (NO_2) rose by 106.25% as a result of the E40 result, but emissions of hydrocarbons (HC), carbon monoxide (CO), and carbon dioxide (CO_2) decreased by 52.88, 73.66, and 9.72%, respectively.

Research on ethanol/gasoline blends in spark-ignition engines has shown that E20 offers the best balance between lower emissions and sustained engine power. According to the research, E20 is an environmentally friendly option because of its higher combustion efficiency, which lowers emissions of hydrocarbons (HC) and carbon monoxide (CO) (Rimkus et al., 2024).

Although they pointed out that extended use may necessitate ethanol-compatible materials due to increased corrosion risks, the lubricating effects of E30 and E40 blends were investigated, and it was discovered that these blends could improve engine lubrication by lowering operating temperatures (S. Karimi et al., 2021). The study looked at E10, E20, and E30 fuel mixtures and discovered that E20 was the most efficient and produced the least pollution, while E30 and E40 needed changes in ignition timing to maintain efficiency. It was observed that to maximise its performance, E40 needed specific adjustments (Badawy et al., 2024).

When engine performance was evaluated at different ethanol levels, it was discovered that E20 reduced emissions with increased engine power and fuel consumption while maximising engine efficiency without requiring major changes. Due to ethanol's lower energy density, E30 and E40 used more fuel, but they also significantly decreased exhaust emissions and displayed phase separation (Deshmukh et al., 2022).

This study looked at fuel economy and found that E20 balanced emissions and fuel efficiency. Because ethanol is oxygenated to improve combustion, higher blends such as E40 resulted in significant emission reductions, especially in NO_x and HC levels (Faraji et al., 2021).

Due to the high oxygen content of ethanol, it was discovered that E30 and E40 blends offered lower-level emission benefits; however, fuel consumption at these levels was unsustainable. However, with only minor adjustments, E20 proved to be efficient for the four-stroke SI engine and offered a more cost-effective alternative to higher blends (Singhal et al., 2023).

With minimal adjustments to conventional petrol engines, E20 was found to achieve stable combustion and efficient fuel use. However, an increase in ignition delay was observed at E30 and E40, requiring modifications to ignition timing to increase combustion efficiency (Yesilyurt et al., 2023).

Tests between E10 and E40 were conducted using a commercial petrol engine. It was found that E20 enhanced engine power and combustion quality, while E40 optimised emission benefits. As a result, E20 was advised for everyday use, and E40 was only suggested for modified engines that could handle higher ethanol levels (Baratian et al., 2021)

This study discovered that although E10 and E20 improved emissions and combustion efficiency, blends above E30 needed to have their ignition timing adjusted for maximum efficiency, particularly when engine loads were higher. Without significant changes, E20 was determined to be the most effective balance between emissions and efficiency (Yakın & Behçet, 2021).

An analysis of the effectiveness of higher ethanol blends in lowering greenhouse gas emissions found that while E30 and E40 significantly reduced emissions, they may have an effect on fuel economy if used in engines that aren't modified. However, E20 demonstrated its viability for conventional engines by offering a balanced performance and reduced emissions (Karmakar et al., 2024).

2.10 A Synopsis of the Procedure for Bioethanol Techno-Economic Analysis (Tea)

To determine if making bioethanol is practical, Techno-Economic Analysis (TEA), which provides important details about costs, investment potential, and how well it can grow from small labs to large factories is used (Kosamia et al., 2022). To determine how profitable, sustainable, and competitive bioethanol production is compared to fossil fuels and other renewable energy sources, TEA merges technical performance information with economic factors. Using this method, decision-makers can estimate possible returns on investment, optimize processing conditions, and locate cost bottlenecks (Kumar et al., 2023). TEA is especially crucial for new bioethanol production technologies, like Deep Eutectic Solvent (DES) pretreatment, where economic feasibility and operational effectiveness are critical to widespread adoption. The stepwise approach is the most thorough and flexible TEA method available, bridging the gap between industrial application and laboratory research (Wunderlich et al., 2021).

2.10.1 Examining Techno-Economic Analysis Techniques

TEA in the production of bioethanol has developed numerous approaches, each with specific benefits and drawbacks. The most popular methods are the stepwise approach, process simulation, sensitivity analysis, Monte Carlo simulations, and discounted cash flow (DCF) analysis (Karpagam et al., 2021).

2.10.2 Discounted Cash Flow (DCF) Analysis

Using variables like capital investment, operating expenses, and anticipated revenue over the plant's lifetime, DCF analysis calculates the present value of future cash flows produced by bioethanol production (Buttignon, 2020). This method is useful for evaluating long-term profitability and determining net present value (NPV) and internal rate of return (IRR). However, DCF is less flexible in response to changing economic conditions because it is extremely sensitive to assumptions about market prices, feedstock costs, and discount rates (Sutjipto et al., 2020). Furthermore, it may produce erroneous estimates since it fails to adequately consider process improvements or technology breakthroughs that could affect future expenses and income. Due to these drawbacks, it is less applicable to new bioethanol production technologies that are still undergoing cost and process optimizations (Vayas-Ortega et al., 2020).

2.10.3 Simulation Using Monte Carlo

By taking input variables' uncertainties into account, Monte Carlo simulations bring probabilistic modelling to TEA. By executing numerous simulations with different parameter values, this technique enables researchers to assess the variety of potential economic outcomes (Barahmand & Eikeland, 2022). Monte Carlo analysis is useful for large-scale projects because it offers a thorough risk assessment. A study found that making the plant bigger could lower financial risks even when ethanol prices are lower, based on Monte Carlo simulations that looked at the economic risks of producing bioethanol from wheat straw (Behera & Paramasivan, 2021). Similarly, Monte Carlo simulations showed that the costs of feedstock greatly affect how profitable a biorefinery is in an analysis of bioethanol production from engineered energy cane. Likewise, Monte Carlo simulations revealed that the costs of raw materials greatly affect how much money a biorefinery makes in a study of bioethanol production from specially designed energy cane (Cortés-Peña et al., 2024). Its intricacy and dependence on large datasets, however, may restrict its use in preliminary studies or laboratory-scale analyses. Furthermore, this approach is less feasible for small-scale feasibility assessments due to the computational intensity needed to model complex bioethanol production systems (Rodgers, 2024).

2.10.4 Analysis of Sensitivity

Sensitivity analysis looks at how changes in important economic factors, like the price of ethanol, feedstock, and enzymes, affect the overall profitability of producing bioethanol. This method pinpoints important cost factors and offers information about areas that need optimization (Chuenphan et al., 2022). For instance, a study on the production of bioethanol from plant materials in China found that the costs of raw materials and enzymes are key factors affecting the lowest price at which ethanol can be sold, showing how important it is to focus on reducing these costs. (Zhang et al., 2024). Although sensitivity analysis is simple and efficient, it can produce oversimplified results because it ignores the interdependencies between variables. It is therefore less useful for making comprehensive decisions since it might not adequately represent the intricate relationships that affect the overall economic feasibility of bioethanol production (Goffart & Woloszyn, 2021). For this reason, sensitivity analysis is frequently combined with other techniques, like Monte Carlo simulations, to offer a more thorough comprehension of economic risks and uncertainties (Huang et al., 2024).

2.10.5 Process Simulation Models

To forecast material flows, equipment costs, and mass and energy balances, process simulation tools like Aspen Plus and SuperPro Designer combine TEA with process modelling. According to Somoza-Tornos et al., (2021), these models play a crucial role in bringing laboratory-scale experiments to an industrial scale. Process simulation's main benefit is its capacity to offer a thorough and numerical evaluation of the technical and financial facets of bioethanol production. However, these models are less accessible for early-stage research because they require a significant amount of expertise and high-quality input data (Castro et al., 2022). Furthermore, inaccurate input data can produce deceptive outcomes, which reduces the validity of process simulation models. Despite its benefits, process simulation alone cannot effectively show the changes in costs and operational challenges that happen in real time when scaling up (Sunwoo et al., 2023).

2.10.6 Stepwise Approach Tea

A new TEA methodology called the stepwise approach methodically assesses the production of bioethanol from lab-scale trials to industrial uses. Experimental data collection, process modelling, cost estimation, economic feasibility assessment, and scalability evaluation are some of the sequential steps that make up this approach (Ntimbani et al., 2021). The stepwise approach is especially beneficial because it ensures a more accurate representation of production feasibility by combining economic projections with real-time experimental data. The stepwise approach yields more accurate economic estimates than purely theoretical models because it incorporates actual experimental data (Kocsi et al., 2020). By assessing the financial implications at every stage, it facilitates a seamless transition from laboratory research to industrial implementation. To increase efficiency, stepwise TEA complements optimisation strategies like the Response Surface Methodology (RSM) and machine learning models (Zappi et al., 2023). The stepwise approach is better suited for new bioethanol technologies than DCF or sensitivity analysis because it considers changes in processes and new technology developments (Vasilakou et al., 2023). It also guarantees economic scalability and optimises process efficiency, making it a strong framework for evaluating the feasibility of producing bioethanol. Future studies should concentrate on improving this approach's predictive accuracy by combining cutting-edge computational tools and real-world case studies (Hasanly et al., 2021).

2.11 Optimization of Research Work

A key component of scientific and engineering research is optimisation, which makes it possible to choose the best option among a number of workable alternatives. It is essential for increasing productivity, cutting expenses, and enhancing process performance. The simplex method, gradient-based approaches, linear programming, and nonlinear programming are examples of traditional optimisation techniques (Yang et al., 2022). Numerous fields, such as environmental modelling, material science, and chemical engineering, make extensive use of these techniques. However, complex, multi-modal, and high-dimensional problems are frequently beyond the scope of traditional optimisation techniques.

Metaheuristic algorithms such as Genetic Algorithms (GA), Particle Swarm Optimisation (PSO), Simulated Annealing (SA), Differential Evolution (DE), and Ant Colony Optimisation (ACO) have gained popularity because of these challenges (Roostaee et al., 2024). By performing global searches instead of depending on local optima, these algorithms efficiently handle multi-objective, nonlinear, and constraint-heavy problems. For example, Kumar et al., (2023) showed how GA outperforms conventional techniques in maximising aircraft fuel efficiency by emphasising its capacity to traverse intricate search spaces.

Chemical reaction optimisation, machine learning model tuning, and industrial process control have all benefitted greatly from hybrid optimisation models that combine GA and Response Surface Methodology (Singh et al., 2023). In a similar vein, Zaini et al., (2023), showed that by optimising critical operational parameters, PSO-based optimisation decreased energy consumption in wastewater treatment. These methods' increasing significance in optimisation-based research is demonstrated by their use in a variety of fields.

2.11.1 Design Expert Software and Experimental Design Techniques

One of the best statistical programs for creating experiments, examining interactions, and streamlining procedures is called Design Expert. It was created by Stat-Ease Inc. and is a favourite tool in engineering, pharmaceutical sciences, and materials research because it can be used for factorials, response surfaces, and mixture designs (Montgomery, 2021).

With this software, researchers can assess how several independent variables affect a response variable, minimising the number of experiments needed. Akram & Garud, (2021) used Design Expert to optimise tablet formulation parameters, improve dissolution profiles, and lower production costs. Similar to this, DesignExpert has been used in the food industry to maximise flavour enhancement, nutrient retention, and the extraction of bioactive compounds (Huang et al., 2021).

2.11.2 Response Surface Methodology (Rsm)

RSM is a set of mathematical and statistical methods for creating, refining, and streamlining processes. It is widely used to examine the connections between various input variables and output responses in scientific research, engineering, and manufacturing (Habibi et al., 2021). Complex interactions and quadratic relationships, which might not be captured by simpler designs, can be modelled using RSM techniques like CCD and BBD (Abubakar et al., 2022).

Applications of RSM include materials engineering Chaturvedi et al., (2022), and chemical process optimisation Rajewski & Dobrzyńska-Inger, (2021),where precise control over variables leads to better performance and efficiency, where exact control over variables improves performance and efficiency. According to recent research, combining RSM with AI methods for hybrid modelling can improve prediction accuracy in advanced material synthesis and biomedical applications (Patel et al., 2023)).

2.11.3 Central Composite Design (Ccd)

The popular response surface methodology (RSM) technique, known as CCD, includes factorial points, axial points, and centre points. It works well for process optimisation and quadratic modelling. CCD was used by Pashaei et al., (2020) to optimise the biodegradation of organic pollutants, showing that it decreased experimental trials while preserving predictive accuracy.

Because of its exceptional ability to capture nonlinear interactions between variables, CCD is perfect for engineering applications that require accurate modelling. (Mahmoudi et al., 2023) showed that CCD greatly decreased experimental errors and increased the accuracy of models for nanoparticle synthesis. However, when working with high-dimensional systems, CCD can become computationally costly, requiring the use of alternative techniques like machine learning-based optimisation or Box-Behnken designs (BBD).

2.11.4 Full Factorial Design (FFD)

Full Factorial Design (FFD) is perfect for identifying higher-order interactions because it assesses every possible combination of factor levels. Compared to the Taguchi designs. Shukla et al., (2022) used FFD to optimise catalytic processes, revealing deeper insights into reaction mechanisms.

When dealing with multiple factors, FFD necessitates a significant number of experiments, but it is very robust in detecting interaction effects. According to Brown, (2023) FFD is still better for applications like aerospace material optimisation, where a thorough grasp of factor interaction is essential. However, due to their computational intensity, large-scale problems frequently choose heuristic approaches or fractional factorial designs.

2.11.5 Box-Behnken Design (Bbd)

BBD is a substitute for CCD that captures nonlinear relationships with fewer experimental runs; its effectiveness in optimising enzyme production was shown by Sharma et al., (2023), lowering expenses without sacrificing model accuracy.

In fields like biotechnology and material science, where accurate modelling is necessary and experimental costs are high, BBD works especially well. Salamat et al., (2024) emphasised BBD's resilience in the synthesis of nanomaterials, showing that it offered superior predictive powers in contrast to traditional DOE methods. However, BBD is less effective under extremely variable conditions because it does not have the axial points that CCD does.

2.11.6 Machine Learning Applications in Optimization and Modeling

Because of its capacity to process massive datasets, identify patterns, and enhance decision-making, machine learning (ML) has emerged as a crucial tool in optimisation and predictive modelling. ML algorithms are highly applicable in a variety of fields, such as engineering, healthcare, and finance, because they adaptively learn from data in contrast to traditional statistical methods (Wu et al., 2023). ML is a preferred method for resolving high-dimensional, multi-objective, and nonlinear optimisation problems due to its universality.

2.11.7 Artificial Neural Networks (ANN)

Widely used in prediction, classification, and optimisation, artificial neural networks (ANNs) are computational models modelled after biological neural networks. In predicting the compressive strength of concrete, Fadare, (2023) showed that ANN performed better than RSM and was better at capturing nonlinearities.

ANN is a better method for big data analysis, chemical modelling, and biomedical applications because of its universal applicability (Smith, 2023). Notwithstanding their adaptability, ANNs need a lot of data and processing power, which may be a drawback in some situations.

2.11.8 Boosted Regression Trees (Brt)

BRT is an ensemble learning technique that uses boosting techniques to increase the accuracy of decision trees. It has been effectively used in scientific and engineering applications, such as optimising bioethanol production. Recent research by Torres-

Martínez et al., (2024) showed that BRT outperformed conventional regression techniques in accurately modelling nonlinear relationships for bioethanol yield prediction.

Because BRT can handle complex, nonlinear dependencies between variables, it produced the best results when predicting bioethanol production yields. In contrast to conventional regression models, BRT balanced variance and bias while successfully lowering prediction errors. BRT has also been used in biomedical research to predict disease risk and in environmental engineering to optimise systems for controlling air pollution (Swaroop et al., 2023). this approach is very useful for process optimisation in applications involving sustainable energy and industrial biotechnology because of its resilience.

2.11.9 Random Forest (RF)

A popular ensemble learning technique called RF builds several decision trees and combines their results to increase prediction accuracy. It has been used in a number of fields, such as material science, biomedical research, and environmental modelling. When looking at traditional statistical models, RF provided more accurate predictions for material failure rates (Ahangari Nanehkaran et al., 2022). It is a fantastic option for complex, high-dimensional data because of its resilience to overfitting.

2.11.10 Support Vector Machines (Svm)

SVM is a potent supervised learning model that performs exceptionally well in tasks involving regression and classification. It has been effectively used to solve engineering problems in areas like chemical process monitoring, bioinformatics, and fault detection. SVM's effectiveness in nonlinear problem solving was highlighted in recent studies by

Liu et al., (2024), which showed that it outperformed conventional regression techniques in optimising polymeric material compositions.

2.11.11 Polynomial Equation Modeling

One popular mathematical method for explaining intricate relationships between variables is polynomial equation modelling. It is especially helpful when modelling nonlinear systems, where simple linear regression fails to capture complex interactions (Montgomery, 2020). Engineering, environmental sciences, and materials research commonly use polynomial models, such as quadratic, cubic, and higher-order equations (Rostyslav et al., 2024).

Polynomial equations have been widely used in chemical engineering to maximise catalyst performance and reaction kinetics. According to Chohan et al., (2022) polynomial models outperformed traditional linear regression techniques in terms of accuracy when forecasting biofuel conversion rates. Similarly, in mechanical engineering, using higher-order polynomial models to predict stress-strain relationships in composite materials has led to better evaluations of how well structures perform (Nguyen et al., 2023).

Polynomial models are also helpful in biomedical research because they help improve pharmacokinetic parameters and understand how drugs are released (Jayakumar et al., 2024). When applied to high-dimensional data, polynomial models may experience overfitting despite their adaptability. To counteract this, researchers frequently use machine learning methods like ANN or Random Forest in conjunction with polynomial regression to improve predictive accuracy (Singh et al., 2025).

2.12 Analysis of Variance (ANOVA)

ANOVA is a basic statistical technique used in experimental optimisation to determine whether changes in a response variable are due to random chance or controlled experimental factors. ANOVA helps improve processes by breaking down the overall variation into parts related to different sources, allowing us to assess how independent variables and their interactions affect the response (Montgomery, 2020).

The F-value is one of the crucial metrics in ANOVA and represents the ratio of systematic variance (variance caused by factors) to random variance (error variance) (Lee, 2023). A high F-value (usually ≥ 4) indicates a meaningful effect since it indicates that the factor or model accounts for a sizable amount of the variation. On the other hand, a low F-value (near 1) means that the factor acts like random variation and does not significantly affect the response variable (Olusanya et al., 2024). By calculating the likelihood that the observed effect was the result of chance, the p-value further quantifies statistical significance. While a p-value > 0.05 implies that the factor is not statistically significant and does not significantly affect the response, a p-value ≤ 0.05 indicates that the factor has a significant influence (Lakens et al., 2020).

Another crucial ANOVA parameter is the coefficient of determination (R^2), which shows how much of the variability in the response variable can be accounted for by the model. According to (DeForest et al., 2023), a well-fitting model typically has $R^2 \geq 0.80$, meaning that it adequately describes the data. However, if the R^2 value is less than 0.50, it indicates that the model should be improved by adding more factors or changing the experimental design in order to better capture the relationship between the independent variables and the response (Purwanto & Sudargini, 2021). A high R^2 value by itself, however, is insufficient to validate a model; in order to account for the number of predictors and prevent overfitting, adjusted R^2 should also be taken into consideration (Sharma et al., 2021).

The interpretation of the ANOVA table also depends on where significance appears. The model is appropriate for predictive applications since it indicates that the chosen independent variables taken together have a significant impact on the response variable ($p \leq 0.05$), as shown at the top of the table. A model that is not significant ($p > 0.05$) means that the chosen variables do not explain the differences in the responses well enough, which means changes to the model are needed (Rasoolimanesh et al., 2021). The ANOVA table's lack of fit test evaluates how well the model matches the observed data. Since it shows that the model adequately explains the data, a non-significant lack of fit ($p > 0.05$) is preferred. A significant lack of fit ($p \leq 0.05$) means that the model doesn't properly represent the data, which suggests that changes are needed in how the data was collected or which factors were chosen (Khatoon & Rai, 2020).

R^2 is typically directly impacted by ANOVA results. A high R^2 frequently accompanies a significant model ($p \leq 0.05$) with a high F-value, indicating that the model accounts for the majority of the variation in responses. Though some variables have a statistically significant effect, a model that is significant but has a low R^2 (< 0.50) suggests that not all influential factors are captured by the model, necessitating additional refinement. However, if the model has a high R^2 but is not significant ($p > 0.05$), it might be overfitting, which means the model is capturing random noise instead of actual relationships. To guarantee a balanced and trustworthy model fit in these situations, validation using adjusted R^2 is required.

2.13 Gaps and Challenges

Although bioethanol production has drawn a lot of interest as a renewable energy source, there are still a number of unmet needs in terms of process optimisation and implementation. This study found several research gaps, especially in the areas of choosing the best solvent combinations, using Zambian corn stover as a bioethanol feedstock, and using Deep Eutectic Solvents (DES) for biomass pretreatment. These gaps should be filled to advance bioethanol production technology and make it economically and environmentally viable for widespread use.

The little investigation of lactic acid and choline chloride as DES components for biomass pretreatment is one of the main research gaps. Ionic liquids have been extensively researched, but their practical use is restricted by their high cost, toxicity, and environmental concerns. Because they are less expensive, less toxic, biodegradable, recyclable, and have a lower density than ionic liquids, lactic acid and choline chloride offer an alluring substitute. Furthermore, studies have demonstrated that DES pretreatment with lactic acid and choline chloride significantly increases cellulose yield while using less energy than ionic liquids. To fully grasp their potential and limitations in the production of bioethanol, more research is necessary, as their application is still primarily in the research and development stage. More experiments are needed to find the best mix because little information is known about how changing the amount of lactic acid and choline chloride affects how well lignin is removed and how accessible cellulose is.

The absence of information regarding Zambian corn stover's structural makeup and suitability for the production of bioethanol represents another significant gap. Substantial regional and climatic variations in the structural carbohydrates in lignocellulosic biomass affect the overall yield of bioethanol (Tse et al., 2021). This study sought to ascertain the structural makeup of Zambian corn stover and evaluate its effectiveness in generating fermentable sugars and ethanol, as no previous research has been done on it as a bioethanol feedstock. Future studies must establish this data to optimise its use and assess its economic feasibility for large-scale production. Another research gap that could increase the productivity of bioethanol is the unexplored possibility of genetic or agronomic modifications to increase its biomass yield and sugar content.

Furthermore, there hasn't been much process standardisation in the optimisation of DES pretreatments for bioethanol production. Many of the studies that are currently available rely on conventional statistical techniques, which are not very accurate at predicting complex biomass compositions. Although machine learning methods like gradient-boosted regression trees (BRT) have demonstrated promise for increasing optimisation accuracy, little is known about how they can be applied to DES-based pretreatment. To develop flexible optimisation strategies that can be used with different biomass sources, more research is required to improve these models' predictive abilities. Extending these predictive models could enhance the scalability of bioethanol production, increase process efficiency, and reduce experimental costs. Also, studies are needed on use of stable and reusable DES are for pretreatment, and how they impact the efficiency of fermentation and enzymatic hydrolysis over time.

The limited assessment of ethanol-gasoline blends for engine performance represents another research gap. Although ethanol emissions and combustion efficiency have been the subject of much research, there are still few real-world engine performance assessments available. The majority of studies have concentrated on laboratory-scale evaluations without considering the long-term durability and performance effects of ethanol blends. Determining whether higher ethanol blends are viable for broad use requires an understanding of how various ethanol-gasoline blends impact emissions, engine performance, and fuel economy over time. To offer thorough insights into practical applications, future research should involve field testing and performance monitoring of ethanol-fuelled engines under various operational and climatic conditions. Furthermore, little is known about how ethanol fuel blends affect ignition timing, fuel injector clogging, and long-term engine wear.

Moreover, integrated techno-economic and environmental feasibility studies for the production of bioethanol based on DES are severely lacking. A complete method that includes both techno-economic analysis (TEA) and life cycle assessment (LCA) is needed to fully understand how practical it is to produce bioethanol from DES, since many studies look at economic costs and environmental effects separately. To support the widespread adoption of bioethanol, more research is needed to examine supply chain logistics, government incentives, and industrial implementation strategies. Investment in sustainable fuel technologies and increased market acceptance of bioethanol could result from a comprehensive policy framework that supports its production. There is also room for more research because the contribution of government subsidies and carbon credit incentives to lowering the cost of producing bioethanol has not been fully assessed.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Introduction

This chapter describes the material tools, materials, and equipment methods used to produce bioethanol from Zambian corn stover by pretreatment with Deep Eutectic Solvent (DES). The collection and preparation of lignocellulosic biomass was the first step in the study's systematic, multi-stage experimental design. A solution made of lactic acid and choline chloride was used to treat the biomass; it was meant to break apart the complex structure of lignocellulosic material and make it easier for enzymes to digest. Using mathematical and statistical modelling techniques implemented in the Design Expert software through Central Composite Design (CCD), the DES pretreatment and enzymatic hydrolysis stages were optimised to maximise efficiency. These models allowed for a thorough assessment and prediction of key factors that affect sugar yield and conversion speed. After that, microbial fermentation was applied to the hydrolysate to produce ethanol. We purified the recovered ethanol and mixed it with petrol in various ratios to assess its performance.

In addition to exhaust emissions such as CO, NO_x, HC, and CO_2, engine tests were performed to evaluate brake thermal efficiency, brake specific fuel consumption, and power output. These evaluations revealed the practicality of ethanol-gasoline blends as renewable fuel alternatives. The chapter's conclusion explains the methods used to evaluate the entire process for being environmentally friendly and economically feasible, including the techno-economic analysis and life cycle assessment (LCA). To guarantee scientific rigour and reproducibility, every procedure, piece of equipment, and material was meticulously documented.

3.2. Experimental Materials

3.2.1 Reagents and Standards

To conduct this research, the following standards and reagents were manufactured and purchased: cellulase enzymes (SAE0050), saccharomyces cerevisiae yeast 424A (LNH-ST), distilled water, lactic acid (LA-AR ≥85% purity), choline chloride (ChCl-AR ≥98% purity), and glucose G8270.

3.2 Equipment

Various tools were employed throughout the study to grind the corn stover, dry it and perform pretreatment, incubate enzymatic hydrolysis, ferment, distil, test for fermentable sugar, and assess the purity of bioethanol. See equipment pictorial details as used in the experiments in APPENDIX 1

Table 3. 1: Equipment Used During Experiments

Equipment	Model	Purpose
Edibon model computer-controlled bioethanol processing unit	EBEC	1. Enzymatic hydrolysis 2. Fermentation 3. Distillation
High Performance liquid Chromatograph (HPLC)-RID	SHIMADZU Nexera XR	Fermentable sugar determination
Gas Chromatograph Mass Spectrometry (GC-MS)	SHIMADZU GCMS-TQ8040	Bioethanol Alcohol level
Atico Computer Controlled Hybrid Test Bench with fuel flow meter, airflow meter, torque tester and speed sensors	ATE-987	Engine performance parameters
Gas Analyzer	Viskor Va 500	Determination of emission levels during engine tests
Grinding Mill	AL-KO	Grinding corn stover
Sieve	Gilson	Separation of solid biomass from the liquid
Magnetic stirrer	F20500570F1	Agitating mixtures
Digital scale	PR224	Weighing of lab materials
Fischer Scientific Oven	650-58	Drying corn stover
Atico Tiltable Crucible Electrical Furnace	ATE-123	Pretreatment of corn stover
Thermo Fisher Scientific stirrer	Max Q 6000	Agitating Slurry
Sterile, conical flasks, glass funnels graduated cylinders, pitets	Assorted	Various measurements during experiments

3.3 Experimental Procedures

The study used very detailed methods that involved statistical, along with mathematical models and machine learning, to explore the process of turning corn stover into bioethanol. First, the corn stover was thoroughly physiochemically characterised, including its ash, moisture content, extractives, and structural carbohydrates (cellulose and hemicellulose). Next, lignin was added. The pretreatment and enzymatic hydrolysis procedures were optimised using the Response Surface Methodology (RSM). The two processes were optimised, and Design of Experiment (DOE) was carried out using Design Expert-13 software via Central Composite Design (CCD). To determine the relationship between process parameters and the yields of fermentable sugar and cellulose, this method required the use of second-order polynomial regression models. To better understand how different process factors affected the amounts of fermentable sugar and cellulose produced, machine learning models were used, especially Boosted Regression Trees (BRT), to enhance the accuracy of the predictions by analysing complex relationships and focussing on the experimental data from the pretreatment. During the study, corn stover was pretreated using the Deep Eutectic Solvent (DES), which consists of lactic acid and choline chloride. These substances were chosen due to their eco-friendly characteristics and their efficiency in delignifying biomass. To increase the yield of fermentable sugars, enzymatic hydrolysis was then carried out using ideal pretreated corn stover and ideal conditions.

Before the enzymatic hydrolysis, the optimal hydrolysate yield was fermented, and bioethanol was obtained through distillation. In the other set of experiments, gasoline/bioethanol blends were tested and evaluated for engine performance and emission characteristics. In order to evaluate the bioethanol product's suitability as fuel, blends of petrol and bioethanol were conducted and carried out tests of their emission characteristics and engine performance. The last assessment was a techno-economics study of the bioethanol production process using DES optimisation from laboratory scale to commercial scale for blending from E10, E20, E30 and E40. It also assessed the gains in carbon credits and the price of petrol at the pump using Zambia's 2023 petrol consumption as a baseline.

3.3.1 Collection of Biomass

The corn stover sample, purchase, and gathering took place at Satnum Farm in Lusaka, Zambia. Prioritising maintaining sample integrity and avoiding contamination or degradation, the collected corn stover samples were dry cleaned to remove sand and then stored in a lockable cabinet in breathable, sterile Ziplock bags to prevent microbial growth(Davis et al., 2022).

3.3.1.1 Grinding Biomass

The gravimetric method was used to dry the corn stover prior to grinding. In order to achieve this, the biomass was put in a Fischer Scientific oven that was set to 40°C. It was then weighed every 30 minutes until the weight stayed constant, signifying that the moisture content had stabilized. Despite its apparent simplicity, the method worked well for figuring out the consistent weight of corn stover and the moisture content at that point, which was thought to be between 10 and 20 per cent, which is within the range for grinding (Wright, 2023).

Following drying, the corn stover was ground in an AL-KO model hammer mill, which was set up for particles ranging from 1 to 2 mm because this size was perfect for pretreatment and could improve the corn stover's ability to extract cellulose (Paul et al., 2024).

3.3.2 Characterization of Corn Stover

3.3.2.1 Proximate Analysis

To ascertain the basic composition of corn stover, proximate analysis had to be performed. This comprised extractives, ash content, and moisture content. With an eye towards bioconversion, specifically bioethanol, these parameters gave the corn stover's energy potential. The following was the method used to determine the moisture content

i. Moisture Content

Dried corn stover were sampled and weighed 3.0 g. After that, the corn stover was kept in an oven set to 105°C for 24 hours. The sample was dried, cooled, and then weighed again (E1755-01, 2007). The Moisture content was calculated using the following formula, noting all equations were summarized in APPENDIX 2.

$$Mosture\ Content\% = \frac{W_i - W_f}{W_i} \times 100 \qquad 3.1$$

Where:

- W_i = Initial weight (before drying)
- W_f = Final weight (after drying

ii. Determination of Ash Content

The ash content procedure was as follows:

After being weighed in a clean crucible, 2.0g of the dried corn stover sample was added. In order to completely burn off organic matter, the sample was then placed in a crucible and heated to 550°C for four hours in a furnace. The crucible was burnt, allowed to cool, and then weighed again (Shon et al., 2021).

The ash content was calculated using the formula:

$$Ash\ Content\% = \frac{W_f}{W_i} \times 100 \qquad 3.2$$

Where:

- W_i = Initial weight (before burning)
- W_f = Final weight (after burning)

iii. Determination of Extractives

Dried corn stover weighing 1.5 g was placed in a previously weighed crucible to identify the extractives. Then, a furnace heated it to 950°C and held it there for seven minutes (Emrahi et al., 2021) The sample was weighed again after it cooled. The following formula was used to calculate the volatile matter content (Woźniak et al., 2021).

$$Extractives\ Content\% = \frac{W_i - W_f}{W_i} \times 100 \qquad 3.3$$

Where:

- W_i = Initial weight (before burning)
- W_f = Final weight (after burning)

3.3.2.2 Determination of Structural Carbohydrates and Lignin

The structural carbohydrate analysis of the biomass was done to determine the amounts of cellulose, hemicellulose, and lignin present. To give a general idea of what to expect from the pretreatment results, this was done prior to pretreatment.

i. Determination of Cellulose Content

The amount of cellulose was measured, a significant structural carbohydrate in biomass and the primary extraction resource during pretreatment. It contains glucose monomers connected by β (1→4) bonds. The acid hydrolysis chosen, which broke down cellulose to form glucose monomers. The cellulose matrix of corn stover was identified using the acid hydrolysis method (Hafid et al., 2021). 10 mL of 72% sulphuric acid (1:10 ratio) was added to a 1.0 g sample of corn stover that had been placed in an acid-proof crucible. To ensure adequate hydrolysis incubation time, the crucible was subsequently placed in an oven set to 30°C for two hours. After this time, the sulphuric acid concentration was diluted to 4% (v/v) by adding distilled water, but the original solution was kept (Almashhadani et al., 2022). Subsequently, the mixture was placed in an oven set at 100°C for three hours, completing the hydrolysis process while being manually stirred once every hour. After cooling to room temperature, the mixture was filtered through Whatman No. 1 filter paper to get rid of any solid residues. High-performance liquid chromatography (HPLC) was then used to analyse the filtrate and determine the glucose concentration (Reymond et al., 2020).

The formula for determining cellulose content:

$$Cellulose\ content\% = \frac{Y_g}{W_s} \times 0.9 \quad 3.4$$

Where:

- Y_g = Glucose yield (mg)
- W_g = Initial sample weight (mg)
- 0.9 = Conversion factor from glucose to cellulose

iv. Determination of Lignin Content

Lignin content is determined using a gravimetric method following acid hydrolysis, commonly referred to as the Klason lignin method. In this approach, a 1.0 g sample of corn stover is treated with 72% sulfuric acid at 30°C for two hours, similar to the cellulose method. The purpose of this step is to hydrolyze and solubilize the polysaccharides (cellulose and hemicellulose), leaving lignin largely intact due to its resistance to acid degradation. (Van Soest et al., 2020).

10 mL of 72% sulphuric acid was added to a 1.0 g sample in an acid-proof crucible at a 1:10 ratio. The acid concentration is then diluted to 4% using distilled water, and the sample is heated at 100°C for three hours to ensure complete breakdown of carbohydrates. After cooling, the mixture is filtered to separate the acid-insoluble residue, which is assumed to be primarily lignin (Tang et al., 2020).n. The residue is then dried and weighed. Lignin content is calculated as the percentage of the residue weight relative to the initial sample weight. Unlike cellulose analysis, no chemical quantification (like HPLC) is used, as the method relies solely on the physical mass of undigested lignin.(Yulia et al., 2024).

The lignin content was calculated using:

$$Lignin\ Content\% = \frac{W_r}{W_s} \times 100 \qquad 3.5$$

Where:

- W_r = Residue weight (mg or g)
- W_s = Initial sample weight (mg or g)

v. Determination of Hemicellulose Content

By subtracting the remaining identified characterisation components of the biomass from 100, we calculated the hemicellulose content using the mass balance approach (see equation 3.6). Because there was no standard for xylose and arabinose, High-Performance Liquid Chromatography (HPLC), which was used to quantify cellulose, could not be used to determine hemicellulose (Puițel et al., 2024). Therefore, the hemicellulose content was estimated using mass balance. This helped guarantee that the total biomass composition included hemicellulose (Zhang et al., 2021).

When direct quantification is not possible, the mass balance method has always been a viable alternative in the event that one element is lacking in biomass compositional studies. It has also shown itself to be a trustworthy estimation technique. In this case, a careful analysis of moisture, ash, extractives, cellulose, and lignin identified hemicellulose as the remaining fraction (Durán-Aranguren et al., 2024) in this case. When access to comprehensive calibration standards was restricted, this approach was especially important.

Even in cases where changes in biomass structure may introduce small uncertainties, lignocellulosic biomass analysis still widely uses this technique. This method provided a trustworthy estimate of the hemicellulose content and is dependent on precise measurements of the remaining components (Kumar et al., 2021). The mass balance approach offered a reliable way to measure hemicellulose in biomass research when it is properly standardized and methodologically consistent (Piazza et al., 2024).

$$\text{Hemicellulose (\%)} = 100-(M+A+E+C+L) \quad 3.6$$

Where:

- M = Moisture content (%)
- A = Ash content (%)
- E = Extractives (%)
- C = Cellulose (%)
- L = Lignin (%)

3.4 Optimization of Deep Eutectic Solvents (DES) Pretreatment for Enhanced Cellulose Yield Ffom Corn Stover

Three crucial steps were used in the methodical pretreatment of corn stover using Deep Eutectic Solvents (DESs): (1) drying the stover to remove excess moisture, (2) creating the DES solvent by mixing lactic acid (LA) and choline chloride (ChCl) in molar ratios, and (3) carrying out the pretreatment experiment under controlled conditions under the direction of a methodically planned experimental framework. These procedures ensured that the corn stover was adequately prepared for the lignocellulosic breakdown that followed, allowing for the identification of ideal conditions that maximized cellulose yield. According to the National Renewable Energy Laboratory (NREL), each step of the pretreatment process was described in detail in the sections that followed

i. Drying of Corn Stover

To prepare it for uniformly distributed interaction with the deep eutectic solvents (DESs), the corn stover was dried. The corn stover was further dried to make sure that the moisture content wouldn't interfere with the pretreatment process itself, impeding the efficiency of pretreatment conditions like temperature and, most importantly, diluting the DES (Sluiter, 2021).

- Procedure for Drying Corn Stover

1. A Fisher Scientific electric oven set to 60°C was used to further dry the previously dried corn stover. The biomass was tested for dryness at 1-hour intervals and recorded the weight of each interval. The drying process was continued until the static weight was reached, which meant that the corn stover weight remained constant over two consecutive weighing attempts.

2. The drying procedure was carried out until the static weight was attained, meaning that the weight of the corn stover did not change during two successive attempts at weighing.

This technique ensured that the moisture content didn't hinder the DES's ability to soak into the biomass and complete the necessary chemical reactions for effective pretreatment (Nahar et al., 2021).

ii. Synthesis of Deep Eutectic Solvents (DESs)

One of the preparatory steps in the pretreatment process of corn stover was the synthesis of Deep Eutectic Solvents (DESs). Using precise ratios determined by each pretreatment reagent's molar requirements, the two were synthesised (see APPENDIX 2). Choline chloride was a solid powder, whereas lactic acid was a liquid. The necessary molar ratios were computed using information from the literature (Ge et al., 2023), based on the ratios chosen for the pretreatment procedure. Table 3.2 displays the ratios for the DES synthesis (lactic acid to choline chloride). To guarantee that the choline chloride was completely dissolved, this mixture was stirred at regular intervals throughout the procedure, and eventually a homogeneous liquid phase was produced. The deep eutectic solvent (DES) in this study consists of a hydrogen bond donor (HBD) and an acceptor (HBA), and they need to work together for the DES to form(Altamash et al., 2020). Thus, lactic acid (LA) was determined to be the HBD and choline chloride (ChCl) to be the HBA during this investigation. This combination is well-known and valid because it works well for pretreatment of lignocellulosic biomass. To encourage hydrogen bonding and produce a solid and uniform DES, the mixture was appropriated using molar ratios (Sazali et al., 2023).

Table 3. 2: Deep Eutectic Solvents Ratios(Kwon et al., 2020)

No.	Ratio	Choline Chloride Solution	Lactic Acid
1	1:2	100ml	117.2ml
2	1:6	100ml	351.8ml
3	1:10	100ml	586.0ml

3.4.1 Design of Experiment (DOE) for Corn Stover Pretreatment

A thorough Design of Experiment (DOE) framework, using statistical guidance, was employed to determine the ideal pretreatment conditions for corn stover valorization as a lignocellulosic (biomass) feedstock for bioethanol production (Pashaei et al., 2020). Using the Response Surface Methodology (RSM) to traverse the intricate response surface through the use of the Design Expert-13 software interface for experimental design sequence and analysis, this investigative phase concentrated on the empirical optimization of cellulose yield, the principal response variable (Njoku & Otisi, 2023). The Face-Centred Central Composite Design (FCCD) was selected from various Response Surface Methodology (RSM) options because it is easy to use in experiments and works well for estimating second-order (quadratic) models. Axial points, which correspond to an alpha (α) value of 1, are strategically placed in the center of each face of the factorial cube when using FCCD (Nurmitasari & Mahfud, 2021). This setup lowered the chances of the model becoming unstable and making predictions beyond realistic experimental conditions by ensuring all experimental levels stayed within the defined limits, which should be within the factor range (Szpisják-Gulyás et al., 2023).

Four important independent variables were chosen for this study based on their essential role in affecting biomass deconstruction efficiency. These variables were each coded at three levels: low, center, and high (Maraphum et al., 2022). To estimate their individual and combined effects, these variables—which include linear and curvature quadratic—were integrated into the FCCD framework. There were 30 distinct runs in the experimental design matrix, including factorial, axial, and CenterPoint replicates (Jankovic et al., 2021). To increase the model's robustness and evaluate system reproducibility, center points were incorporated into the experimental design. By carefully managing the process settings, the pretreatment tests were done according to the planned experiment (Vollmer et al., 2022).

After each experiment, the amount of cellulose was measured using the gravitational method, once the solid part of the pretreated biomass was separated from the liquid chemicals by sieving. To find the cellulose yield compared to the original dry mass, the fibrous leftover had to be dried until it reached a stable weigh (Fagundes et al., 2021). To calculate the cellulose yield as a ratio of the initial dry mass, the fibrous residue had to be dried to a constant weight. The gravimetric method provided an accurate and verifiable indicator of each pretreatment condition's effectiveness. For modelling purposes, each cellulose yield result was documented as response data (Danesh et al., 2020). The design's structured framework made effective exploration of the multi-dimensional process space and the creation of accurate predictive models possible. Models serve as the foundation for determining the ideal pretreatment parameters that would maximize the yield of fermentable sugar during later processing phases. The experimental protocols and investigative design were consistent with the larger body of literature in biomass conversion and bioethanol research, as well as the best practices used in recent lignocellulosic feedstock optimization research, ensuring scientific rigor (de Almeida Moreira et al., 2023).

Input Factors were:

1. Residence Time (hours): 6, 10.5, and 15 hours
2. Reaction Temperature (°C): 60, 105, and 150°C
3. ChCl:LA Molar Ratio: Varying ratios of ChCl and LA (stoichiometric calculations for mixing).
4. Corn Stover: Solvent Ratio (weight basis): 1:08, 1:20, and 1:32

The level of factors that were studied are shown in Table 3.3.

Table 3. 3: Independent Variables and their levels used in the Central Composite Experimental Design

Variables		Levels		
Uncoded	Coded	-1	0	+1
LA/ChCl	X_1	117.2	351.8	586.0
Temp (°C)	X_2	60	105	150
Time (hrs)	X_3	6	10.5	15
Solvent: Solid Ratios	X_4	8	20	32

Procedures:

The process started by treating 1.0 gramme of biomass with the made DES solution for each test, as shown in Table 3.4. The different pre-treated biomass are shown in APPENDIX 3

Table 3. 4: Experimental Design-Pretreatment

Experiment	Process Parameters		Fact+E1:F26 or 3	Factor 4	Responses	
	A: Residence time	B: Reaction temperatures	ChCl:LA	corn stover: solvent	Pretreatment Yield	
	Hours	Degree Celsius	Ratio	Ratio	Actual (g)	Predicted (g)
1	10.5	105	100:351.8	1:32		
2	6	150	100:117.2	1:08		
3	10.5	105	100:351.8	1:20		
4	6	60	100:117.2	1:08		
5	10.5	105	100:351.8	1:20		
6	15	60	100:117.2	1:08		
7	15	150	100:586.0	1:08		
8	10.5	105	100:351.8	1:20		
9	6	150	100:117.2	1:32		
10	10.5	105	100:351.8	1:20		
11	6	150	100:586.0	1:32		
12	10.5	105	100:351.8	1:20		
13	15	150	100:117.8	1:20		
14	6	105	100:117.8	1:32		
15	10.5	105	100:586.0	1:20		
16	10.5	105	100:351.8	1:08		
17	10.5	105	100:351:8	1:20		
18	10.5	105	100:117.2	1:20		
19	15	150	100:117.2	1:32		
20	15	150	100:586.0	1:32		
21	6	60	100:586.0	1:08		
22	10.5	60	100:351.8	1:20		
23	6	60	100:586.0	1:32		
24	6	150	100:586.0	1:08		
25	15	60	100:586.0	1:32		
26	15	60	100:117.2	1:32		
27	15	60	100:586.0	1:08		
28	6	105	100:351.8	1:20		
29	10.5	150	100:351,8	1:20		
30	15	105	100:351.8	1:20		

1. Then, the biomass and DES mixture were heated to a specific temperature (60°C, 105°C, or 150°C) for a set amount of time (6, 10.5, or 15 hours). The biomass and DES mixture were then exposed to a controlled reaction temperature (60°C, 105°C, or 150°C) for a predetermined residence time (6, 10.5, or 15 hours). To guarantee consistent heating during the pretreatment procedure, an electric tiltable crucible furnace was employed.

2. After finishing the pretreatment, the mixture was allowed to cool to room temperature. A sieve was then used to separate the DES from the pretreated corn stover.

3. The biomass was dried once more at 60°C to a constant weight after being thoroughly cleaned with distilled water to get rid of any remaining DES.

4. The entire procedure was repeated twice

This Design of Experiment (DOE) approach targeted reliable and repeatable outcomes in line with NREL's biomass pretreatment protocols, serving as a guide for the optimization process and playing a crucial role in determining the best pretreatment conditions for corn stover (Dowe, 2023)

A second order polynomial model equation was further used to predict the pretreatment results with the following order:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i<j}^{4} \beta_{ij} X_i X_j \qquad 3.7$$

Where:

Y is the response (cellulose yield),

X_1, X_2, X_3 and X_4 represent the independent variables (LA/ChCl, Temperature, Residence Time, and Solvent: Solid Ratio),

B_0 is the intercept,

βi are the linear coefficients,

βii are the quadratic coefficients, and

βij are the interaction coefficients.

3.4.1.1 Analysis Of ANOVA

The statistical uniqueness of the models used for corn stover pretreatment was examined using analysis of variance (ANOVA). The experimental results were analyzed using the Two-Factor Interaction (2FI) model, and predictions were made using the quadratic model. The ANOVA results, which included F-values, p-values, and lack-of-fit tests, were used to determine which model was the most suitable.

3.4.1.2 Statistical Analysis

The ratio of lactic acid (LA) to choline chloride (ChCl), reaction temperature, reaction time, and the ratio of corn stover to solvent all had an impact on how well the DES pretreatment worked. The effects of these four independent variables on cellulose yield were investigated using Central Composite Design (CCD). The following variables were taken into account: residence time (X_3), reaction temperature (X_2), the LA/ChCl ratio (X_1), and the ratio of corn stover to solvent (X_4). These factors were optimized to improve the cellulose yield (Y), the chosen response variable. An accurate response surface model was created by exploring the factor space using the particular coded and uncoded levels for each variable, as indicated in Table 3.3. To ensure the accuracy and dependability of the experimental results, this strategy adhered to accepted optimization techniques (Smith et al., 2021). By following the NREL standard, the designer minimized experimental runs while guaranteeing an organized and effective optimization process.

3.4.1.3 Cellulose Yield Determination

Each run's cellulose yield was calculated using the gravitational method. The process involved determining the biomass's initial weight prior to pretreatment and its final weight following pretreatment. The cellulose yield is the final biomass weight following pretreatment. In each experiment, the cellulose yield were measured using the gravimetric method to see how effective the pretreatment was (Cariaga et al., 2023).

Deep eutectic solvents (DES), particularly lactic acid and choline chloride, aided in the removal of lignin and hemicellulose during the pretreatment. After dissolving the hemicellulose and breaking up the lignin, the cellulose fraction was removed from the biomass. The accessibility of cellulose for enzymatic hydrolysis was enhanced by this procedure.

The gravimetric method has been widely applied in biomass pretreatment studies. Researchers have calculated cellulose yield as a measure of a solvent's effectiveness in delignification and hemicellulose removal (Sinquefield et al., 2020). Following pretreatment, the technique guarantees precise quantification of cellulose recovery.

The cellulose yield percentage was calculated using the following formula:

$$\text{Cellulose yield} = \frac{\text{Final weight of biomass}}{\text{Initial weight of biomass}} \times 100$$

This formula measured the amount of cellulose that was still present in the biomass following pretreatment, which was an essential sign of how well the pretreatment had eliminated non-cellulosic substances. The following is a summary of the approach, which is frequently used in biomass research to evaluate cellulose recovery following different pretreatment techniques:

$$Cellulose\ Yield = \frac{Y}{X} \times 100 \qquad 3.8$$

where:

X = Initial weight of biomass (before pretreatment)

Y = Final weight of biomass (after pretreatment

3.5 Optimization of Enzymatic Hydrolysis of Cellulose Using Central Composite Design for Enhanced Bioethanol Production

This section presents the process for optimising the enzymatic hydrolysis of cellulose to produce fermentable sugar yield. To determine the ideal conditions for the highest fermentable sugar yield from the corn stover slurry, the optimisation process used the Central Composite Design (CCD) model of the Design Expert-13 software. The temperature and time were optimised by maintaining a constant hydrolysate. The procedure was carried out using the Edibon Computer Controlled Bioethanol Processing Unit, which has exact controls for monitoring during the enzymatic hydrolysis phase. Following enzymatic hydrolysis, the hydrolysate was added to the fermentation tank for fermentation and distillation, marking the final step in the production of bioethanol. Effective processing under controlled conditions was made possible by the Edibon system's integration of fermentation and distillation processes. Prior research demonstrated the efficacy and efficiency of employing CCD to enhance enzymatic hydrolysis procedures. The benefits of employing sophisticated bioprocessing units in the production of bioethanol were illustrated by Chen, Wang, et al., (2021) who demonstrated the advantages of using advanced bioprocessing units in bioethanol production and supported by (Gao et al., 2020). By optimizing the enzymatic hydrolysis step, this all-encompassing strategy aimed to increase bioethanol production efficiency (Sharma et al., 2020).

i. Preparation of Biomass

Boosted Regression Trees (BRT) forecasted the best conditions, and the biomass for enzymatic hydrolysis was sourced from a separate pretreatment experiment. The central input parameters produced the best yield according to the BRT prediction. The ideal yields from the real experiments and the outcomes predicted by the polynomial model equation were lower than the ideal prediction of 0.461 g. To achieve the actual optimal figure, or something very close to it, a series of four additional runs based on the BRT optimal conditions central were carried out. Even though the ideal yield couldn't be achieved, the closest yield of 0.4601g, was used for further processing. The pretreated biomass underwent enzymatic hydrolysis and cellulose yield analysis.

ii. Slurry Preparation

The first step after finishing the pretreatment process was to prepare the slurry for enzymatic hydrolysis. Cellulose fibres are submerged in distilled water, and cellulase enzymes are used to create a slurry (Ren et al., 2020). This procedure made it possible for the biomass to be properly hydrated and distributed with enzymes, both of which were essential for effective hydrolysis. Thereafter, the mixture was incubated for 24 hours to allow for the first enzyme activity, which increased the cellulose discharge and made the mixture homogenous for subsequent processing (Martínez-Trujillo et al., 2020).

iii. Mixing Biomass, Distilled Water, and Enzymes

Sample preparation was done using the best cellulose yield of 0.4601 g, which was reached through several experiments to find the best conditions for Boosted Regression Trees (BRT). The necessary ratios of biomass, distilled water and liquid cellulase enzyme (SAE0050) were combined. To guarantee adequate liquid availability for enzymatic action while avoiding excessive dilution, a ratio of 10 mL of distilled water per gram of cellulose solids was employed (Sohail et al., 2023). Also, to ensure the right amount of enzyme for effective breakdown, 10 mg of cellulase enzyme was added for every gramme of biomass (Pimentel et al., 2021). The method balanced the enzyme performance. To ensure uniform dispersion and avoid settling, the mixture was gently agitated. The mixture became isomorphic after the slurry was incubated for 24 hours (Pulunggono et al., 2022).

iv. Sieving and Separation of Hydrolysate

A 150-micron mesh sieve was used to remove any remaining solids from the slurry after a 24-hour incubation period. Weighing the liquid hydrolysate considered the wet solids' absorption of water and enzymes. In line with earlier findings, the sieved hydrolysate's final weight was 4.302 g, meaning that roughly 15% of the liquid mass was lost to the residue solids (Padierna-Vanegas et al., 2022).

v. Transfer to Edibon Bioethanol Processing Unit

The filtered liquid hydrolysate was then transferred to the Edibon Computer Controlled Bioethanol Processing Unit for enzymatic hydrolysis, completing the process. To avoid clogging and guarantee smooth operation, only the liquid slurry was moved into the processing unit for this step. Based on the experimental setup, the hydrolysate was kept in the processing unit for 60, 66, or 72 hours at temperatures of 45°C, 47.5°C, or 50°C (San Martin et al., 2021). To effectively break down cellulose into sugars that can be fermented, the controlled incubation conditions ensured that the enzymes had plenty of time to work on the substrate. (Tsegay et al., 2024).

3.5.1 Design of Experiments (DOE) Using Central Composite Design (CCD)

The Central Composite Design (CCD) model in the Design Expert 13 software (Stat-Ease 360) domain was used to integrate a structured Design of Experiments (DOE) method for optimizing the enzymatic hydrolysis of pretreated corn stover for fermentable sugar yield. To streamline process optimization with a sufficient and low number of experimental runs, this statistical method was chosen for its dependability in validating the quadratic effects and interactions of multiple process variables.

Temperature and time were the independent variables under investigation. These variables were chosen because of their well-studied effects on resilience, enzyme activity, and total saccharification efficiency. Three specific temperature ranges—45°C, 47.5°C, and 50°C—were identified as effective for enzymatic hydrolysis, as they improved enzyme performance while keeping thermal stability intact (López-Gutiérrez et al., 2021). Additionally, three time ranges—60, 66, and 72 hours were selected —based on prior research demonstrating their effective sugar release (Sinquefield et al., 2020).

To evaluate experimental reproducibility and improve model reliability, a total of 13 experimental matrices of runs were created, including replicates at the center point (47.5°C, 66 hours). To guarantee consistency in the slurry composition and enzyme concentration across all runs, a constant hydrolysate mass of 4.302 g was maintained for each experiment. Separating the effects of time and temperature on the hydrolysis pathway required this level of control. Generally, the CCD-based DOE facilitated a thorough and statistically sound analysis of the process parameters governing fermentable sugar yield. Table 3.5 illustrates how the design was made.

Table 3.5: Design of Experiment Matrix -Enzymatic Hydrolysis

Experiment	Temperature (ºC)	Time (hours)	Fermentable Sugars Yield (%)
1	47.5	60	
2	45	60	
3	47.5	66	
4	47.5	66	
5	50	72	
6	47.5	72	
7	50	60	
8	47.5	66	
9	47.5	66	
10	45	66	
11	50	66	
12	45	72	
13	47.5	66	

The response of the system (fermentable sugar yield) was modeled using a second-order polynomial equation 3.2

$$Y=\beta_o+\beta_1A+\beta_2B+\beta_{12}AB+\beta_{11}A^2+\beta_{22}B^2 \qquad 3.9$$

Where:

Y is the response (fermentable sugar yield),

β is the intercept

A is the temperature

B is the time

AB represents the interaction between temperature and time

A^2 and B^2 are the squared terms that account for nonlinear effects

3.5.1.1 Coded and Uncoded Levels of the Independent Variables

To study how temperature and residence time affect the amount of fermentable sugars produced from breaking down corn stover biomass with enzymes, the experiment was designed using a Central Composite Design (CCD)(Sunar, Oruganti, et al., 2024). The variables were tested at three different levels: low (-1), Centre (0), and high (+1), along with their actual measurements in degrees Celsius for temperature and hours for time (Khaled, 2024).

Table 3.6 shows the three selected levels, to maximise the enzyme's stability and activity within a realistic operating range. All tests remained within the recommended conditions for cellulase activity because the temperature (45°C to 50°C) and residence time (60 hours to 72 hours) were chosen based on earlier studies and practical limits (Shangdiar et al., 2023).

Table 3. 6: Independent Variables and their levels used in the Central Composite Experimental Design

Variables		Levels		
Uncoded	Coded	-1	0	+1
Temp (°C)	A	45	47.5	50
Time (h)	B	60	66	72

3.5.1.2 ANOVA For Model Validation

The quadratic model used to optimise enzymatic hydrolysis was subjected to an analysis of variance (ANOVA) to assess the significance of its components. ANOVA made it possible to identify important factors influencing the yield of fermentable sugars by helping to partition the variability in the response data (Yildirim et al., 2023).

Time and temperature, as well as their interaction, were two independent variables in the model.

3.5.1.3 Calibration and HPLC Analysis of Fermentable Sugars

The hydrolysate's fermentable sugars were measured using High-Performance Liquid Chromatography (HPLC). This is the most dependable technique for precise sugar analysis in the hydrolysis of lignocellulosic biomass. A calibration curve for glucose was created using standard solutions with concentrations ranging from 100 to 1500 ppm, in accordance with NREL standards for sugar quantification (Piñón-Muñiz et al., 2023)

In order to guarantee a linear relationship between concentration and detector response, the HPLC system was calibrated by injecting known concentrations of glucose (Ristović et al., 2024). To measure the sugar, a 20 µL sample of the hydrolysate was put into the HPLC system that had a refractive index detector (RID). A mobile phase consisting of acetonitrile and water (80:20 ratio) was used to elute the column at a flow rate of 0.5 mL/min. The sugars in the hydrolysate were identified using the glucose retention time (Sganzerla et al., 2022).

3.5.1.4 Determination of Fermentable Sugar Yield

High-Performance Liquid Chromatography (HPLC) was used to ascertain the ideal fermentable sugar yield in the hydrolysate following completion of the enzymatic hydrolysis process (Barbosa et al., 2021). Fermentable sugars were released as a result of the cellulase enzyme breaking down the cellulose in pretreated corn stover. Based on the initial biomass weight, the fermentable sugar yield was computed using the sugar concentrations.

The formula for determining the fermentable sugar yield is as follows:

$$Fermentable\ sugar\ yield\% = \left(\frac{Total\ fermentable\ sugars(g)}{Initial\ bionass(g)}\right) \times 100 \qquad 3.10$$

Where:

Total Fermentable Sugars (g) were obtained by multiplying the concentration of fermentable sugars (g/L) by the total volume (L) of the hydrolysate.

3.5.1.5 Data Analysis

Fermentable sugar concentrations were measured using High-Performance Liquid Chromatography (HPLC) with a refractive index detector, a highly accurate technique frequently used to assess sugar content in biomass hydrolysates (Chatzifragkou et al., 2021). To provide a reliable reference point and enable precise interpretation of sugar concentrations across samples, the HPLC system was calibrated using a number of sugar standards (Santana Junior et al., 2021).

As is customary in biomass conversion studies, the chromatograph's results were then examined to determine the concentrations of fermentable sugar as a percentage of the original biomass (Eom et al., 2021). Enzymatic hydrolysis efficiency can be directly and meaningfully compared across different experimental runs and conditions thanks to the method of expressing sugar yield as a percentage. This method provided a thorough understanding of how the enzyme functions in various hydrolysis scenarios and demonstrates how ideal conditions can improve biomass-to-sugar conversion (Chen et al., 2023).

3.5.1.6 Fermentation And Distillation

The two last interconnected steps in the production of bioethanol are fermentation, in which yeast converts fermentable sugars following enzymatic hydrolysis into ethanol and carbon dioxide, and distillation, which separates the fermentation broth from water and other leftover substances to produce bioethanol. To guarantee high yields and ideal ethanol purity, these interrelated processes need to be closely regulated and observed, especially when producing bioethanol for industrial or fuel-blending uses.

The computer-controlled Edibon Bioethanol Process Unit, which was used for this study, combined the fermentation and distillation phases, allowing for a smooth transition between the two procedures. Saccharomyces cerevisiae, the yeast strain that turns the sugar-rich hydrolysate into ethanol, was cultivated during the fermentation stage. After fermentation was finished, the broth was sent straight to the distillation unit, where it was heated and vaporized to separate the ethanol from the remaining liquids.

Temperature, pH, and fermentation time were all precisely controlled thanks to the combination of computer-controlled systems and automated monitoring. This integration made it possible to produce bioethanol efficiently and scalable while reducing the possibility of contamination or ethanol loss during stage transitions. The steps and technologies used for each stage of the fermentation and distillation processes were described in detail in the sections that followed.

3.5.1.7 Fermentation Process

Saccharomyces cerevisiae, a type of yeast, breaks down fermentable sugars during the biological process of fermentation, producing carbon dioxide and bioethanol. The production of bioethanol was significantly impacted by the conditions under which fermentation took place, and to achieve the best results, a number of variables, including temperature, pH, agitation, and yeast strain, had to be carefully managed. The Edibon Computer-Controlled Bioethanol Process Unit kept these parameters within the ideal ranges for the highest bioethanol yield. 3.52 grammes of hydrolysate were prepared at the start of the procedure, and the presence of fermentable sugars was first determined through pre-analysis using high-performance liquid chromatography (HPLC). The Edibon unit, which processes bioethanol at every stage, had its fermentation tank filled with this hydrolysate. According to Almeida et al., (2023) Saccharomyces cerevisiae was pre-activated in a 10% glucose solution at 35°C for 30 minutes to guarantee the yeast's metabolic readiness. In accordance with NREL's suggested yeast-to-substrate ratio of 1 gramme of yeast per 10 grammes of hydrolysate, 0.0352 grammes of yeast were added to the hydrolysate after activation (Huang & Reardon, 2022).

The ideal temperature for Saccharomyces cerevisiae activity, 40°C, was chosen for the fermentation process. As advised by Juneja & Kumar, (2024) a water-heating bath with a regulated temperature circulates around the fermentation tank to maintain a constant temperature throughout the process. Regularly the pH of the fermentation broth was checked, and the Edibon Computer bioethanol processing unit maintained it within the ideal range of 4.5 to 5.0. In order to ensure that fermentation proceeded without inhibition, the Edibon unit's acid actuators were programmed to automatically add acid to the broth in order to adjust the pH if it deviated from this range (Ma et al., 2021). Throughout the process, the fermenting broth was continuously mixed to ensure even sugar distribution and avoid the development of bioethanol gradients. As suggested by (Khan et al., 2023) , the agitation also helped to lessen the inhibitory effects of bioethanol on yeast growth. The machine unit controls were timed to shut down at the designated time, and the fermentation was scheduled to run for 72 hours. To ensure exact control over the fermentation duration and avoid over-fermentation, the computer-controlled system of the Edibon unit automatically stopped fermentation after the predetermined time. The computer system was routinely checked for pH and temperature during the fermentation period. The pH range was within the range, and if the level strayed outside of it, acid could be injected. Crucially, during the fermentation process, there was no way to directly check the amount of bioethanol. The broth was prepared for the distillation stage after the 72-hour fermentation period was completed.

3.5.1.8 Distillation Process

The last stage of producing bioethanol was distillation, which separated the bioethanol from the fermentation broth. Distillation was used to purify the bioethanol by eliminating water and other impurities, resulting in a high-concentration ethanol product that could satisfy fuel-grade requirements. Instead of draining the fermentation broth after the process, the Edibon Bioethanol Process Unit design moved it to the heating mantle via a connecting pipe so that distillation could begin. A pump was used to move the broth to the heating mantle; it was activated by a computer and stopped when it displayed a pressure drop and signaled that it had been transferred completely. After placing the broth on the heating mantle, the heating elements were turned on, and the distillation process started.

After fermentation was finished, the broth was moved to the distillation unit, where it was heated to 78.5°C, the boiling point of bioethanol, and it started to boil. The bioethanol started to boil at 78.5°C after the temperature progressively increased. Evaporation began at the boiling point, leaving the water on the heating mantle while the alcohol gathered at the machine's condenser. The temperature settings at the boiling point remained at 78.5°C until the entire evaporation process was finished (AbdElhafez et al., 2022).

When vapor was produced during the separation process, it flew upward along the distillation column and came into contact with a number of trays or packing materials. The materials improved the separation of bioethanol from water and heavy impurities by increasing the contact surface area between the liquid and vapor. The residual liquid, which mostly consisted of water and other heavier substances, stayed at the bottom of the column while the bioethanol vapor rose through it (Prado et al., 2023). The bioethanol vapor flowed through the condenser on top of the distillation column, where it was cooled and condensed back into a liquid state. The receiver flask was used to collect the purified bioethanol. The condensation step ensured the separation of ethanol from remaining water and contaminants, producing a high-concentration bioethanol distillate (Oke, 2023). To control any potential intermittency during the distillation process, the temperature was frequently checked. Since the distillation process is carried out in a transparent glass assembly, the entire process was visible. Following distillation, the bioethanol was gathered and transported to a third-party facility for Gas Chromatography-Mass Spectrometry (GC-MS) quality analysis. The purpose of this analysis was to determine the quality of the bioethanol produced, make sure it was bioethanol of a specific grade, and find any remaining impurities. The Results and Discussion section will include comprehensive yield results and discussions.

3.5.1.9 Process Integration And Optimization:

The Edibon Bioethanol Process Unit's integration of the fermentation and distillation processes reduced the possibility of contamination or ethanol loss by ensuring a seamless transition between stages. The computer-controlled operation of the system ensured that the distillation conditions were optimized for maximum ethanol yield while also enabling precise regulation of important fermentation parameters like temperature, pH, and fermentation time.

The automated system closely monitored both phases, and an internal pump allowed the fermentation broth to be smoothly moved from the fermentation tank to the distillation unit, reducing the possibility of contamination. By ensuring that every step of the process—from fermentation to distillation—was effective and carefully managed, this integration eventually produced high-purity bioethanol.

3.6 Gasoline/Bioethanol Blends and Engine Performance Parameters

Following the completion of the bioethanol production process, the bioethanol from corn stover had to be blended with petrol to test it on an engine and look at various engine performance and operation parameters. For engine testing, the process required the production of enough bioethanol to mix with petrol. The NREL standards served as a guide for all of this work, ensuring that the methods employed are respectable (Abel et al., 2021). The strategy used a methodical procedure to assess the emissions and performance of different blends of bioethanol and petrol. Making the bioethanol and petrol was the first step in this study. The first step in this study involved carefully blending bioethanol and petrol at various ratios to produce fuel blends. After blending, the engine was calibrated and the measurement systems were connected to it using established standards to ensure precise data collection. This process involved calibrating sensors for gas emissions, fuel flow, exhaust temperature, torque, and brake power. Following calibration, the engine was tested using various fuel blends, and performance metrics like power output, fuel consumption, and exhaust emissions were continuously recorded in comparison to established benchmarks. Based on the information gathered, the performance metrics—such as brake power, fuel efficiency, and thermal efficiency—were then computed. Additionally, exhaust emissions were measured to evaluate each blend's environmental impact. These procedures were designed to ensure reliability and consistency in all tests, allowing for a thorough analysis of how bioethanol blending affects emissions and engine performance.

3.6.1 Blending Procedure

To ensure homogeneity and uniformity in the engine testing samples, bioethanol and petrol were blended to create various fuel mixtures. The following procedure describes the steps involved in preparing each fuel blend.

3.6.1.1 Preparation of Bioethanol and Gasoline:

To ensure homogeneity and uniformity in the engine testing samples, bioethanol and petrol were blended to create various fuel mixtures. The following procedure describes the steps involved in preparing each fuel blend (Konur, 2023).

I. **Blending Process**: Petrol and bioethanol were combined in different amounts to make the following blends (Kroyan et al., 2022):

   - **G100**: 100% gasoline (reference fuel)
   - **E10**: 10% ethanol, 90% gasoline
   - **E20**: 20% ethanol, 80% gasoline
   - **E30**: 30% ethanol, 70% gasoline
   - **E40**: 40% ethanol, 60% gasoline

II. **Mixing**: To avoid phase separation, the bioethanol and petrol were carefully mixed in a laboratory setting. To guarantee homogeneity and uniform ethanol distribution throughout the petrol, each blend was carefully mixed with a stirrer. To achieve uniformity and avoid fuel inconsistencies during experimental procedures, such mixing protocols were crucial (Kalinoski et al., 2021). Each blend was stirred for a further fifteen minutes after mixing to guarantee total homogenisation. This stage made sure there were no irregularities in the final fuel composition and that the ethanol was dispersed uniformly throughout the petrol (Liu et al., 2023).

III. **Storage**: To prevent contamination or evaporation, the fuel mixtures were kept in sealed containers after they were homogenised. Given that biofuel stability was a critical factor in the testing environment, this step was essential for preserving the fuel blends' durability until they were used in the engine tests (Mahajan et al., 2022).

3.6.2 Calibration and Maintenance Procedures

The same car engines used in laboratories must be calibrated before being used in research to produce accurate results. These engines have precision instrumentation, which needs to be rigorously calibrated on a regular basis to maintain the accuracy and reproducibility of the results. Since these lab engines are used for in-depth performance analysis of alternative fuels, like blends of bioethanol, they require more stringent calibration procedures.

3.6.2.1 Engine Calibration: Iso 1585:2020

The Atico Computer Controlled Hybrid Test Bench Engine was calibrated to ensure that all of the sensors and data recording devices were operating correctly. To ensure that the torque meter, brake power indicator, exhaust temperature sensors, and fuel flow meter provided accurate readings, calibration required adjusting the engine's sensors. When testing alternative fuels, this procedure was essential for getting accurate and trustworthy performance data (Clark et al., 2021)

The torque meter was calibrated by subjecting the engine to known reference loads and comparing the readings with the anticipated outcomes. According to biofuel engine testing protocols, torque calibration was required for precise power output measurements (Alsenas, 2022).

The brake power and torque were verified by comparing the engine specifications and manufacturer's data. An important component of engine test accuracy, this approach made sure the engine was running within anticipated bounds (Tucki, 2021).

3.6.3 Gas Analyzer Calibration:

To ensure the analyser provided accurate readings of exhaust gas emissions for each fuel blend, certified gas mixtures with known amounts of carbon monoxide (CO), hydrocarbons (HC), carbon dioxide (CO_2), oxygen (O_2), and nitrogen oxides (NO_x) were used to calibrate the VISKOR VG-500 Gas Analyser (NOx) (Neely et al., 2020). To ensure precise exhaust gas emission readings for every fuel blend, the gas analyser was calibrated before biofuel combustion testing (Clark et al., 2021).

3.6.4 Fuel Flow Meter Calibration:

To calibrate the fuel flow meter, a known volume of fuel was passed through the system, and the readings were compared to the anticipated flow rate. To ensure that the meter provided accurate fuel consumption information throughout the tests, modifications were made. To calculate fuel efficiency and guarantee consistent results, accurate fuel flow measurement was essential (Lommele et al., 2024).

3.6.5 Air Flow Meter Calibration:

To ensure that the flow rate of air intake was accurately recorded, the air flow meter was calibrated by measuring known air volumes and modifying the sensor readings. Accurate evaluation of air-fuel ratios during engine testing, which has a substantial impact on combustion and emissions performance, depends on properly calibrated air flow meters.

3.6.6 Testing Procedure for Each Blend

The testing process proceeds as follows after calibrating the engine and related systems and preparing the fuel blends:

a) Engine Starting: The first fuel blend (e.g., G100) was used to start the engine and then gave it time to stabilise. The testing software recorded baseline data for fuel consumption, power output, exhaust emissions, and other important parameters. To ensure accurate comparisons between the various fuel blends, consistent starting conditions were crucial.

b) Continuous Monitoring and Data Logging: The computer system recorded data during the test. Fuel flow rate, air intake, exhaust temperature, torque, brake power, and emissions were among the variables monitored by the system. With each fuel blend, these measurements offer a thorough understanding of the engine's performance and behaviour. Accurate performance data collection during biofuel testing required the use of data logging systems (Dagle et al., 2022).

c) Repeat Testing for Every Blend: Each blend was tested three times to ensure its repeatability and dependability. The procedure was repeated for the other blends (E10, E20, E30, and E40) after testing with G100. To guarantee uniformity throughout all tests, the engine was operated under the same circumstances for every blend, keeping the speed at 2500 RPM. Reliability and experimental error were reduced through repeated testing (Köten et al., 2020).

d) Post-test Analysis: After gathering the data for each blend, the engine's power output, fuel consumption, emissions, and thermal efficiency were assessed. The performance of each blend could be thoroughly compared thanks to a post-test analysis, which also offered insightful information about how engine behaviour is impacted by varying ethanol concentrations (Leach et al., 2022).

1. Brake Power (BP)

$$BP=\frac{2\pi NT}{60} \qquad 3.11$$

Where:

N=Engine speed (2500 RPM)

T=Measured torque (Nm)

2. Brake Specific Fuel Consumption (BSFC)

$$BSFC=\frac{\dot{m}f}{BP} \qquad 3.12$$

Where:

$\dot{m}_f$=fuel mass flow rate (Kg/h)

BP=Brake power (kW)

3. Indicated Power (IP)

$$IP=\frac{2\pi NPindV}{60} \qquad 3.13$$

Where:

N=Engine speed (2500 RPM)

P_{ind}=Indicated mean effective pressure (bar)

Cylinder displacement volume (L)

4. Brake Thermal Efficiency (BTE)

$$\text{BTE}=\frac{BP}{\dot{m}f\times LHV}\times 100 \tag{3.14}$$

Where:

BP=Brake power (kW)

$\dot{m}_f$=fuel mass flow rate (Kg/h)

LHV=Fuel lower heating value (MJ/Kg)

5. Torque (T)

$$\text{T}=\frac{BP\times 60}{2\pi N} \tag{3.15}$$

6. Heat Balance

$$\text{Heat input}= \dot{m}_f\times\text{LHV} \tag{3.16}$$

Heat output=Useful work (BP)+Heat losses to coolant, exhaust gasses and friction

It should be noted that the ASTM D4809-20 standard, which is typically used to calculate where LHV is applied, states that the LHV for bioethanol is 26.8 megajoules per kilogram (MJ/kg (Gonzalez et al., 2020).

3.6.7 Data Compilation

Data from emissions and engine performance tests were put together to see how different mixtures of bioethanol and gasoline influenced key operational factors. Five fuel blends were examined in the study: E10 (10% bioethanol, 90% petrol), E20 (20% bioethanol, 80% petrol), E30 (30% bioethanol, 70% petrol), E40 (40% bioethanol, 60% petrol) and G100 (pure petrol). Fuel flow rate, air intake, exhaust temperature, torque, brake power, brake specific fuel consumption (BSFC), indicated power (IP), brake thermal efficiency (BTE), heat balance, and emissions data (CO, HC, CO_2, and O_2) were all included in the collected data.

Air intake, which was required for combustion analysis, and fuel flow rate, which was used to calculate the amount of fuel consumed per hour, were among the parameters measured during testing. The exhaust temperature was measured to evaluate heat losses and combustion efficiency. Engine performance was assessed by measuring torque and brake power, and fuel efficiency was ascertained by calculating brake specific fuel consumption (BSFC). Engine speed and indicated mean effective pressure were used to calculate indicated power (IP), which provided information about the engine's total power output. The lower heating value (LHV) of the fuel was used to calculate brake thermal efficiency (BTE), which measures how well fuel energy was transformed into useful work.

The distribution of energy input from fuel combustion was examined using heat balance calculations. While the heat output was divided into useful work (BP), coolant losses, exhaust gas losses, and frictional losses, the total heat input was calculated using the rate of fuel mass flow and its LHV. This approach made it easier to comprehend how energy is used and lost in various ethanol-gasoline blends.

To evaluate the effects of ethanol blending on the environment, emission data were examined. Carbon monoxide (CO), hydrocarbons (HC), carbon dioxide (CO_2), and oxygen (O_2) were among the emissions that were measured. While the CO_2 and O_2 concentrations offered information on oxygen availability and combustion efficiency, the CO and HC emissions showed levels of incomplete combustion.

To compare the performance of various ethanol blends and identify the best fuel mixture for increased efficiency and decreased environmental impact, this data was further examined under the results and discussion section.

3.7 Techno-Economics Analysis of Bioethanol Production Process

The techno-economic analysis (TEA) in engineering studies is an essential tool for determining whether it is feasible to scale bioethanol production from laboratory to industrial levels. This methodology made sure that research was an integrated approach that assessed both technical and financial viability, rather than just an academic exercise. TEA evaluated the sustainability of a bioethanol production facility that can produce 50,000 litres per day by combining capital expenditure (CapEx), operational expenditure (OpEx), and economic analysis. Production capacity, cost-effectiveness, revenue generation, and sensitivity analysis to take market swings and process optimisations into account were among the important parameters considered in the analysis. This thorough framework offered insightful information for formulating policies and making investment decisions.

For this TEA, a step-by-step methodology was employed, starting with the estimation of capital and operating expenses and progressing to revenue projections and economic performance indicators(Karnaouri et al., 2022). Estimates of costs were derived from previous research on the production of bioethanol, industry benchmarks, and supplier quotes. Net Present Value (NPV), payback period, and unit production costs were all included in the economic evaluations. To provide a realistic evaluation of long-term profitability, the financial projections also took tax and inflation into account (de Oliveira Azevêdo et al., 2020).

3.7.1 Capital Costs

The capital costs represent the one-time expenditures necessary to set up the bioethanol production facility. Land acquisition, equipment purchases, electrical wiring, piping, construction, and other amortised costs were all included. The necessary capacity of the fermentation tanks (Vc) were estimated using Equation 3.16, which takes into account the plant's daily production and operational characteristics:

$$V_c = \frac{Q.D}{t_w} \qquad 3.17$$

Where:

Q is the total bioethanol production volume per year (L/year).

D is the number of operational days per year (300 days/year).

t_w are the plant's working hours per day (24 hours/day).

The calculated Vc value was used to estimate the construction costs (CC) using Equation 3.17:

$$CC = V_c . C_p \qquad 3.18$$

Where:

The unit cost of building a fermentation tank is C_p, which we derived from supplier quotes. Fermentation tanks, distillation columns, molecular sieves, ethanol storage tanks, pumps, and steam boilers are among the equipment and machinery expenses. These expenses were computed using supplier quotes and the plant's necessary production capacity.

Coefficients from earlier research were used to account for additional expenses like site preparation, piping, and electrical wiring. An estimated 1.5% of the total costs of the capital investment went towards land acquisition. As percentages of the overall construction cost, contingencies, contractor fees, and engineering consulting fees were calculated (Nesamvuni et al., 2022).

3.7.2 Operational Costs

Recurring expenses like feedstock procurement, electricity, water, taxes, salaries, and maintenance were all included in the operational costs. The bioethanol plant required 200 tonnes of corn stover per day as feedstock. Electricity consumption was evaluated based on the energy demands of critical equipment such as pumps, distillation units, and boilers. Water consumption was evaluated based on its application in the fermentation, cooling, and feedstock preparation processes. The energy needs of essential machinery like boilers, distillation units, and pumps were also considered

The amount of water needed for process operations, such as the preparation of feedstock, fermentation, and cooling, was used to compute the costs of water consumption. Government taxes and employee salaries were estimated to be 1% and 2% of the total capital costs, respectively, while maintenance and repair expenses were estimated to be 1.5% of the total (Vijesandiran, 2022).

Reducing feedstock costs through bulk purchase discounts and lowering electricity costs through the use of energy-efficient technologies were two ways to optimise operating costs.

3.7.3 Revenues, Net Present Value and Payback Period

To provide a thorough understanding of the project's investment costs and benefits, a number of economic evaluation tools were considered. Sales of bioethanol were the main source of income, with additional revenue coming from by-products like carbon dioxide, which is used in beverages, and leftover biomass, which is used as animal feed. Based on current market trends, the estimates took into account the bioethanol sale price as well as the financial gains from reusing treated process water.

As shown in eqs. (3.18) and (3.19), the basic instruments for a project's value include NPV, PB, and production cost per unit (Zhang, 2019). A project's net present value (NPV) indicates its value. The time it takes to recoup an investment's cost is known as the payback period. It is calculated by dividing the average cash flows by the initial investment. An investment with a shorter payback period is more appealing than one with a longer one. Based on Zambia's average inflation rate, which was 11.04% between 2005 and 2024 (Alkhalidi et al., 2024).

$$NPV = \sum_{t=1}^{n} \frac{CF_t}{(1+r)^i} - \text{Initial Investment} \quad 3.19$$

Where:

- CF_t is the cash flow at time t.
- *r* is the discount rate (11.04% or 0.1104).
- *n* is the project lifespan (20 years).
- Initial Investment is the total capital cost.

Despite not taking the time value of money into account, the payback period is helpful when choosing investments (Kiran, 2022). The payback period is calculated using Equation 3.20 and computed the annual profits by deducting operating costs from total revenues.:

$$\text{Payback Period} = \frac{\text{Total Capital Costs (\$)}}{\text{Annual Profits (\$/year)}} \quad 3.20$$

The analysis showed that using optimised processes, such as efficient distillation and dehydration technologies, increased profitability.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.0 Chapter Introduction

This chapter presents the experimental findings and analysis that pertain to the optimisation and assessment of bioethanol production from Zambian corn stover. Starting with biomass characterisation, the study followed a systematic procedure that included pretreatment, enzymatic hydrolysis, fermentation, distillation, and blending. Proximate and ultimate analyses verified the energy potential of corn stover demonstrating favorable elemental composition and calorific value. By using Central Composite Design (CCD) and Response Surface Methodology (RSM) in Design Expert software, the steps for pretreatment and hydrolysis were improved to find the best conditions for getting the most cellulose and sugar. Cellulose yield was accurately predicted using machine learning models, including Boosted Regression Trees (BRT), Support Vector Machines (SVM), Random Forests (RF), and Artificial Neural Networks (ANN). The BRT model performed the best ($R^2 = 0.80$). To facilitate scale-up and process control, mathematical models were also created and verified using ANOVA.

Saccharomyces cerevisiae was used to ferment the sugars under ideal conditions, and the resulting ethanol was distilled to an 80% concentration. E10, E20, E30, and E40 blends of bioethanol and petrol were tested for engine performance and emission characteristics. E20 demonstrated the most encouraging outcomes among the blends, with lower emissions and improved fuel efficiency. The financial feasibility of the production process, including the acquisition of feedstock, processing expenses, and possible market impact, was assessed through a techno-economic analysis. The results demonstrate that it is feasible to produce bioethanol on a large scale from corn stover, providing Zambia with an economically and environmentally sound way to lessen its reliance on fossil fuels.

4.1 Characterization of Corn Stover

Prior to pretreatment, Zambian corn stover was characterised to assess its chemical characteristics and structural makeup. Understanding its chemical makeup was crucial for deciding if it could be used to make bioethanol and for developing an effective pretreatment plan using Deep Eutectic Solvent (DES) technology. Its moisture content, ash content, extractives, cellulose, hemicellulose, and lignin were all thoroughly examined to comprehend its physicochemical characteristics and how they affected the processes of thermochemical conversion, enzymatic hydrolysis, and fermentation efficiency.

Moisture content is crucial for biomass storage and conversion efficiency, as excessive moisture hinders combustion and enzymatic processes. The substances in the biomass affected how well it could be turned into biofuel, while the ash content influenced how well the reactor worked and created unwanted leftovers. The potential fermentable sugar yield was determined by the structural carbohydrates, cellulose and hemicellulose, and these variables directly affected the efficiency of bioethanol production. On the other hand, lignin, a significant component that was resistant to enzymatic hydrolysis, necessitated pretreatment to improve the accessibility of carbohydrates.

This study offered a thorough analysis of these compositional factors, highlighting their importance in maximising bioethanol production. The study showed that Zambian corn stover can be a good source for biofuel and ensured the creation of effective pretreatment methods by understanding how these factors worked together. Table 4.1 below contains the test results.

Table 4. 1: Proximate and Structural Carbohydrates Results

Component of Corn Stover	% composition
Moisture Content	4.3
Ash Content	2.4
Extractives	12
Cellulose	46
Hemicellulose	22.1
Lignin	13.2

4.1.1 Moisture Content

The moisture content of biomass influences its conversion, storage, and transportation efficiency. Within the permissible range of 4–15%, the analysis found a moisture content of 4.3%. This comparatively low moisture content improves the efficiency of fermentation and enzymatic hydrolysis and lowers the energy needed for drying, which is advantageous for the production of bioethanol. Generally speaking, lignocellulosic biomass can have a moisture content of 4–15%; lower values are preferred for higher energy yield. Low moisture content optimised processing costs by increasing combustion efficiency and reducing the need for pre-drying (Banda, 2020).

4.1.2 Ash Content

Ash content, the inorganic fraction of biomass, plays a crucial role in determining its usability in biofuel production. The Zambian corn stover sample produced an ash content of 2.4%**,** which is within the acceptable range of 1–10% for lignocellulosic biomass. Lower ash content reduces slag formation in thermal processes and minimizes the accumulation of unwanted residues in biochemical conversions (Kasoma et al., 2021).

4.1.3 Extractives

The amount of biomass's inorganic fraction, or ash content, is a key factor in determining how useful it is for producing biofuel. The ash content of the Zambian corn stover sample was 2.4%, falling between the permissible range of 1 and 10% for lignocellulosic biomass. Reduced ash content minimises the accumulation of undesirable residues in biochemical conversions and lessens the formation of slag in thermal processes (Kpalo et al., 2020).

4.1.4 Cellulose Content

Extractives, which include non-structural elements like fats, resins, and phenolics, influence the properties of biomass and processing efficiency. Test results showed that 12% of Zambian corn stover contained extractives, which is consistent with values found in studies pertaining to biofuel. Depending on the biomass source, extractives can range from 5 to 20%. These substances can affect the efficiency of enzymatic hydrolysis and aid in the synthesis of useful biochemicals (Siankwilimba et al., 2023).

4.1.5 Hemicellulose Content

The flexibility and breakdown efficiency of biomass are affected by hemicellulose, which is a mixed type of polymer made from five and six-carbon sugars. The sample's hemicellulose content of 22.1% fell within the usual range of 20–35% for lignocellulosic feedstocks. Hemicellulose can break down into useful C5 sugars that can be used for biochemical processes and is important for providing fermentable sugar needed to make bioethanol (Uzoagba et al., 2024).

4.1.6 Lignin Content

The complex aromatic polymer lignin offers resistance to microbial degradation and structural rigidity. The lignin content of the Zambian corn stover sample was 13.2%, which falls within the reported range of 10-25% for biomass used in biofuel applications. Lignin has the potential to produce bioproducts like bio-based adhesives, chemicals, and energy, even though it prevents enzymatic hydrolysis (Kalala et al., 2022).

4.1.7 The Potential Of Zambian Corn Stover For Bio-Conversion

Zambian corn stover's compositional analysis confirmed that it is a viable feedstock for bio-conversion processes, especially those that produce bioethanol. The low moisture and ash content made it easier to process energy-efficiently, ensuring that there were minimal losses during heating and chemical changes, based on the analysis results. Furthermore, the moderate extractive content indicated the possibility of recovering valuable biochemicals

The stover had levels of cellulose and hemicellulose similar to those in good lignocellulosic feedstocks, showing it could be used to make bioethanol. However, to increase the amount of fermentable sugar and improve enzymatic hydrolysis, an effective pretreatment strategy was needed because of the lignin. However, to improve fermentable sugar yield and enzymatic hydrolysis, the presence of lignin required an efficient pretreatment strategy. These results showed that Zambian corn stover could be a valuable and renewable energy source that offers important economic and environmental benefits, and this was supported by reliable research on making biofuel from lignocellulosic biomass.

4.2 Optimization of the Pretreatment of Corn Stover

This section covers the use of Deep Eutectic Solvents (DES) to optimise the pretreatment procedure for improving cellulose recovery from corn stover. The results are presented in three main critical areas: predictive model performance evaluation, comparison of experimental and predicted results, and the impact of pretreatment variables on cellulose yield. As indicated in Table 4.2, each of these areas has optimal results that offer insightful information about the effectiveness of various pretreatment conditions. All these were obtained from

Table 4. 2: Measured and Predictable Cellulose Yield-Pretreatment

Item	Independent Variables				Dependent Variables		
Exp.	Residence Time (Hours)	Reaction Temperature (Degrees Celsius)	ChCl: LA (g)	Corn Stover: Solvent (g)	Actual Cellulose Yield (g)	Model Equation Validated Yield (g)	Predicted Pretreatment Yield (g) by the BRT model
1	10.5	105	100:351.8	1:32	0.3339	0.3482	0.3667
2	6	150	100:117.2	1:08	0.3471	0.3096	0.3855
3	10.5	105	100:351.8	1:20	0.3428	0.3484	0.3443
4	6	60	100:117.2	1:08	0.4067	0.4081	0.3418
5	10.5	105	100:351.8	1:20	0.4289	0.3484	0.3818
6	15	60	100:117.2	1:08	0.3649	0.3787	0.3818
7	15	150	100:586.0	1:08	0.3872	0.3415	0.3612
8	10.5	105	100:351.8	1:20	0.4241	0.3484	0.3663
9	6	150	100:117.2	1:32	0.3623	0.3430	0.2789
10	10.5	105	100:351.8	1:20	0.4009	0.3484	0.4610
11	6	150	100:586.0	1:32	0.4599	0.4219	0.3469
12	10.5	105	100:351.8	1:20	0.2729	0.3484	0.3818
13	15	150	100:117.2	1:20	0.2712	0.2757	0.2566
14	6	105	100:117.2	1:32	0.2984	0.3416	0.3385
15	10.5	105	100:586.0	1:20	0.326	0.3341	0.2893
16	10.5	105	100:351.8	1:08	0.2891	0.3193	0.2626
17	10.5	105	100:351.8	1:20	0.3884	0.3484	0.3315
18	10.5	105	100:117.2	1:20	0.274	0.3059	0.3463
19	15	150	100:117.2	1:32	0.2629	0.2752	0.3818
20	15	150	100:586.0	1:32	0.4242	0.4369	0.3818
21	6	60	100:586.0	1:08	0.3413	0.3026	0.3333
22	10.5	60	100:351.8	1:20	0.3372	0.3739	0.3649
23	6	60	100:586.0	1:32	0.3204	0.3323	0.4254
24	6	150	100:586.0	1:08	0.2567	0.3212	0.2999
25	15	60	100:586.0	1:32	0.367	0.3804	0.3101
26	15	60	100:117.2	1:32	0.3862	0.3358	0.3868
27	15	60	100:586.0	1:08	0.3441	0.3560	0.3217
28	6	105	100:351.8	1:20	0.3666	0.3790	0.2716
29	10.5	150	100:351.8	1:20	0.3065	0.3530	0.4063
30	15	105	100:351.8	1:20	0.3443	0.3791	0.3818

The optimal conditions for cellulose yields were determined through the optimisation process. The experimental model showed how specific process conditions affected cellulose recovery, achieving the highest cellulose yield of 0.4599 g in run 11. The accuracy of the mathematical model in matching the experimental results was shown when the quadratic model predicted the best cellulose yield in run 20 at 0.4369 g. The BRT model, which had the highest predictive accuracy, demonstrated a strong ability to capture complex variable interactions by achieving its optimal result in run 10 at 0.4610 g. These results are consistent with earlier studies on the use of DESs for lignocellulosic biomass pretreatment, where it was demonstrated that cellulose yield was greatly increased by optimising reaction parameters (Sun et al., 2023).

Each model's predictive power was assessed using the coefficient of determination (R^2). With an R^2 of 0.44, the laboratory model (experimental results) indicated moderate predictability but significant experimental outcome variability. With an R^2 of 0.90, the quadratic model outperformed this, showing a significantly higher relationship between cellulose yield and process variables. The BRT model had the highest R^2 of any machine learning model, at 0.80. It was followed by the ANN (R^2 = 0.78), SVM (R^2 = 0.74), and RF (R^2 = 0.63). These results indicate that machine learning models, especially the BRT model, were successful in forecasting cellulose yield even though the quadratic model offered a good mathematical fit. Research has shown that machine learning algorithms, like ANN and BRT, can handle the intricate relationships in biomass pretreatment and increase the accuracy of predictions (Cronin et al., 2020).

A more thorough examination of the trends across input parameters identifies a number of significant cellulose yield patterns. Moderate reaction temperatures (105°C) and residence times (10.5 hours) were generally associated with higher cellulose recovery, especially when the solvent-to-solid ratio was balanced. Significant variation was observed in the lab results across various input conditions. The highest cellulose recovery was obtained at a high ChCl:LA ratio (100:586.0), a short residence time (6 hours), and a high reaction temperature (150°C) in Run 11 (0.4599 g). High reaction temperatures combined with ideal solvent ratios have also been shown in other studies to improve cellulose accessibility and lignin removal (Lin et al., 2021). It's intriguing to note that run 4 (0.4067 g) produced a low ChCl:LA ratio (100:117.2), a relatively high yield at a lower temperature (60°C), and a shorter residence time (6 hours), indicating that extreme parameter values are not always required to maximise cellulose recovery. Although the quadratic model was effective at predicting general trends, it was not very good at handling extreme values or replicated input conditions where there was more experimental variability. Its prediction of the highest yield in run 20 (0.4369 g) with a high solvent-to-solid ratio (1:32) and high reaction temperature (150°C) highlights the importance of balancing reaction conditions rather than relying on extreme input values.

The most successful model at capturing the intricate relationships between process variables was the BRT model, which had the highest predictive power ($R^2 = 0.80$). Run 10, which had moderate parameter levels (temperature of 105°C, residence time of 10.5 hours, and ChCl:LA ratio of 100:351.8), produced the highest BRT-predicted yield (0.4610 g). This finding suggests that the BRT model preferred balanced parameter levels over extreme values. The BRT model was better at dealing with real-world differences in experiments because it responded more to repeated input parameters and small changes in reaction conditions than the quadratic model, which tends to average out differences. According to other research, BRT models are effective at optimising biomass, especially when it comes to capturing both linear and nonlinear dependencies (Baraka et al., 2024).

When replicated input conditions were considered, several runs had different cellulose yields but the same reaction settings. For instance, despite having identical input conditions, runs 3, 5, 8, 10, and 17 produced actual yields that ranged from 0.3428 g to 0.4289 g. For these runs, the quadratic model predicted a constant yield of 0.3484 g, indicating that it was unable to adequately capture experimental variability. The BRT model, on the other hand, generated different predictions during these runs, demonstrating its capacity to identify minute variances and inconsistent experimental results. Furthermore, there were noticeable differences between runs that combined lower and higher input parameter levels. For instance, runs 6 and 7 had different reaction temperatures (60°C and 150°C, respectively) but the same residence time (15 hours). Higher residence times did not always translate into higher yields unless they were paired with ideal temperatures, as evidenced by the actual yields of 0.3649 g and 0.3872 g. The BRT model made predictions that were more in line with the actual results, showing it can handle complex relationships, while the quadratic model predicted Run 6 at 0.3787 g and Run 7 at 0.3415 g, which was a bit off from the trend.

The results suggest that while using a lot of solvent and higher temperatures increased the amount of cellulose produced, the best results didn't always come from using the highest levels. The quadratic model ($R^2 = 0.90$) provided consistent estimates but didn't respond much to small changes in experiments, while the experimental model reflected real-world differences but wasn't as accurate in predictions. The BRT model was the top-performing machine learning model, handling both linear and non-linear relationships with ease. The discrepancy between the expected and actual outcomes emphasises how crucial it is to optimise cellulose recovery using a variety of strategies to produce reliable predictions that can be used for industrial bioethanol production. These results support the growing significance of predictive modelling in biomass optimisation and are consistent with recent research on DES pretreatment techniques for biofuel production (Gundupalli et al., 2021).

4.2.1 Laboratory Results: CCD Model Performance

Accurate predictive models that optimise process parameters are essential for the efficient conversion of lignocellulosic biomass to valuable products. Many models have been created using Response Surface Methodology (RSM) and Central Composite Design (CCD) to understand how key process factors are related. This study evaluated how accurately two models the quadratic model and the Two-Factor Interaction (2FI) model predicted the amount of cellulose produced from biomass pretreatment experiments.

ANOVA, fit statistics, cellulose yield curve comparisons, and 3D surface analysis were among the statistical methods used to evaluate the model's dependability. These evaluations demonstrated each model's predictive advantages and disadvantages in terms of capturing process interactions and cellulose yield estimation. The study determined the best method for process optimisation and emphasised the drawbacks of the weaker model by contrasting the actual cellulose yield data with the model's predictions.

4.2.1.1 ANOVA For 2FI Model And Quadratic Model

The analysis of variance (ANOVA) for the two-factor interaction (2FI) model showed that the model didn't have a significant effect (F-value = 1.49, p-value = 0.2189), indicating that the differences explained by the model were not significantly different from random variation. The result is displayed in Table 4.3. The model only included BC as a significant interaction term (p-value = 0.0280), suggesting a relationship between the ChCl:LA ratio and reaction temperature.

Table 4. 3: ANOVA for Quadratic model Results

Response 1: Cellulose (13)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0372	10	0.0372	0.0372	0.2189	not significant
A-Residence Time	0.0016	1	0.0016	0.0016	0.4378	
B-Reaction Temperature	0.0000	1	0.0000	0.0000	0.9459	
C-ChCl:LA	0.0001	1	0.0001	0.0001	0.8164	
D-Corn Stover: Solvent	0.0092	1	0.0092	0.0092	0.0702	
AB	0.0036	1	0.0036	0.0036	0.2431	
AC	0.0071	1	0.0071	0.0071	0.1082	
AD	0.0049	1	0.0049	0.0049	0.1760	
BC	0.0141	1	0.0141	0.0141	0.0280	
BD	2.128E-06	1	2.128E-06	2.128E-06	0.9770	
CD	0.0052	1	0.0052	0.0052	0.1650	
Residual	0.0475	19	0.0475			
Lack of Fit	0.0238	12	0.0238	0.5852	0.8021	not significant
Pure Error	0.0237	7	0.0237			
Cor Total	0.0846	29				

The absence of additional important terms, however, indicated that the model had trouble capturing important factors influencing cellulose yield. Moreover, the non-significant lack of fit test (F-value = 0.59, p-value = 0.8021) indicated a good fit with the experimental data. However, the overall insignificance of the model constrained its predictive power, making it unsuitable for trustworthy process optimisation (Dargahi et al., 2021). On the other hand, the quadratic model successfully captured important factors and was highly significant (F-value = 10.80, p-value < 0.0001). Strong model fit was shown by significant terms like C (p = 0.0010), D (p = 0.0064), AB (p = 0.0032), AC (p = 0.0015), BC (p < 0.0001), BD (p = 0.0017), and CD (p = 0.0023). The strength of the quadratic model was also supported by the fact that it didn't show any significant lack of fit (p = 0.9397), which showed that it predicted cellulose yield better than the 2FI model (Ao et al., 2024).

4.2.1.2 Fit Statistics

The fit statistics as shown in Table 4.4, further highlighted the shortcomings of the 2FI model. Only 43.91% of the variability in cellulose yield was explained by the model, according to the low R^2 value (0.4391), leaving a significant amount of variation unexplained. This low value indicated that nonlinear relationships and important interactions in the experimental data could not be accounted for by the model.

Table 4. 4: Fit Statistics

Std. Dev.	Std. Dev.	R^2	0.4391
Mean	Mean	Adjusted R^2	0.1439
C.V. %	C.V. %	Predicted R^2	-0.6056
		Adeq Precision	5.6909

Additionally, the negative predicted R^2 (-0.6056) indicated that using the average as a predictor could have been more reliable than the current model, while the adjusted R^2 value (0.1439) showed that the model's ability to predict was weak. The reliability of the model was further diminished by its coefficient of variation (C.V. = 14.37%), which indicated high variability in predictions. While the adequate precision value of 5.69

suggested that the model could still navigate the design space, its poor fit to experimental data made it suboptimal for cellulose yield estimation. On the other hand, the quadratic model provided a significantly better fit, explaining the majority of the variation in cellulose yield with an adjusted R^2 value above 0.9. This suggested that non-linear relationships and higher-order interactions were important in cellulose yield prediction, which the 2FI model was unable to adequately capture (Zhang et al., 2021).

4.2.1.3 Cellulose Yield Curve Analysis

Figure 4.1 shows the cellulose yield curve, which illustrates the model's predictive limitations, especially its incapacity to capture intricate interactions. With differences between actual and predicted values, the yield curve showed notable variation across various experimental conditions. Several instances of overestimation or underestimation resulted from the 2FI model's poor alignment with experimental data.

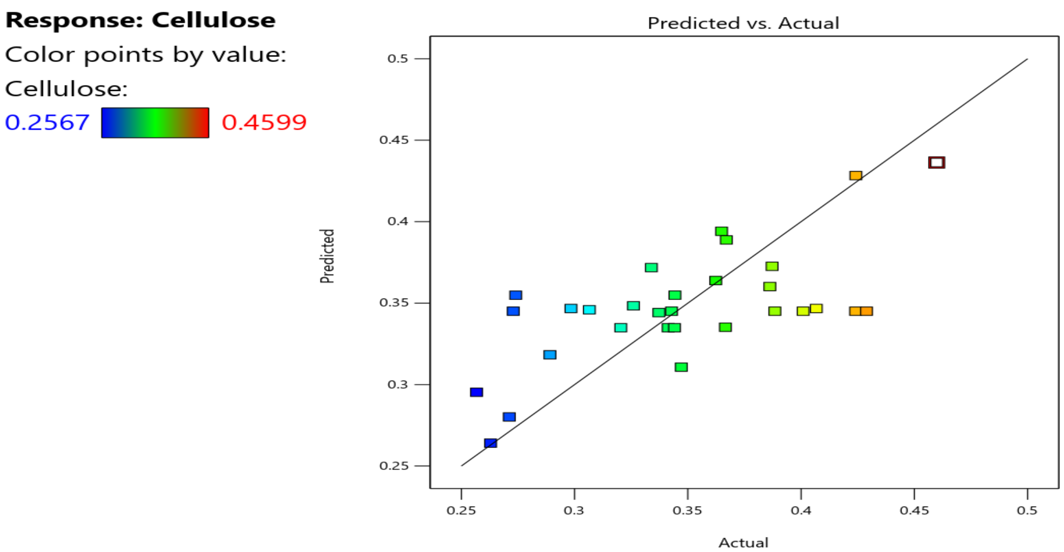

Figure 4. 1: Cellulose Yield Curve

This inconsistency showed that better models are needed because cellulose yield was affected by complex factors like reaction temperature and solvent ratios. In contrast, the quadratic model matched actual cellulose yields better and followed the experimental trend more closely. In contrast, the quadratic model showed a stronger correlation with actual cellulose yields, following the experimental trend more closely. According to the data, the 2FI model lacked the depth required to make precise predictions about outcomes, while the quadratic model was more appropriate for determining ideal conditions (Han et al., 2024).

4.2.1.4 3d 2fi Surface Analysis

A thorough illustration of the effects of reaction temperature, residence time, lactic acid-to-choline chloride (ChCl:LA) ratio, and solvent-to-biomass ratio on cellulose yield during the pretreatment of corn stovers was given by the 3D response surface plot. With 30 experimental runs spanning a reaction temperature range of 60–150°C and residence times ranging from 6–15 hours, the study was based on a Central Composite Design (CCD). As shown in Figure 4.2, the quadratic model successfully illustrated the complex relationships between these process parameters, highlighting specific areas of high and low cellulose yield across the response surface.

Critical trends in cellulose recovery were emphasised by color-coded surface gradients and contour distributions; lower yields were indicated by blue-green zones, while optimal conditions were represented by yellow-to-red zones. The necessity for exact process optimisation was highlighted by the steep transitions in some areas, which implied that even slight changes in reaction conditions would result in notable changes in cellulose yield. The comparison of the quadratic and 2FI models also showed that the 2FI model better represented the complex relationships affecting cellulose recovery (Fernandez et al., 2024).

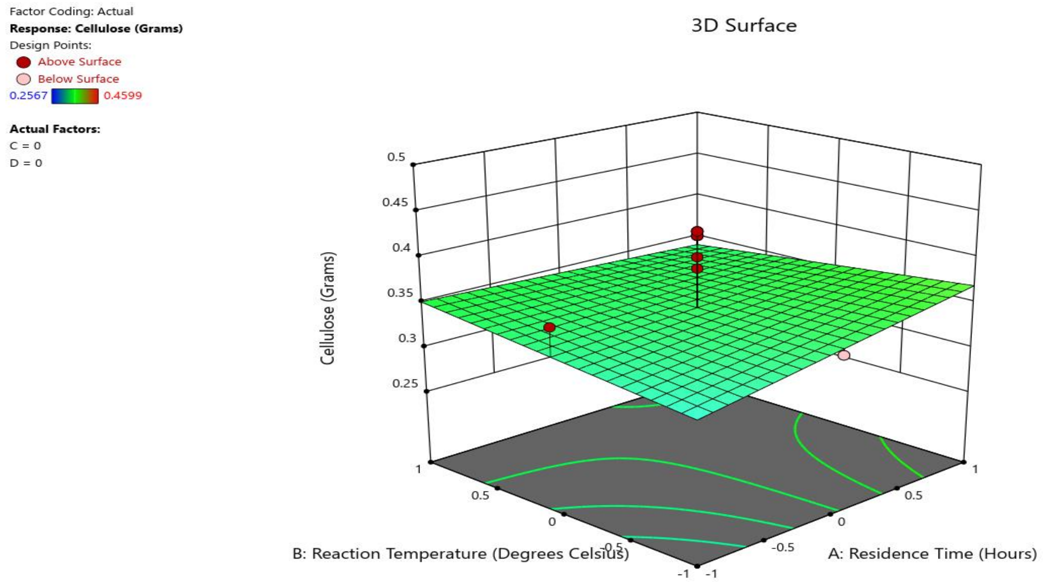

Figure 4. 2: 3D 2FI Surface

The 3D response surface analysis successfully illustrated how changes in solvent and catalyst composition, as well as reaction temperature and residence time, affected the yield of cellulose. Important information about the ideal circumstances for optimising cellulose recovery and the thresholds beyond which degradation occurred was revealed by the distribution of color-coded areas across the response surface.

Low cellulose yield was associated with the blue-green areas of the response surface, suggesting that the pretreatment conditions were either too mild or too severe to promote efficient biomass solubilisation. As demonstrated by Runs 4 (60°C, 6h, 0.4067 g) and 21 (60°C, 6h, 0.3413 g), these zones were found at low reaction temperatures (60–80°C) with brief residence times (6–8 hours). Under these circumstances, poor enzymatic digestibility resulted from limited solvent penetration and lignin removal due to a lack of thermal energy (Santos et al., 2023). According to studies, the cellulose-lignin complex maintains its structural integrity at low temperatures, which lowers the efficiency of hydrolysis (Weimer, 2022).

Interestingly, run 13 (150°C, 15h, 0.2712 g) and Run 19 (150°C, 15h, 0.2629 g) both showed low cellulose yields at the upper extremes of temperature and residence time, especially at 150°C with extended residence times (15 hours). Given that high temperatures have been shown to decompose sugar and produce unwanted degradation byproducts, this decline implied that excessive heat exposure caused cellulose degradation (Martínez-Hernández et al., 2024). The sharp color changes from green to blue in these areas demonstrated that, in extreme situations, even small temperature or reaction time increases resulted in sharp yield decreases, highlighting the significance of avoiding overprocessing.

The response surface progressively changed from green to yellow as reaction conditions improved, indicating a moderate cellulose yield in circumstances that improved lignin removal but did not completely maximise cellulose recovery. Runs 22 (60°C, 10.5h, 0.3372 g) and 28 (105°C, 6h, 0.3666 g) demonstrated this trend, with yield improving with increasing reaction temperature and residence time. It was confirmed that moderate heating (100–120°C) and reaction times (10–12 hours) allowed for effective lignin disruption while preventing cellulose loss by the widely spaced contour lines in these regions, which showed that cellulose yield remained relatively stable over a wide range of conditions (Sunar, Bhattacharyya, et al., 2024).

The best pretreatment conditions were represented by the yellow-to-red areas of the response surface, which correlated with the highest cellulose yields. Run 5 (105°C, 10.5h, 0.4289 g) and Run 10 (105°C, 10.5h, 0.4009 g) both showed these high-yield regions at moderate reaction temperatures (105–120°C) and residence times of 10.5–12 hours. The abrupt change in color from yellow to red indicated that even minor adjustments to the reaction conditions within this range led to a notable boost in the recovery of cellulose. Studies have shown similar patterns, with deep eutectic solvents (DES) effectively removing lignin at moderate temperatures while optimising cellulose retention for enzymatic hydrolysis (Sooch et al., 2023).

Some areas showed abrupt reversions from red to yellow and back to green, especially at prolonged residence times and high solvent concentrations, even though these regions produced high yields. Run 20 (150°C, 15h, 0.4242 g), for instance, demonstrated that although yield was still high, extended exposure ran the risk of decreasing recovery efficiency. Given that prolonged residence time has been associated with partial cellulose solubilisation and increased sugar degradation, this trend supported the idea that overprocessing resulted in cellulose loss through secondary reactions (Lima-Sousa et al., 2023). High process sensitivity was further supported by the closely spaced contour lines in these areas, where cellulose yield was significantly impacted by even small changes in parameters.

Additionally, the response surface revealed areas of relative stability where slight changes in reaction parameters had little effect on the yield of cellulose. Widely separated contour lines in these zones suggested that process optimisation could be somewhat flexible. It is crucial to strike a balance between efficiency and economic and energy considerations because studies have shown that after cellulose reaches a stable recovery threshold, additional process intensification offers diminishing returns (Torres-Sciancalepore et al., 2023).

Overall, the 3D response surface analysis verified that the best residence times (10.5–12 hours) and moderate temperatures (105–120°C) were found to balance the removal of lignin and the preservation of cellulose. The identification of stable regions indicated that some flexibility was possible under particular operating conditions, while the presence of steep transition zones in some regions highlighted the necessity of precise process control. These results demonstrated that, when appropriately optimised, deep eutectic solvent pretreatment greatly improves the performance of enzymatic hydrolysis, which in turn leads to increased biomass conversion efficiency.

4.2.2 Evaluation of CCD Polynomial Model in Predicting Cellulose Yield

Optimising the pretreatment of lignocellulosic biomass depends heavily on the precision of predictive models. In this study, cellulose yield was predicted using the Central Composite Design (CCD) polynomial model equation 3.7, allowing for an assessment of how well it captured experimental trends. The model's performance was assessed using ANOVA, fit statistics, 3D surface analysis, and a comparison of actual versus predicted cellulose yield. This section presents the results and discusses the strengths and limitations of the model in relation to process optimization.

4.2.2.1 ANOVA For Quadratic Model

Optimising the pretreatment of lignocellulosic biomass depends heavily on the precision of predictive models. In this study, cellulose yield was predicted using the Central Composite Design (CCD) polynomial model equation 3.7, allowing for an assessment of how well it captured experimental trends. ANOVA, fit statistics, 3D surface analysis, and a comparison of the actual and predicted cellulose yield—which is displayed in Table 4.5—were used to evaluate the model's performance. Deeper understanding of the model's accuracy and capacity to improve process optimisation was made possible by these analyses. Reducing waste, increasing biomass conversion efficiency, and guaranteeing reproducibility in cellulose extraction procedures all depend on an understanding of these predictive capabilities.

An F-value of 10.80 and a p-value of less than 0.0001 were found in the analysis of variance (ANOVA) results, indicating that the quadratic model was statistically significant and that it successfully explained variations in cellulose yield. The significance of these factors in influencing cellulose yield was confirmed by the identification of several model terms as significant, including C ($p = 0.0010$), D ($p = 0.0064$), AB ($p = 0.0032$), AC ($p = 0.0015$), BC ($p < 0.0001$), BD ($p = 0.0017$), CD ($p = 0.0023$), A^2, B^2, and C^2. These findings highlight the strong dependency of cellulose yield on solvent composition and reaction conditions. The model's ability to accurately forecast yield trends and its excellent alignment with the experimental data were demonstrated by the non-significant lack of fit test (F-value = 0.31, p-value = 0.9397).

Table 4. 5: ANOVA for Quadratic model

Response 1: Cellulose (13)

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	0.0361	14	0.0026	0.0026	< 0.0001	significant
A-Residence Time	0.0002	1	0.0002	0.0002	0.3591	
B-Reaction Temperature	0.0003	1	0.0003	0.0003	0.2540	
C-ChCl:LA	0.0040	1	0.0040	0.0040	0.0010	
D-Corn Stover: Solvent	0.0024	1	0.0024	0.0024	0.0064	
AB	0.0029	1	0.0029	0.0029	0.0032	
AC	0.0036	1	0.0036	0.0036	0.0015	
AD	0.0000	1	0.0000	0.0000	0.8180	
BC	0.0084	1	0.0084	0.0084	< 0.0001	
BD	0.0035	1	0.0035	0.0035	0.0017	
CD	0.0032	1	0.0032	0.0032	0.0023	
A^2	0.0016	1	0.0016	0.0016	0.0197	
B^2	0.0011	1	0.0011	0.0011	0.0448	
C^2	0.0037	1	0.0037	0.0037	0.0014	
D^2	0.0003	1	0.0003	0.0003	0.2638	
Residual	0.0036	15	0.0002			
Lack of Fit	0.0009	8	0.0001	0.3076	0.9397	not significant
Pure Error	0.0027	7	0.0004			
Cor Total	0.0397	29				

Nevertheless, some variables, like reaction temperature (B) and residence time (A), were found to have less of an effect, indicating that solvent composition was more important in the recovery of cellulose. The findings implied that yield could be further optimised by fine-tuning reaction conditions (Nguyen et al., 2023).

4.2.2.2 Fit Statistics

Table 4.6 shows that the quadratic model accounted for about 91% of the differences in cellulose yield, showing it is very good at making predictions with an R^2 value of 0.9098. The high adjusted R^2 (0.8256) strengthened the model's dependability across various experimental conditions by indicating that it remained strong even after controlling for the number of predictors. But the predicted R^2 (0.6244) was less than anticipated and differed from the adjusted R^2 by over 0.2, indicating that there might be overfitting or unidentified factors affecting the yield of cellulose.

Table 4. 6: Fit Statistics

Std. Dev.	0.0155	R^2	0.9098
Mean	0.3481	Adjusted R^2	0.8256
C.V. %	4.44	Predicted R^2	0.6244
		Adeq Precision	14.6648

The coefficient of variation (C.V. = 4.44%) indicated low experimental variability, while an adequate precision ratio of 14.6648 confirmed a strong signal-to-noise ratio, making the model useful for navigating the design space. However, the difference between the adjusted and predicted R^2 values showed that more testing was needed to make sure the model could accurately predict outcomes. Model refinement through data transformations or process parameter adjustments could enhance its performance further (Patel, 2023).

4.2.2.3 3d Response Surface Analysis

The 3D response surface plot of Figure 4.4, showed how factors like reaction temperature, time, the ratio of lactic acid to choline chloride (ChCl:LA), and the amount of solvent compared to biomass affected the predicted cellulose yield when treating corn stover. The study employed a Central Composite Design (CCD) with 30 experimental runs, covering a reaction temperature range of 60–150°C and residence times between 6 and 15 hours. The response surface helped to thoroughly examine how the input factors influenced predictions of cellulose yield, showing areas with both low and high expected yields

The quadratic model generated a well-defined response surface, which exhibited strong nonlinear interactions between process variables. The surface featured color-coded gradients that indicated variations in yield; blue-green regions corresponded to low cellulose yields, and yellow-red regions represented optimal conditions. The presence of steep color transitions and dense contour lines in specific areas suggests that minor fluctuations in input parameters cause significant variations in cellulose yield predictions. The results confirmed that precise control of reaction conditions played a crucial role in optimising cellulose recovery (Vijayan et al., 2023).

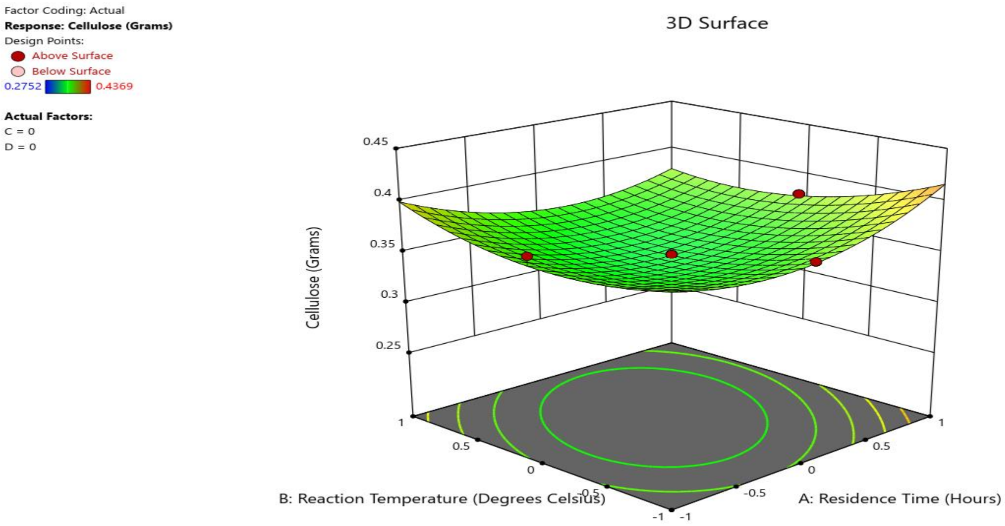

Figure 4. 3: 3D Response Surface

The 3D response surface plot graphically displayed the effects of temperature, residence time, solvent-to-biomass ratio, and ChCl:LA ratio on the model-predicted cellulose yield. This cellulose yield sensitivity to process conditions was demonstrated by the distribution of colored regions and contour patterns, which highlighted areas where input parameters were either optimised or resulted in unfavorable reactions.

The blue-green areas on the response surface showed low predicted cellulose yields, indicating that the conditions were insufficient for efficient biomass breakdown. According to Runs 4 (60°C, 6 h, 0.4081 g) and 21 (60°C, 6 h, 0.3026 g), these low-yield regions were mostly seen at low reaction temperatures (60–80°C) and brief residence times (6–8 hours). It was found that the reaction conditions in this range were not good enough for removing lignin because the flat patterns in these areas showed that changing the input factors did not significantly affect the amount of cellulose produced. According to earlier research, lignin and hemicellulose mostly held their structure at low temperatures, which restricted the amount of cellulose that could be hydrolysed by enzymes (Wendt et al., 2022).

Furthermore, as demonstrated by Runs 13 (150°C, 15 h, 0.2757 g) and 19 (150°C, 15 h, 0.2752 g), low predicted cellulose yields were seen at the upper extremes of reaction temperature and residence time, especially at 150°C and 15 hours. These areas showed steep gradient shifts from green to blue, suggesting that even slight increases in temperature or residence time led to precipitous drops in cellulose yield. This pattern suggested that being exposed to high temperatures for a long time broke down cellulose, which matched findings that temperatures over 150°C led to the breakdown of polysaccharides, making it harder to recover cellulose(Rennison et al., 2024). Longer reaction times at high temperatures sped up the breakdown of sugars, leading to harmful byproducts like furfural and hydroxymethylfurfural (HMF), which made it harder for enzymes to work effectively(García Martín et al., 2020).

The response surface changed from green to yellow between the low-yield zones, indicating predictions for a moderate cellulose yield. The slow change indicated that solvent penetration increased as reaction conditions approached ideal levels, improving lignin solubility and cellulose exposure. Runs 22 (60°C, 10.5 h, 0.3739 g) and 28 (105°C, 6 h, 0.3790 g) demonstrated these trends, with yield predictions improving as temperature and residence time increased. Moderate temperatures (100–120°C) and residence times (10–12 hours) allowed for the best lignin removal without breaking down cellulose, as confirmed by the more widely spaced contour lines in these areas, which showed that cellulose yield remained stable over a wider range of conditions (Zafar et al., 2024).

The most successful pretreatment conditions were represented by the yellow-to-red areas of the response surface, which matched the highest anticipated cellulose yields. As demonstrated in Runs 5 (105°C, 10.5 h, 0.3484 g) and 10 (105°C, 10.5 h, 0.3484 g), these ideal conditions were found at moderate reaction temperatures (105–120°C) and residence times of 10.5–12 hours. The abrupt change from yellow to red demonstrated that even minor adjustments to the reaction conditions within this range significantly enhanced the predictions of cellulose yield. Prior research found that under these circumstances, deep eutectic solvents (DES) effectively eliminated lignin, maximising cellulose retention and minimising hemicellulose loss (Singh et al., 2024).

Particularly at extended residence times and high solvent concentrations, some regions of the response surface showed sudden reversions from red to yellow and then back to green, despite the high anticipated yields. For example, run 20 (150°C, 15h, 0.4369 g) indicated that although the expected yield was still high, there was a risk of cellulose degradation if the reaction time was increased further. Previous research showed that excessive exposure to pretreatment conditions resulted in increased sugar loss from secondary reactions and partial cellulose solubilization (Mockdeci et al., 2023). The closely spaced contour lines in these areas emphasised the necessity of exact process control to prevent degradation losses, showing that even minor changes in reaction conditions had a substantial impact on cellulose yield.

Additionally, the 3D response surface showed areas of process stability where minor changes in parameters had little effect on the expected cellulose yield. Widely separated contour lines identified these zones, suggesting that process optimisation was flexible under specific operating conditions. Studies have shown that after cellulose yield stabilises, increasing process conditions result in decreasing returns, highlighting the significance of striking a balance between efficiency, cost-effectiveness, and energy consumption (Duque García et al., 2023).

The model's predictions for cellulose yield showed that moderate temperatures (105–120°C) and residence times (10.5–12 hours) were best for balancing lignin removal and keeping cellulose intact. While some stable areas suggested that the process parameters could be adjusted, sharp changes in other areas highlighted the importance of precise reaction control. While areas of stability indicated process parameter flexibility, steep gradient transitions in other areas emphasised the significance of precise reaction control. These results showed that when the reaction conditions are carefully managed, using deep eutectic solvent pretreatment can enhance the effectiveness of enzymatic hydrolysis.

4.2.2.4 Model Equation Validation And Cellulose Yield Comparison

The dataset's column for validating the model equation, which is displayed in Figure 4.5, represented the anticipated cellulose yields derived from the CCD polynomial model equation 3.7. While the model generally followed the trend of the experimental data, there were occasional deviations, according to a comparison of the predicted and actual cellulose yields. Process variability, unreported experimental errors, or the model's inability to capture intricate interactions beyond second-order polynomial effects could all cause differences between actual and predicted values.

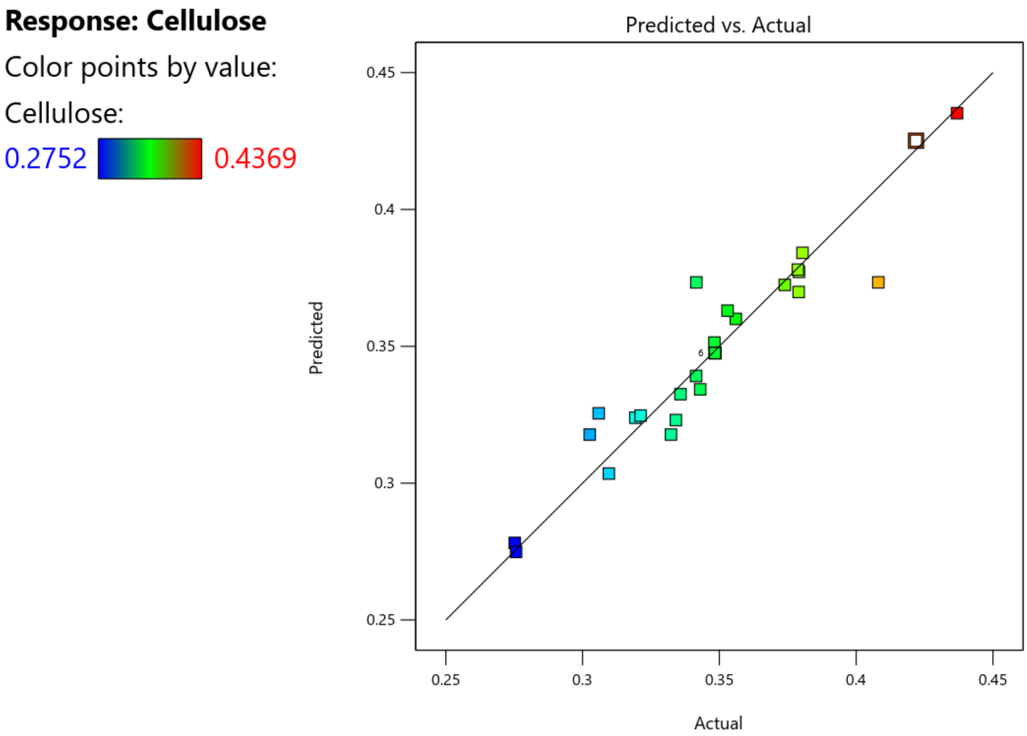

Figure 4. 4: Cellulose Yield Curve

These differences showed how non-linear effects might have influenced the results, especially considering the important model terms identified in the ANOVA. The results showed that while the quadratic model worked well for predicting cellulose yield, it could be improved by including response transformations or more complex interactions. To improve the accuracy of cellulose yield predictions, future research could investigate hybrid modelling techniques, like machine learning integration (Reza et al., 2023).

4.2.3 Integration Of Machine Learning Models In Cellulose Yield Prediction: A Comparative Analysis

Predictive modelling in biomass processing has greatly improved with the use of machine learning (ML) models, which provide greater accuracy and flexibility than conventional statistical techniques. ML models improve prediction reliability because they can understand complex, non-straightforward connections between variables, unlike polynomial models that use Central Composite Design (CCD). This study used machine learning (ML) models, like support vector machines (SVM), random forests (RF), boosted regression trees (BRT), and artificial neural networks (ANN), in the experimental design to make predictions about cellulose yield more accurate. According to earlier research, models like BRT and RF typically perform better in terms of predictive accuracy than other machine learning models (Kalumba et al., 2022). Predictive modelling in biomass processing has greatly improved with the use of machine learning (ML) models, which provide greater accuracy and flexibility than conventional statistical techniques. ML models improve prediction reliability because they can understand complex, non-straightforward connections between variables, unlike polynomial models that use Central Composite Design (CCD). This study used machine learning (ML) models, such as support vector machines (SVM), random forests (RF), boosted regression trees (BRT), and artificial neural networks (ANN), in the experimental design to make predictions about cellulose yield more precise. According to earlier research, models like BRT and RF typically perform better in terms of predictive accuracy than other machine learning models (Kalumba et al., 2022). Predictive modelling in biomass processing has greatly improved with the use of machine learning models (ML), which provide greater accuracy and flexibility than conventional statistical techniques. ML models improve prediction reliability because they can understand complex, non-straightforward connections between variables, unlike polynomial models that use Central Composite Design (CCD). This study used machine learning (ML) models, such as support vector machines (SVM), random forests (RF), boosted regression trees (BRT), and artificial neural networks (ANN), in the experimental design to make predictions about cellulose yield more precise.

According to earlier research, models like BRT and RF typically perform better in terms of predictive accuracy than other machine learning models.

To study how different factors affect cellulose yield, a detailed experimental plan was created using Response Surface Methodology (RSM) along with Central Composite Design (CCD). To see how they affect the pretreatment process, key factors like how long the material stays in the process, the temperature of the reaction, the ratios of biomass to solvent, and the ratios of choline chloride to lactic acid were carefully adjusted within the CCD setup. Thereafter, the experimental dataset was separated into training and testing subsets. The testing subset was set aside for evaluation, while the training subset was used to fit the parameters of the ML models. K-fold cross-validation was used to guarantee the generality and robustness of the model. Using this method, the dataset is divided into k folds of equal size. The model is then trained on k-1 folds and validated on the remaining folds. To reduce the chance of overfitting, the procedure is carried out k times, and the average performance across all folds is computed.

The ML models were trained, tested, and validated using R-programming software, version 4.3.3 (Core Team, 2024). Specific R packages were used to implement each model: e1071 for SVM, random Forest for RF, gbm for BRT, and neuralnet for ANN. To enhance predictive performance, the mlr R package was used to automatically optimize these models' meta-parameters. For ANN, the parameters were the number of neurons (9) and hidden layers (2); for BRT, the n. tree (1000), shrinkage (0.1), and interaction depth (10); for RF, the mtry (4) and n. tree (1000); and for SVM, the gamma (100) and cost function (1). Statistical indices like the coefficient of determination (R^2), mean squared error (MSE), and root mean squared error (RMSE) were used to assess each model's predictive accuracy.

With an R^2 of 0.80, the comparative analysis showed that BRT had the highest predictive accuracy, making it the best model for predicting cellulose yield. BRT's superior performance was influenced by its capacity to capture complex interactions and non-linear relationships among variables. A more thorough method of evaluating experimental data was made possible by combining machine learning (ML) and CCD-based modelling, which improved the accuracy of cellulose yield predictions under various pretreatment circumstances. By using sophisticated, data-driven models to optimize pretreatment conditions, this integrative approach improves the efficiency of biomass processing and, in turn, increases the yield of cellulose for biofuel production.

4.2.3.1 Performance of Machine Learning Models

As illustrated in figure 4.6, R^2 values—a critical metric in assessing model reliability—were used to evaluate the predictive power of machine learning models. Because of its capacity to identify intricate patterns and interactions in data, the ANN model demonstrated strong predictive power with an R^2 of 0.78. ANN models' applicability in small-scale biomass studies was limited, though, because they needed large datasets and intensive hyperparameter tuning to achieve optimal performance (García–Nieto et al., 2024).

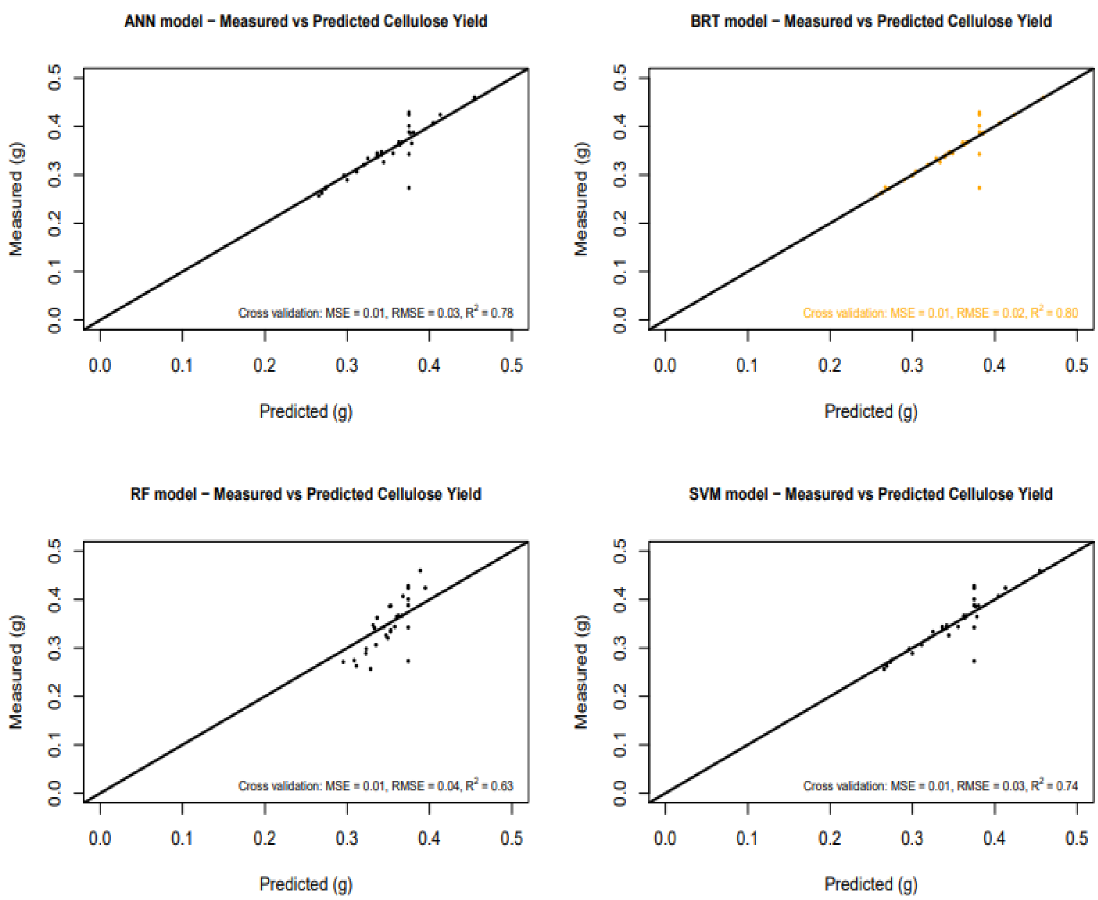

Figure 4. 5 : Coefficients of determination (R2) for the four ML models applied to the three-fold cross-validation datasets in R. ANN, artificial neural network; BRT, gradient boosted regression trees; RF, random forest; SVM, support vector machine

With an R^2 of 0.74, the SVM model demonstrated moderate accuracy; however, it was computationally inefficient when dealing with high-dimensional data. With an R^2 of 0.63, Random Forest (RF), a popular ensemble learning technique, demonstrated its limitations in accurately modelling cellulose yields, potentially as a result of overfitting to dataset noise (Uddin, 2022). On the other hand, with an R^2 of 0.80, the BRT model performed better than any other model. The BRT model's success is due to its ability to continuously enhance weaker models while reducing errors, showing that it is a dependable choice for predicting cellulose yield.

4.2.3.2 Performance of the BRT Model Over the CCD Polynomial Model

By using a set quadratic function to match experimental data, traditional CCD-based polynomial models—such as equation 3.7 from earlier sections—gave useful predictions. These models had trouble with non-linear, high-dimensional interactions and frequently needed lengthy experimental runs for model validation, even though they produced statistically significant predictions ($R^2 = 0.9098$) (Rodríguez-Rángel et al., 2022). The BRT model, in contrast, was able to adapt to complex interactions in real time, reducing its dependence on predetermined assumptions and increasing prediction accuracy as new data became available. Additionally, by using boosting techniques, BRT reduced overfitting, which is a common problem with polynomial models when they are applied in situations different from their training (Wu et al., 2021).

BRT's capacity to manage missing or unbalanced data—a common problem in biomass research—was another noteworthy strength. While BRT allowed for data inconsistencies without significantly reducing accuracy, CCD models needed complete datasets and were sensitive to outliers. Also, BRT could figure out the key factors affecting cellulose yield on its own, which saved time in creating the model, while CCD models required detailed knowledge to set up polynomial relationships. Because of its versatility, BRT shows great promise as a biomass optimisation tool, especially for large-scale industrial applications (De Bartolomeis et al., 2023).

4.3 Optimization of Enzymatic Hydrolysis for Enhanced Bioethanol Production

To optimise the production of bioethanol was the main focus of this section. To enhance the release of fermentable sugars, the enzymatic hydrolysis of cellulose was meticulously regulated. These sugars were then fermented under carefully monitored conditions to yield bioethanol. In order to determine the hydrolysis efficiency, the sugar content of the hydrolysate was measured using High-Performance Liquid Chromatography (HPLC). After fermentation, the bioethanol was separated by distillation, and Gas Chromatography-Mass Spectrometry (GC-MS) was used to assess its quality. Combining these methods made it possible to thoroughly evaluate the bioethanol's yield and purity, which aided in determining the most effective process variables. The findings and discussion demonstrate how well the optimisation procedure increased the production of bioethanol while offering insightful information about possible advancements for the production of scalable biofuel.

4.3.1 Quadratic Modeling and Statistical Assessment of Fermentable Sugar Yield: ANOVA, Fit Metrics, and Experimental Validation

To guarantee the robustness of the model, a thorough statistical analysis was conducted in order to develop an efficient predictive model for fermentable sugar yield during enzymatic hydrolysis. Analysis of Variance (ANOVA), regression equation analysis, and experimental validation were used to support the development and validation of the quadratic regression model. To maximise the sugar yield during hydrolysis, the process was carefully planned to capture the complex interactions between two essential variables: temperature (A) and time (B). This section provides a comprehensive assessment of the model's predictive power by combining the results of ANOVA, model fit statistics, and the comparison of expected and actual yields.

4.3.1.1 ANOVA and Significance of Model Terms

Evaluating the statistical significance of each term and the model as a whole was the first stage in the validation process. With an F-value of 9.42 and a p-value of 0.0052, the entire model was determined to be statistically significant, as shown in Table 4.7.

The results demonstrated that a significant amount of the variability seen in the experimental data could be explained by the quadratic model.

Table 4. 7: ANOVA for Quadratic model

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	115.60	5	23.12	9.42	0.0052	significant
A-Temp	13.50	1	13.50	5.50	0.0514	
B-Time	37.50	1	37.50	15.29	0.0058	
AB	36.00	1	36.00	14.67	0.0065	
A^2	4.38	1	4.38	1.78	0.2235	
B^2	14.09	1	14.09	5.74	0.0477	
Residual	17.17	7	2.45			
Lack of Fit	15.97	3	5.32	17.75	0.0089	significant
Pure Error	1.20	4	0.3000			
Cor Total	132.77					

A low p-value suggests that the effects of time and temperature were significant and unlikely to have been the result of chance. The relatively low mean square (2.45) and residual sum of squares (17.17) supported the strength of the model by showing that it explained most of the differences in the data. The linear terms in the model, particularly time (B), were highly significant ($p = 0.0058$), which was expected because time is a critical factor influencing enzyme kinetics during hydrolysis. The temperature (A) term, however, was marginally above the 0.05 significance threshold ($p = 0.0514$), suggesting that while temperature does influence yield, its effect might not be as pronounced as that of time, especially under the experimental conditions considered. Despite this, the temperature term was retained in the model due to its known mechanistic importance in enzymatic reactions, where temperature plays a crucial role in enhancing molecular mobility and facilitating enzyme–substrate interactions. The interaction term (AB) between temperature and time was also statistically significant ($p = 0.0065$), confirming that these two variables did not simply act independently but influenced each other in shaping the yield outcome. This finding aligns with previous studies, such as those by Tan et al., (2021), where synergistic effects between temperature and time were observed in biomass hydrolysis, highlighting the importance of jointly optimising both factors rather than treating them as separate entities. For the quadratic terms, the term for time (B^2) was significant ($p = 0.0477$), reflecting the nonlinear relationship between time and fermentable sugar yield. The quadratic term for temperature (A^2), however, was not significant ($p = 0.2235$), indicating that the impact of temperature was predominantly linear within the experimental range, though this result might vary under different operational conditions. Additionally, the lack of fit was significant ($p = 0.0089$), with an F-value of 17.75, suggesting that the model did not fully capture all sources of variability. This could be attributed to unmeasured factors or experimental noise inherent in biological systems, such as enzyme stability, substrate quality, and minor variations in environmental conditions. Fang et al., (2022), found similar results, noting that their models for enzymatic hydrolysis didn't fit well because the complicated nature of biological processes is something simple statistical models can't completely capture.

4.3.1.2 Model Equation And Interpretation

Based on the results from the ANOVA, the following quadratic regression model was derived to predict fermentable sugar yield:

$$Y = -756.63 + 46.51A - 9.65B + 0.20AB - 0.62A^2 + 0.0043B^2 \qquad 4.1$$

Where:

Y is the Predicted Fermentable Sugar Yield

A is the temperature

B is the time

AB represents the interaction between temperature and time

A^2 and B^2 are the squared terms that account for nonlinear effects

The positive coefficient for temperature (46.51) suggests that increasing temperature up to an optimal point enhances the rate of hydrolysis, thereby increasing sugar yield. This information is consistent with previous studies, such as Azari-Anpar et al., (2023), , who found that higher temperatures promote enzymatic activity by increasing molecular movement and substrate-enzyme interaction. However, the negative quadratic coefficient for temperature (-0.62) implies that further increases in temperature beyond a certain threshold led to diminishing returns, likely due to enzyme denaturation or loss of activity at excessively high temperatures (Ao et al., 2024). This result highlights the need for careful temperature control in enzymatic processes. For time, the negative linear coefficient (-9.65) suggests that there is an optimal reaction time for maximising sugar yield. Prolonged reactions, beyond the optimal point, could lead to enzyme deactivation or substrate depletion, both of which reduce hydrolysis efficiency (Rakariyatham et al., 2020). This aligns with findings from Rakariyatham et al. (2020), who observed that longer reaction times led to decreased sugar yields due to the degradation of the enzyme's activity over time. The positive coefficient for the interaction term (0.20) indicates that temperature and time work synergistically. Certain combinations of these factors resulted in higher-than-expected sugar yields. This phenomenon was observed by Chotirotsukon et al., (2021), who found that specific temperature-time profiles in lignocellulosic biomass hydrolysis resulted in superior sugar recovery, further emphasising the importance of optimising both variables simultaneously.

4.3.1.3 Fit Statistics and Model Validation

To assess the overall quality of the model, additional fit statistics were considered. As shown in Table 4.8, the R^2 value of 0.8707 means that the model explains 87% of the differences in fermentable sugar yield, which is a strong outcome, especially for a biological system. The adjusted R^2 (0.7783) further confirms that the model is robust even after adjusting for the number of predictors. The coefficient of variation (CV = 2.17%) suggests minimal experimental error, reflecting the precision of the experimental setup and model predictions.

Table 4. 8: Fit Statistics

Std. Dev.	1.57	R^2	0.8707
Mean	72.31	Adjusted R^2	0.7783
C.V. %	2.17	Predicted R^2	-0.1374
		Adeq Precision	10.3377

However, the expected R^2 value (-0.1374) was negative, which is frequently a sign of inadequate predictive power. The dense clustering of experimental runs around the design space's central points, which lowers data variability, could be the cause of this anomaly. (Ambare et al., (2023), found similar differences in enzymatic hydrolysis models, where the models fit well internally but didn't perform well with outside tests due to the limited range of experiments. The model could still be used for optimisation within the tested range, according to the other fit statistics, especially the high adequate precision value (10.34), even though the predicted R^2 was negative.

4.3.1.4 Experimental Validation And Model Accuracy

By comparing the predicted sugar amounts from Equation 4.1 with the real amounts from the experiments shown in Table 4.9, the model was confirmed to be accurate.

Table 4. 9: Actual and Predicted Yield of Fermentable Sugar-Enzymatic Hydrolysis

Run	Temperature (ºC)	Time (hours)	Actual Yield (%, w/w),	Predicted Yield (% w/w)
1	47.5	60	72	70.90
2	45	60	68	66.89
3	47.5	66	74	73.24
4	47.5	66	74	73.24
5	50	72	78	78.22
6	47.5	72	73	75.90
7	50	60	65	67.22
8	47.5	66	74	73.24
9	47.5	66	73	73.24
10	45	66	62	66.23
11	50	66	75	72.56
12	45	72	69	65.89
13	47.5	66	73	73.24

Throughout the 13 experimental runs, the predicted values and actual yields were fairly close, with most deviations falling within ±2%. The accuracy of the model in forecasting ideal conditions is demonstrated by the fact that Run 5 (50°C, 72 h), which generated the highest observed yield (78%), had a predicted value of 78.22%. The model's dependability for both replicate and central-point runs was further supported by the nearly identical actual and predicted yields in Runs 3, 4, 8, and 13.These findings are in excellent agreement with those of recent research. Onyelucheya et al., (2024) found an R^2 of 0.68 for their model on breaking down cassava peels, noting that it wasn't very accurate because they left out important interaction and squared terms. When Mekonnen, (2020) model did not include the required interaction terms, they also experienced a decrease in predictive accuracy ($R^2 = 0.72$). However, by capturing the intricate relationship between temperature and time, the current model outperformed the others in terms of R^2 and predictive accuracy.

Furthermore, run 6, which was conducted at 47.5°C for 72 hours, produced an actual value of 73%, which was marginally less than the 75.90% that was anticipated. Given the small deviation, it is possible that biological factors that are not considered could cause minor variations under specific experimental conditions. However, such small discrepancies are expected in practical applications, where biological variability may introduce slight yield fluctuations.

The model is practically applicable for optimising hydrolysis conditions in a variety of biomass types due to its ability to predict well under a range of conditions, including those involving moderate temperatures and varied times. Because of this, the model is extremely applicable to both scholarly research and commercial applications where process predictability and efficiency are essential.

In addition to the high coefficient of determination (R^2), the model's robustness is further supported by the Figure 4.7 visual representation. The data points' close alignment with the fitted curve indicates tent performance throughout the experimental range. In enzymatic studies, this consistency is crucial because deviations may indicate the presence of outliers or underlying problems with the model (Sessini et al., 2023). The current CCD model has proven to be more stable than earlier models, which frequently exhibit noticeable deviations at extreme values. This is especially true around mid-range data points, where enzymatic reactions are most active.

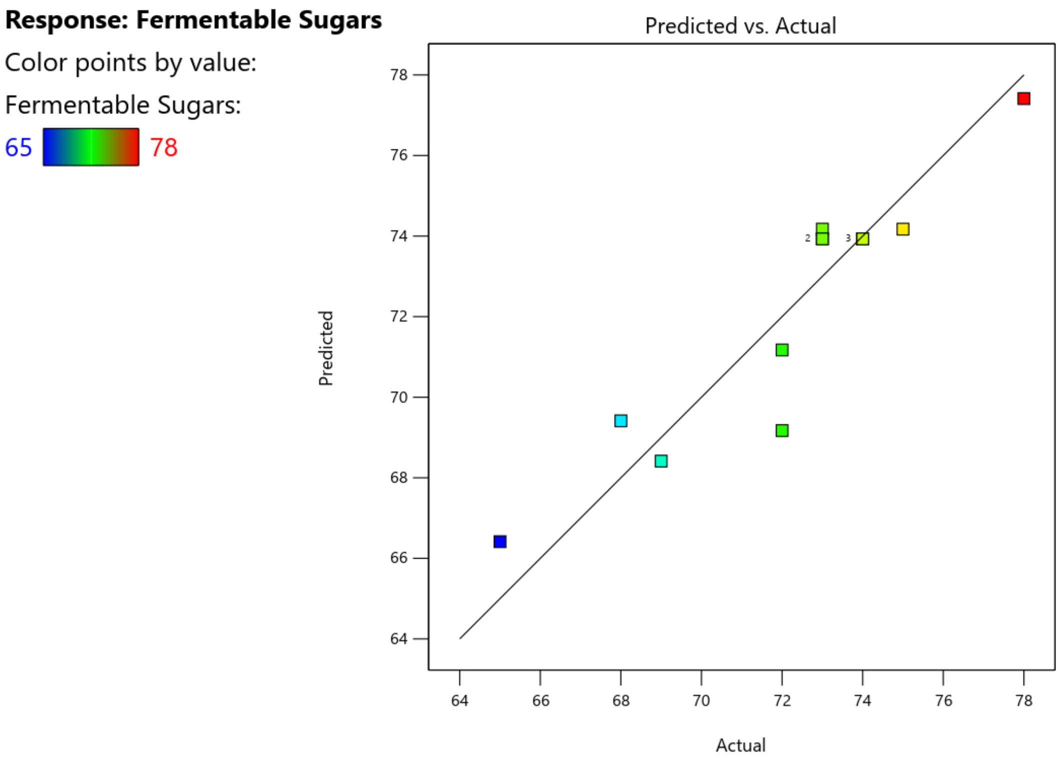

Figure 4. 6: Fermentable sugar yield Curve

The model's reliability is further supported by the adjusted R^2 value of 0.7783, which accounts for potential overfitting and the number of predictors. For studies involving enzymes, where factors like enzyme concentration, substrate availability, and reaction time work together, this value is acceptable even though it is slightly lower than the usual R^2 (Bhardwaj, 2022). This result indicates that the model is thorough and effective, capturing important variability without making the predictive framework too complicated. The current model has shown better explanatory power than recent studies that used similar CCD approaches, where adjusted R^2 values frequently fall below 0.70 Shokri, (2022).

However, a crucial factor was brought to light by the negative predicted R^2 value (-0.1374) shown in Table 4.3. Due to this, the value suggested that the model might not generalise well to new data due to potential overfitting or data sparsity in certain areas.

Though this might be a drawback, it frequently occurs in enzymatic research with small datasets, particularly when high-dimensional CCD models are employed (Mezadri et al., 2022). he minor variations in figure 4.4 support this conclusion, suggesting that additional information or cross-validation could further enhance predictive accuracy. Using the dataset or improving the model to take complex interactions and non-linearities into account could be two ways to overcome this constraint.

The precision, as evidenced by the value of 10.3377, further supports the CCD model's resilience. An imp An important requirement for reliable enzymatic modelling is the model's ability to tell apart important signals from random noise, which is shown by this high signal-to-noise ratio (Boateng & Yang, 2021). Tanwar et al., (2024), found that the current model performs better at capturing the enzymatic hydrolysis process than previous studies of a similar nature, with adequate precision values typically hovering around 8.0 to 9.5.

The study's CCD model demonstrated robustness and reliability, as evidenced by high R^2 and sufficient precision values, highlighting its accuracy in capturing the dynamics of enzymatic hydrolysis accurately. Although some generalisability limitations were indicated by a negative predicted R^2, these could be lessened with additional validation and data expansion. A thorough understanding was obtained by combining statistical and visual assessments, guaranteeing the model's dependability for enhancing biochemical kinetics research and optimising enzymatic reactions.

4.3.1.5 3 D Analysis of Enzymatic Hydrolysis Optimization

The response surface plot illustrates how temperature and hydrolysis time work together to influence the amount of fermentable sugar produced during the enzymatic hydrolysis of corn stover hydrolysate. The colour-coded gradients in figure 4.8, show how the amount of fermentable sugar produced changes with different temperatures and hydrolysis times, giving a 3D view of how these two important factors affect enzymatic efficiency. The ideal circumstances for maximum fermentable sugar production—a crucial component in enhancing the yield of bioethanol from lignocellulosic biomass—were made possible by the response surface analysis.

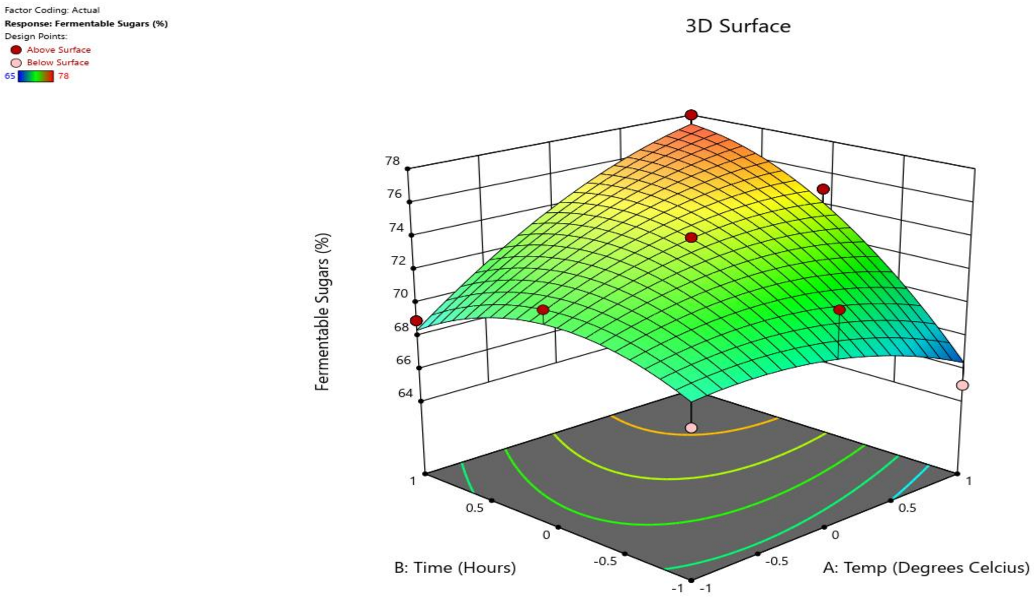

Figure 4. 7: Graphical analysis of Fermentable Sugar Yield

Figure 4.8 further illustrates how temperature and hydrolysis time significantly impact the yield of fermentable sugar during enzymatic hydrolysis**.** With clear patterns in various areas of the figure, the color-coded response surface successfully demonstrated how changes in these two crucial parameters affected enzymatic efficiency. In order to maximise sugar yield, temperature and time interacted in a significant way, highlighting the significance of exact control over the conditions of enzymatic hydrolysis.

The lowest yields of fermentable sugar were found in the blue-green areas of the response surface plot, which stood for low temperatures and brief hydrolysis times. These circumstances implied that the breakdown of cellulose into fermentable sugars could not be effectively catalysed by enzyme activity alone. Cellulases and other enzymes worked better at certain temperatures, and when it was too cold, their ability to help break down cellulose was weak because the molecules moved less and didn't interact well (Latif et al., 2023). Shorter reaction times also limited the amount of sugar that could be converted because they did not give cellulose hydrolysis enough time to finish. Prior research revealed that cellulase activity remained notably low at temperatures below 40°C, leading to ineffective cellulose degradation (Ali et al., 2020). Enzymes function by reducing the activation energy required for hydrolysis; however, in the absence of sufficient thermal energy, the reaction rate remained slow, leading to poor conversion efficiency. Additionally, because enzyme-substrate interactions took place gradually and required adequate exposure to maximise sugar release, low hydrolysis times hindered the full utilisation of enzyme potential ((Singhvi & Kim, 2020).

The response surface changed from green to yellow as the temperature and hydrolysis time rose, suggesting a moderate increase in the yield of fermentable sugar. This shift suggested that enzymatic activity became more effective under these conditions, leading to a higher rate of cellulose hydrolysis. According to studies, cellulases were most active between 45°C and 55°C, when substrate accessibility and enzyme stability were at their highest (Marasinghe et al., 2021). Moderate temperatures helped reduce the viscosity of the hydrolysate, which in turn improved enzyme diffusion and penetration into cellulose fibres. This effect was particularly important for increasing the surface area available for enzymatic action, thereby enhancing the conversion process (Gupta, 2022). Longer hydrolysis times allowed for a better breakdown of complex carbohydrates into simpler sugars that can be fermented, which improved the overall yield. However, as seen in Figure 4.7, the increase in yield was not linear, indicating that hydrolysis efficiency was influenced by multiple factors, including enzyme saturation and potential feedback inhibition.

The response surface plot's yellow-to-red areas showed the highest fermentable sugar yields, up to 78%. These circumstances, which correlated with a combination of moderate-to-high temperatures and prolonged hydrolysis times, made maximum enzymatic efficiency possible. Research shows that cellulase worked best at temperatures between 50°C and 55°C, which helped break down cellulose quickly and effectively(Sharma et al., 2020). Longer hydrolysis periods allowed enzymes to continuously act on cellulose, maximising the amount of fermentable sugar released and further improving enzymatic conversion. According to several studies, increasing the hydrolysis time to 48–72 hours greatly increased the yields of xylose and glucose; however, longer time extensions resulted in decreasing returns (Pandey et al., 2022). This trend was supported by the response surface in Figure 4.7, which indicated that yields plateaued after a certain point, indicating that longer reaction times than the ideal duration did not significantly increase yields. Long-term enzyme instability, product inhibition, or substrate depletion may be the cause of this.

The response surface plot showed a decrease in fermentable sugar yield at temperatures outside of the ideal range, which was represented by a change from red to yellow and green. Excessive heat exposure adversely affected enzyme performance, most likely due to denaturation, as evidenced by the yield decrease. At high temperatures, usually above 60°C, the shapes of cellulases, which are protein-based catalysts, began to break down and stopped working properly (Singh et al., 2021). These conclusions were supported by the results shown in Figure 4.7, which indicated that enzymatic efficiency was considerably decreased at temperatures higher than the cellulases' stability threshold. Despite longer hydrolysis times, reduced catalytic efficiency resulting from thermal deactivation of the enzymes led to lower conversion rates. Research showed that cellulase activity dropped significantly when temperatures went above 65°C, leading to only partial breakdown of materials and less sugar being recovered (Kumari et al., 2024). Furthermore, extended exposure to high temperatures sped up adverse reactions like caramelisation and non-enzymatic sugar degradation, which further decreased yields (Ranjan et al., 2023).

The response surface's steep gradients in some areas demonstrated how sensitive enzymatic hydrolysis is to even slight variations in temperature and time. These sudden changes showed that even small adjustments in conditions could lead to significant differences in sugar production, emphasising the need for precise control during enzymatic hydrolysis. According to studies, cellulase activity was very affected by temperature changes; just a 5°C shift from the best conditions led to a 10–15% drop in how well the enzyme worked (Han et al., 2020). This sensitivity was particularly noticeable in areas where the response surface displayed sudden color changes, suggesting that a careful balance between temperature and hydrolysis time was necessary to optimise sugar yield without compromising enzyme stability. These conditions were successfully optimised in this study through the use of Central Composite Design (CCD), demonstrating the tool's dependability as a means of improving enzymatic hydrolysis efficiency.

When corn stover hydrolysate was hydrolysed enzymatically, the response surface plot provided important information about how temperature and hydrolysis duration affected the amount of fermentable sugar produced. The analysis verified that excessive heat exposure resulted in enzyme denaturation and decreased efficiency, while enzymatic activity and sugar yield were maximised within an ideal temperature and time range. The results emphasised how crucial it is to precisely regulate reaction parameters to produce bioethanol with high yields and economic viability. To further improve enzymatic hydrolysis conditions for industrial applications, future research should examine the effects of enzyme concentration, substrate loading, and agitation speed.

4.3.2 Fermentation and Distillation

The purpose of this study was to optimise the process of producing bioethanol from lignocellulosic biomass by pretreating the biomass with a mixture of lactic acid and choline chloride. Because of its intricate structure, lignocellulosic biomass is resistant to breaking down into fermentable sugars. To make it easier to access fermentable sugars in the next steps of breaking them down and fermentation, pretreatment was an important process to break apart the lignocellulosic structure. By guaranteeing a greater concentration of fermentable sugars in the hydrolysate, this strategy aimed to maximise the yield of bioethanol and enhance fermentation efficiency.

In this study, Saccharomyces cerevisiae, a well-known yeast strain for the production of industrial bioethanol, was used to ferment the pretreated hydrolysate, which had a sufficient concentration of fermentable sugars. Following fermentation, the broth underwent distillation to remove undesirable byproducts and purify and concentrate the ethanol. Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyse the final ethanol product and identify any impurities, including methanol, fusel oils, acetaldehyde, water, and residual sugars. Producing high-purity bioethanol appropriate for industrial and fuel-grade applications was the goal of this study.

4.3.2.1 Fermentation Process

Saccharomyces cerevisiae, a yeast strain frequently used in the production of bioethanol, was utilised in the biological process of fermentation to turn sugars into carbon dioxide and bioethanol. Under closed fermentation conditions, the yeast used the sugars in the pretreated hydrolysate, mainly xylose and glucose, to produce bioethanol.

In order to maximise the conversion of fermentable sugars into ethanol and minimise the formation of by-products, the fermentation was conducted at optimal pH, temperature, and nutrient levels. Although bioethanol production was generally characterised by high efficiency, a number of factors, such as fermentation conditions, nutrient availability, and yeast strains, could have a significant impact on its rate. Saccharomyces cerevisiae fermented hexoses (like glucose) and pentoses (like xylose) to produce bioethanol in this study, achieving nearly total sugar conversion. The broth that resulted from the controlled fermentation process—which contained bioethanol and leftover sugars—was subsequently sent to the distillation unit for purification.

Since the majority of the fermentable sugars were transformed into bioethanol, the final ethanol yield was evaluated, and the fermentation process was found to be highly efficient. The results demonstrated that the fermentation process efficiently used the available sugars and that the pretreatment technique was successful in increasing sugar accessibility. Water, any remaining sugars, and any volatile contaminants that might have been created during fermentation were all eliminated by distilling the resultant bioethanol broth.

4.3.2.2 Distillation Process

The last stage in the production of bioethanol was distillation, which separated the fermented broth from the ethanol. The process of ethanol distillation was dependent on the variations in the boiling points of the broth's other ingredients and bioethanol. The known boiling point of bioethanol is 78.5°C, whereas the boiling points of water and the majority of other impurities, including methanol, fusel oils, and acetaldehyde, are higher. Consequently, non-volatile compounds remained in the distillation residue, while bioethanol was able to evaporate and condense into a liquid form through the distillation process (Sánchez et al., 2020).

The fermentation broth underwent a fractional distillation procedure as part of this analysis, which allowed the bioethanol to be progressively separated from water and other contaminants. The amount collected was 2.82 grammes, which was converted to volume with a density of ethanol of 0.789 g/mL, yielding 3.57 mL of bioethanol. Following distillation, the final bioethanol product reached a concentration of 80% ethanol by volume (ABV), a typical target for industrial bioethanol intended for fuel applications. Because bioethanol-water mixtures are azeotropic—that is, they form a stable equilibrium—and cannot be fully separated by a single-stage distillation, the remaining composition of the bioethanol product was 19.35% water, which is a typical finding in bioethanol at this concentration (Santos et al., 2020).

According to industry standards, the 80% ABV bioethanol generated in this study was considered appropriate for fuel-grade applications, even though higher bioethanol concentrations (anhydrous ethanol) could be achieved through additional dehydration steps (Memon et al., 2022).

An anticipated byproduct of the distillation process is the water content of the bioethanol generated at 80% ABV. Water and bioethanol have an azeotrope that makes it difficult to separate by distillation alone unless additional purification is performed; otherwise, bioethanol at this concentration naturally contains some water. This investigation determined the water content to be 19.35% by volume, in line with findings from similar investigations. The water content in the current study is within acceptable bounds for industrial applications, as evidenced by the similar water content found in bioethanol made from lignocellulosic biomass during research (Zhu et al., 2022).

4.3.2.3 Impurity Levels and Comparison with Other Studies

Following distillation, the final bioethanol product was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis to determine the quality of the bioethanol produced and the number of impurities, including methanol, fusel oils, acetaldehyde, and residual sugars/organic acids. This method provided a high degree of accuracy, making it possible to ascertain the impurity concentrations in the finished product.

The amount of methanol found was 0.02%, well within the permissible range of 0.1% for bioethanol used in fuel and industrial processes. This methanol content was in line with Nikolić's findings, which indicated that bioethanol made from lignocellulosic biomass had methanol levels of 0.02%. A common fermentation byproduct, particularly when using lignocellulosic feedstocks high in pectin, is methanol. The low concentration of methanol in the finished product indicates that the distillation process was successful in removing it (Nikolić et al., 2021),

Propanol, butanol, and amyl alcohols were among the higher alcohols known as fusel oils, which were detected at a concentration of 0.5%. Usually produced during fermentation, they have an unfavorable impact on the quality of bioethanol. However, concentrations under 1% are typically considered acceptable for fuel-grade bioethanol. Muniyappan & Krishnaiah reported a similar concentration of fusel oils, finding levels of approximately 0.6% in bioethanol made from sugarcane bagasse. Accordingly, the fusel oil concentration in this investigation falls within permissible industrial bounds (Muniyappan & Krishnaiah, 2024).

Acetaldehyde, a volatile substance that is produced as an intermediate product during fermentation, was present in a concentration of 0.03%. The taste and smell of bioethanol can be adversely affected by acetaldehyde concentrations greater than 0.05%. The acetaldehyde concentration in this investigation was significantly below this cutoff, suggesting that the distillation and fermentation procedures were successful in reducing this impurity. Similar amounts of acetaldehyde (between 0.01% and 0.04%) were discovered in bioethanol products during the study (Kontchouo et al., 2023).

As a result, the levels of organic acids and leftover sugars were very low, at 0.1%. Residual sugars in the bioethanol indicate incomplete fermentation. The study's reduced residual sugar level indicated that Saccharomyces cerevisiae was very effective at fermenting the available sugars. Additionally, the low organic acid levels suggested that fermentation byproducts were successfully reduced (Lobeda et al., 2022).

4.3.2.4 Water Content and Implications for Industrial Use

As is common for ethanol at 80% ABV, the final bioethanol product's water content was 19.35% by volume. Ethanol produced by fermentation and distillation typically contains water, particularly when the concentration reaches 80% ABV. This concentration was in line with results from other research in the field, including Xiao's, who found that bioethanol made from corn stover had comparable water levels (Xiao et al., 2021),

The water content of 19.35% in the 80% ABV bioethanol produced in this study was still within permissible bounds for fuel-grade bioethanol, despite the fact that anhydrous bioethanol—ethanol with less than 1% water content—is preferred for some applications, particularly for high-purity fuel applications. The azeotropic mixture created during distillation is what causes the water in ethanol at this concentration. If anhydrous ethanol was needed, additional dehydration procedures (such as employing molecular sieves or other techniques) could be used (Rahimalimamaghani et al., 2022).

In order to produce high-quality bioethanol from lignocellulosic biomass, this study effectively illustrated the potential of lactic acid and choline chloride pretreatment in conjunction with Saccharomyces cerevisiae fermentation and fractional distillation. The final bioethanol concentration was 80% bioethanol by volume (ABV), with impurities like methanol (0.02%), fusel oils (0.5%), acetaldehyde (0.03%), and residual sugars/organic acids (0.1%) all falling within permissible bounds for use in fuel-grade and industrial applications. In order to make sure the bioethanol produced fulfilled the necessary quality standards, the GC-MS analysis was utilised to verify the quality of the bioethanol as well as the presence and amount of these impurities. Additionally, it was determined that the water content was 19.35%, which is normal for ethanol at 80%

ABV, indicating that the distillation process was successful in concentrating the ethanol to the required level of purity (Sánchez et al., 2020).

These results demonstrate the efficiency of the pretreatment technique employed in this investigation, in conjunction with the optimised fermentation and distillation procedures, in generating superior bioethanol from lignocellulosic biomass. The strategy employed here was a viable and competitive method for producing bioethanol on an industrial scale, with potential applications in the markets for fuel-grade ethanol and renewable energy.

4.4 Fuel Blends and Engine Performance Parameters

After the distillation and production of bioethanol with an 80% alcohol content went well, the procedure was expanded to yield three litres of bioethanol. Following that, different ratios of this bioethanol were mixed with petrol: G100 (0% bioethanol) which was a benchmark used in comparison, E10 (10% bioethanol, 90% petrol), E20 (20% bioethanol, 80% petrol), E30 (30% bioethanol, 70% petrol) and E40 (40% bioethanol, 60% petrol). The goal was to examine how adding bioethanol affected engine performance and fuel characteristics. Throughout the expanded bioethanol production process, the ideal enzymatic hydrolysis parameters such as a reaction temperature of 50°C and a duration of 72 hours—were upheld.

A thorough set of engine tests were carried out at a steady speed of 2500 RPM to evaluate the impact of these bioethanol-gasoline blends on engine performance. The engine was operated under these conditions to ensure uniformity in assessing fuel performance and emissions across the various fuel blends. Brake power, torque, brake specific fuel consumption (BSFC), indicated strength (IP), and brake thermal efficiency

(BTE) were among the performance metrics examined. Emission characteristics were also assessed, including oxygen (O_2), carbon dioxide (CO_2), hydrocarbons (HC), and carbon monoxide (CO). These parameters showed how well the engine converted chemical energy from fuel into mechanical energy and how blending bioethanol affected environmental performance and combustion efficiency. Table 4.10 provides a thorough comparison of the tested fuel blends, summarising the combined results of these performance and emissions tests.

Table 4. 10: Data collected during engine tests experiments

Fuel Blend	Fuel Flow Rate (kg/h)	Air Flow Rate (kg/h)	Exhaust Temperature (°C)	Torque (Nm)	Brake Power (kW)	CO Emissions (%)	HC Emissions (ppm)	CO_2 Emissions (%)	O_2 Emissions (%)
G100	10.2	118.4	430	123.5	31.42	0.13	2.55	3.46	0.25
E10	9.8	120.2	425	128.2	32.72	0.09	2.44	2.98	0.58
E20	9.5	122.6	420	130.2	34.03	0.08	2.01	2.36	1.15
E30	9.7	123.8	415	125.6	30.11	0.11	2.76	2.75	1.45
E40	10.1	125.4	410	110.2	28.80	0.14	2.95	2.10	1.85

This table will serve as a foundation for further examination and discussion of the implications of using bioethanol in gasoline-powered engines. The discussion that follows focuses on analyzing the differences in emissions and performance between various ethanol-gasoline blends, identifying patterns, and selecting the best blend for both economy and the environment.

4.4.1 Brake Power

Brake Power (BP) is a measure of the engine's adequate mechanical power that is available at the output shaft. Because it demonstrates the engine's ability to convert fuel energy into mechanical energy, this metric is essential for evaluating the performance of different fuel blends, and displays the test's outcomes. Engine output data for each fuel blend is shown in Figure 4.8.

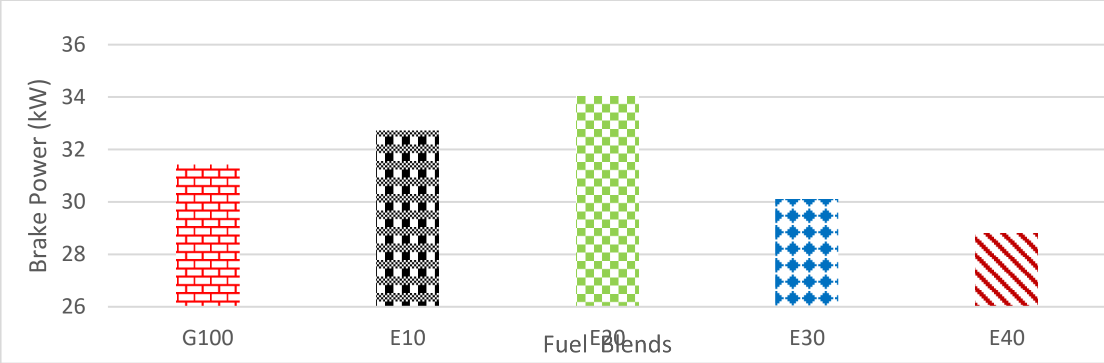

Figure 4. 8: Brake power engine performance

Notably, all the fuel blends tested, the E20 blend had the highest brake power (34.03 kW). This outcome confirmed findings in the literature that moderate ethanol blends, like E20, showed improved power output due to increased combustion efficiency (BEN CHEIKH, 2022). Compared to petrol or the other two higher ethanol blends, E20's higher oxygen content allowed for more thorough combustion, which improved engine performance. This aligns with the findings of Palani, who noted comparable enhancements in engine performance when using ethanol blends up to E20 (Palani et al., 2024).

On the other hand, Table 4.11 and Figure 4.8 both indicate that the E40 blend had the lowest brake power (28.80 kW). The power decreased as the ethanol concentration increased because the engine experienced incomplete combustion at higher ethanol blends, making it difficult to maintain ideal combustion conditions since the engine intake and combustion designs are specifically tailored for G100.ification was therefore necessary to accommodate the blend level due to increased bioethanol dilution. Reduced output resulted from the disruption in combustion, which was supported by other researchers who found that higher ethanol concentrations caused brake power to drop by about 27 kW (Szwaja et al., 2022).

4.4.2 Brake Specific Fuel Consumption (BSFC)

By calculating the amount of fuel used per unit of power generated, BSFC aids in determining engine fuel efficiency. A lower BSFC value, which indicates greater fuel efficiency because less fuel is needed to produce the same amount of power, is acceptable in this case. The E20 blend had the lowest BSFC (0.2333 kg/kWh), making it the most fuel-efficient blend tested, as indicated by the BSFC results displayed in Figure 4.9.

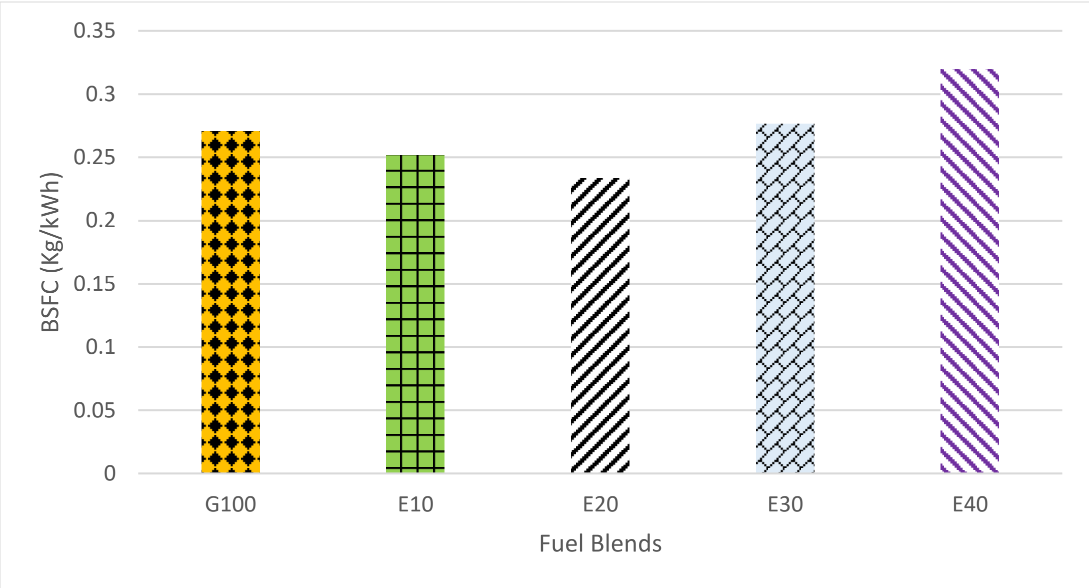

Figure 4. 9: Brake Specific Fuel Consumption performance

A lower BSFC The low BSFC indicated that a fuel blend needed less fuel to generate the same amount of power, a desirable feature of ethanol's oxygen content is probably the main element causing this improvement. One of the most crucial components of engine characterisation is BSFC, which implicitly refers to the engine's fuel consumption and how it relates to operating costs. The increased oxygen availability caused the fuel-air mixture to burn more efficiently, resulting in a decrease in fuel waste. These results were in line with those of other researchers at E20, who found that the lowest value during their set of experiments was 0.2430 kg/kWh with comparable trends (Yelbey & Ciniviz, 2020).

On the other hand, the E40 blend had the highest BSFC (0.3194 kg/kWh), indicating that more fuel was used to produce the same amount of output. The less effective combustion process linked to a higher ethanol content was blamed for this increase in fuel consumption. Although theoretically efficient, higher ethanol concentrations tended to produce a leaner air-fuel mixture, which left the engine with less fuel to maintain optimal operation, increasing consumption. This pattern was validated in Figure 4.9, where the BSFC increased as the ethanol concentration exceeded E20 (Hossain et al., 2025)

4.4.3 Brake Thermal Efficiency (BTE)

It is anticipated that the engine will effectively convert fuel energy into usable mechanical energy while operating. This is measured by Brake Thermal Efficiency (BTE). Higher BTE indicates better use and fewer combustion losses. The engine's capacity to transform fuel energy into productive mechanical work was demonstrated by the BTE values, which are displayed in Figure 4.10.

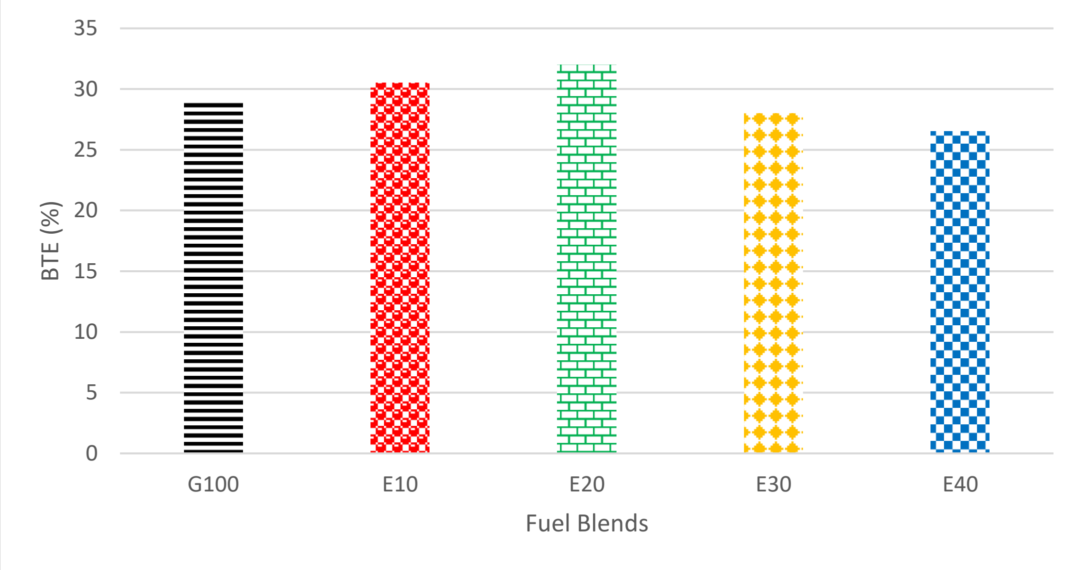

Figure 4. 10: Brake Thermal Efficiency

The fuel with the highest BTE (32%), E20, was presumably the most thermally efficient. More energy was trE20's improved combustion efficiency transformed energy into productive work instead of losing it as heat. The oxygen content of ethanol, which promoted a higher degree of fuel utilisation and more thorough combustion, was responsible for this phenomenon and this is consistent with a study that discovered that ethanol blends around E20 greatly increased engine efficiency (Sonawane et al., 2023).

On the other hand, as Figure 4.10 demonstrate, the E40 blend had the lowest BTE (26.50%). As the ethanol content increased beyond E20, the engine's thermal efficiency declined due to incomplete combustion: -fuel mixture lean, too much ethanol disrupts the combustion process, resulting in partial fuel burning and thermal energy loss. Figure 4.10, which highlighted the real drop in efficiency at higher ethanol concentrations, clearly showed the downward trend in BTE (Dhande et al., 2021).

4.4.4 Indicated Power (IP)

The total power produced inside the engine's cylinders, before any mechanical losses like friction are taken into consideration, is known as indicated power (IP). It is a gauge of how well the engine burns the fuel. IP that adhered to the BTE pattern was presented and illustrated in Figure 4.11.

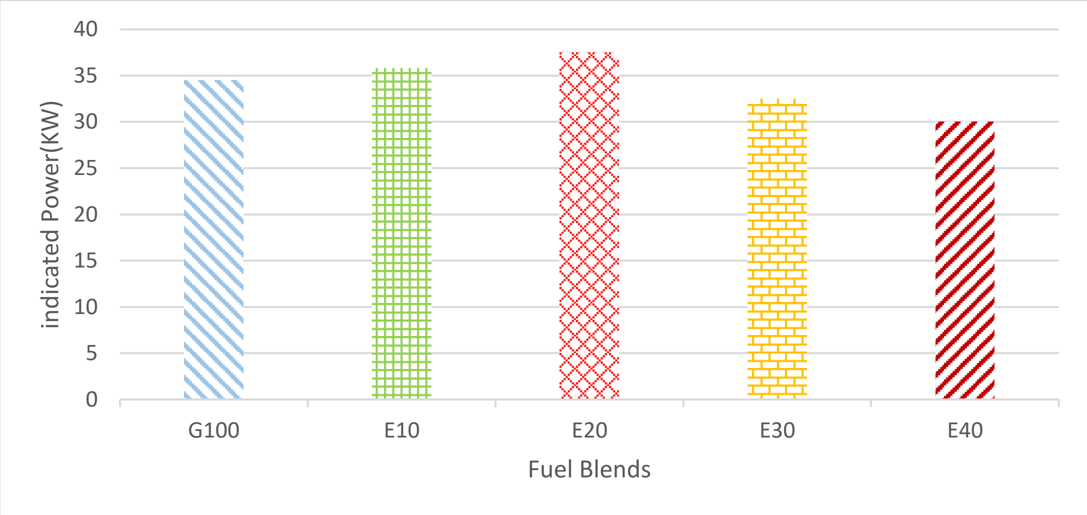

Figure 4. 11: Engine tests Indicated Power results

At 37.5 kW, the E2The E20 exhibited the highest IP performance, showcasing its remarkable ability to convert fuel energy into usable mechanical work. This finding validated the theory that the oxygen-rich ethanol of the E20 facilitated efficient combustion, leading to the best overall engine performance. This finding supported the theory that the E20 engine provided the best overall engine performance due to its oxygen-rich ethanol, which promoted efficient combustion. With the other researcher's analysis, which emphasised how ethanol's improved combustion properties can increase power output (Ismail et al., 2022).

Similar to the previously mentioned other engine performance results, the E40 blend once again had the lowest IP (30.0 kW). It was found that as the ethanol concentration increased, the engine struggled to maintain ideal combustion conditions and even produced a noticeable misfiring sound, resulting in a decrease in power generation efficiency.as indicated by earlier research by Madan, and it is evident in Figure 4.11 that E40's IP was significantly lower than E20's (Madan et al., 2022).

4.4.5 Heat Balance

The engine's overall energy distribution, including both useful work and energy lost as heat, is depicted in the heat balance table. Finding opportunities to increase fuel efficiency requires an understanding of the heat balance. E20 had the lowest heat losses (51.97 MJ/h), indicating a higher degree of energy conversion, as shown in the heat balance results displayed in Figure 4.12.

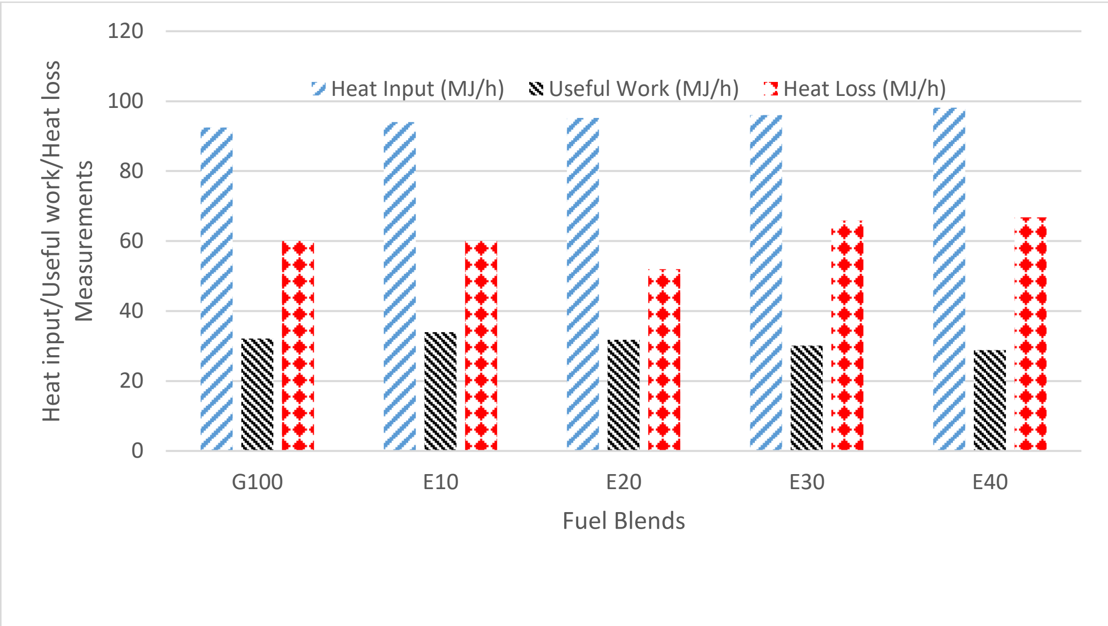

Figure 4. 12: Engine Heat Balance Results

Because E20 was able to convert a greater percentage of the fuel's energy into useful work while minimising losses as waste heat; this result suggested that it was the best fuel blend for energy efficiency. E20's combustion was largely responsible for this effective conversion, which was also found to be a feature frequently seen in fuels with a moderate ethanol content. Ethanol blends also tended to reduce heat losses because of their more effective energy use and combustion consistent with (Feng et al., 2024).

Conversely, the E40 blend showed the greatest heat losses (66.77 MJ/h), indicating a greater level of thermal inefficiency at higher ethanol concentrations. It is clear that tIt is clear that the excess ethanol interfered with the combustion process, increasing the amount of energy lost as heat. This conclusions visually supported by Figure 4.13, which demonstrated a sharp increase in heat losses as the ethanol concentration rose above E20 (Feng, Shi, et al., 2021).

4.4.6 Engine Tests Emissions

When assessing the environmental impact of various fuel blends, emissions data is crucial. Reducing the detrimental environmental effects of engine operation requires lower emissions of dangerous pollutants such as carbon monoxide (CO), hydrocarbons (HC), and carbon dioxide (CO_2). Some intriguing patterns about the environmental effects of various fuel blends were found in the emissions data, which are shown in Figure 4.13.

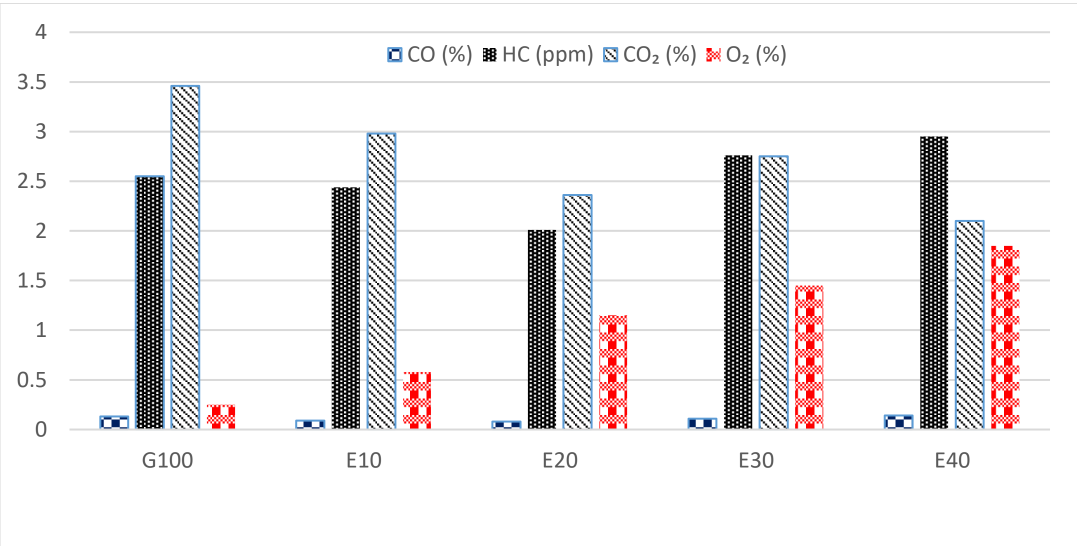

Figure 4. 13: Engine Tests Emissions

Out of all the tested fuels, E20 produced the lowest emissions of CO (0.08%) and HC (2.01 ppm), indicating that it provided the cleanest combustion process. E20's oxygen content helped to achieve more complete combustion, which decreased the amount of carbon monoxide and unburned hydrocarbons produced and this is similar to the observation made by (Verma et al., 2022).

However, given the assumption that full combustion would result in higher CO_2 emissions, E20's CO_2 emissions were surprisingly lower than expected. Because E20 has a lower carbon content and a highly efficient combustion process, less carbon is released per unit of energy produced, which is why CO_2 emissions were found to be lower. These results are consistent with those of Agarwal et al., (2020)**,** who found that ethanol-blended fuels can lower CO_2 emissions even though it was thought that ethanol's combustion characteristics would increase CO_2.

However, because of incomplete combustion brought on by an excessive amount of ethanol, E40 had lower CO_2 emissions (2.10%) than E20. In high ethanol blends that upset the balance of the fuel mixture, the combustion process became less efficient and produced less carbon dioxide as the ethanol concentration rose consistent with (Kurji et al., 2021). Specific numbers for figures 4.8 to 4.13 are shown in APPENDIX 4

4.5 Techno-Economics Analysis

Globally, biofuels are replacing fossil fuels due to the growing demand for renewable and sustainable energy sources. The potential of bioethanol, a renewable fuel made from biomass, to lower greenhouse gas emissions and improve energy security attracted a lot of attention. The FAO report from 2020 (Kojakovic et al., 2022), estimated that 2,777,713 tons of corn stover were produced annually in Zambia. A plentiful and underused lignocellulosic biomass feedstock for the production of bioethanol was corn stover, which was made up of the leaves, stalks and husks left after grain harvest. This biomass source offered a promising chance for the production of bioethanol to augment the use of fossil fuels, given Zambia's growing emphasis on sustainable energy solutions.

Because it could be mixed with petrol to create a cleaner-burning fuel, bioethanol was especially significant. By implementing blending policies, like the E10 mandate (10% ethanol in petrol), nations were able to lower carbon emissions and lessen their reliance on imported petroleum. In 2023, Zambia consumed about 445,044.58 metric tons of petrol annually (Makondo, 2023). An annual production volume of 60,000,000 litres of bioethanol would have been required to meet the E10 blending requirement. This was both an opportunity and a challenge because reaching this goal necessitated a production process that was both scalable and profitable.

A thorough techno-economic analysis was necessary to close the gap between bioethanol production on a laboratory scale and its industrial implementation. Laboratory experiments obtained important information on techniques for pretreating lignocellulosic biomass, fermentation kinetics, yield optimisation, and process efficiency. However, a thorough evaluation of process viability, equipment costs, raw material availability, operating expenses, and economic sustainability was necessary before these findings could be translated into large-scale production. In the absence of such an assessment, there was a chance that investments would be made in technologies that might not be practical or affordable.

This study mainly focused on the techno-economic analysis of a large-scale bioethanol production plant with a daily capacity of 50,000 litres. Determining the economic feasibility of Zambia's bioethanol production from corn stover required this kind of evaluation. Important factors like capital investment, operating expenses, feedstock procurement, energy consumption, and prospective revenue streams were taken into account in the analysis. It also offered information on payback periods, return on investment, and sensitivity analyses under different economic scenarios, which all contributed to the financial viability of establishing large-scale bioethanol production.

Policymakers, investors, and industry stakeholders could make well-informed decisions about the commercial viability of bioethanol production by carrying out this techno-economic evaluation. Additionally, this study helped identify potential challenges such as logistical constraints, feedstock supply chain issues, and technological bottlenecks that might impact large-scale implementation. Such evaluations were essential in directing the development of sustainable biofuels and guaranteeing that the shift from small-scale experimental setups to industrial-scale production was both economically and environmentally feasible, especially in light of the increased emphasis on renewable energy around the world.

4.5.1 Lab Scale Material Costs

The bill of quantities for the supplies and their prices used to carry out this lab-scale study is shown **in Table 4.11** below. Pretreatment reagents, enzymes, and tests were the primary expenses in this table. The biomass was 1g at the lab scale, and a BRT prediction indicated that the optimal amount after pretreatment was 0.461g. The machine received a slurry weighing 3.92161g for enzymatic hydrolysis. After losses from fermentable sugar tests, fermentation began with 3.5216g, and 2.82g of bioethanol was ultimately produced.

Table 4. 11: Materials, test costs during lab scale experiments

	Price in Zambian Kwacha (K)	**Price in US Dollars ($)**
Lactic Acid/ Choline Chloride	40,500	1,500
Corn stover purchase/grind/ transport	5,000	186
Cellulase Enzymes	15,000	556
Saccharomyces cerevisiae yeast	270	10
Fermentable Sugar tests	8,000	300
Alcohol level tests	20,000	800
Purchase of utensils	10,000	400
Support staff wages	20000	800
Total	**118,270**	**4,552**

4.5.2 Financial Viability

An economic analysis was conducted to determine the viability of the expanded bioethanol plant, as indicated in Table 4.12. The analysis calculated capital costs, operating costs, and potential revenue based on the optimised parameters obtained from laboratory-scale experiments, assuming a daily production of 50,000 LL suitable for a large-scale facility with relatively simple equipment. A stepwise approach, as advised by ISO/DTS 14076, was used to methodically evaluate capital and operating expenses, revenue generation, and financial viability. This standard provided a structured methodology for conducting TEA by integrating lifecycle cost analysis, resource efficiency, and investment risks. The structured cost estimation ensured transparency in financial planning, making it a reliable framework for investment decision-making.

Table 4. 12: Techno-Economics of the Bioethanol Production Process (Source: ISO/DTS 14076)

	Description	Justification	Bioethanol		
			%	**Unit**	**Cost**
Capital Costs	Land acquisition	≈ 1.5% of all other capital costs	1.50	$	84,367.50
	Fermentation Tanks	Based on plant capacity (~50,000 liters/day), 4 tanks required.	11.36	$	637,489.20
	Pumps for transferring liquids	Includes pumps for feedstock, mash, and ethanol transfer.	4.87	$	273,695.63
	Ethanol Storage Tanks	Construction of intermediate and final ethanol storage tanks.	3.25	$	182,805.15
	Pre-treatment Tanks	Includes preparation of feedstock (enzymatic hydrolysis).	3.21	$	179,963.28
	Pipes and Valves	Connects tanks, fermenters, and distillation columns.	0.97	$	54,361.13
	Distillation Columns	2 continuous columns for ethanol separation.	22.49	$	1,260,345.98
	Molecular Sieves for Dehydration	Produces fuel-grade ethanol (>99.5% purity).	6.49	$	364,481.03
	Stirrer Motor	Ensures mixing during fermentation. Magnetic Stirrers, Ovens (Various)	3.90	$	218,812.39
	Washing and Filtration Tank	For removing residues from the fermentation broth.	1.94	$	108,732.81
	Steam Boiler	Provides heat for distillation processes.	1.72	$	96,738.69
	Construction works including installations of tanks, piping, valves and electrical works and site works	Includes installation of tanks, piping, electrical works, and site preparation.	12.99	$	727,482.34
	Contractor charges	0.15(CC + CC/0.4)	6.82	$	380,959.47
	Engineering consultancy charges	0.15(0.15(CC + CC/0.4))	7.84	$	438,103.39
	Contingencies and other	0.2(0.15(0.15(CC + CC/0.4)))	12.02	$	674,828.11
	TOTAL CAPITAL COSTS		100	$	5,624,186.00
Operating cost	Corn stover purchase/grinding/transport	Based on 200 tons/day at \$37.5/ton for corn stover.	83.67	$/year	2,250,000
	Lactic Acid/Choline Chloride	Adjusted for industrial-scale production	0.03	$/year	30,000.00
	Cellulase Enzymes	Required for hydrolysis	0.1	$/year	75,000.00
	Saccharomyces cerevisiae yeast	Fermentation process	0.05	$/year	15,000.00
	Electricity consumption	Based on \$0.160/kWh and estimated power use of 1,512 kWh/day.	3.23	$/year	499,670.28
	Water consumption	\$0.47/m^3 for 20 m^3/day over 300 operational days	0.18	$/year`	28,200.00
	Repair and maintenance of all equipment	≈ 1.5% of all other capital costs.	0.68	$/year	82,675.53
	Government taxes	≈ 1% of all other capital costs.	0.46	$/year	56,241.86
	Workers' remuneration	≈ 2% of all other capital costs.	0.93	$/year	112,483.72
	TOTAL OPERATING COSTS		100	$/year	3,169,271.39
	UNIT RUNNING COST	Total operating cost ÷ (50000L/day × 300 days)		$/L	0.21
Revenues	Bioethanol Sales	50,000 liters/day × 300 days/year × \$1.50/liter.		$/year	22,500,000.00
	By-product Sales (CO_2, biomass)	CO_2 for beverages and residual biomass for animal feed.		$/year	750,000.00
	Treated Water Reuse	Estimated savings from reusing water on-site		$/year	150,000.00
	TOTAL REVENUE			$/year	23,400,000.00
	NET PROFIT	Total Revenues (\$/year) – Operating costs (\$/year)		$/year	20,230,7280.6
	PAYBACK PERIOD	Total Capital Costs (\$) ÷ Net Profits (\$/year)		years	2.78 years
	NPV	(cash flow at time t ÷ $\llbracket(1+r)\rrbracket$ ^i)- Initial Investment		$	8,765,000.00

4.5.3 Key Cost Drivers, Production Economics, And Competitiveness Of Gasoline At E10 Blending

The techno-economic analysis of bioethanol production from corn stover using Deep Eutectic Solvents (DES), as presented in Table 4.11, provided an in-depth evaluation of the cost structure, revenue potential, and financial viability of ethanol production for blending with gasoline in Zambia. This analysis was crucial in evaluating the economic viability of bioethanol production, given the growing need to reduce dependence on fossil fuel imports, lower fuel costs for consumers, and enhance energy security. The study was structured around a bioethanol production facility with a capacity of 50,000 litres per day, operating for 300 days per year, leading to an annual ethanol output of 15 million litres per plant. Since Zambia's 2023 gasoline consumption stood at 445,044.58 metric tons, equivalent to 589.1 million liters, the implementation of E10 blending required 58.9 million liters of ethanol annually (Chitandula et al., 2024). To meet this demand, the country would need four bioethanol plants, each operating at full capacity to produce sufficient ethanol for blending.

The cost analysis in Table 4.12 provided a detailed breakdown of the expenses associated with establishing and running a bioethanol plant. The total capital investment for a single ethanol plant was $5.62 million, covering the costs of land acquisition, plant construction, machinery installation, and initial working capital. The annual operating costs, which included feedstock procurement, pretreatment chemicals, enzymatic hydrolysis, fermentation, labor, utilities, and maintenance, amounted to $3.17 million per plant. The largest cost component was corn stover acquisition, handling, and transportation, which accounted for $2.25 million annually, or approximately 83.67% of the total operational costs.

The pretreatment stage, which involved breaking down lignocellulosic structures to improve enzyme accessibility, utilised Deep Eutectic Solvents (DES) such as lactic acid and choline chloride, which were considered a more sustainable and cost-effective alternative to conventional acid-based methods. The annual cost of pretreatment chemicals was $30,000 per plant. Following pretreatment, enzymatic hydrolysis was carried out using cellulase enzymes, which played a critical role in converting cellulose into fermentable sugars. The cost of enzymes was $75,000 per year, making enzyme use one of the most significant biochemical cost elements in ethanol production. The fermentation process relied on Saccharomyces cerevisiae yeast, which cost $15,000 per year for each plant. Additional expenses included utility costs such as water, electricity, and waste management, which contributed to overall operational expenditures. Given these factors, the cost of producing one litre of bioethanol was calculated at $0.21 per litre, making it significantly cheaper than the retail price of gasoline, which stood at $1.1724 per litre.

Comparing this production cost with values from other studies, the cost of bioethanol production had been reported to vary significantly depending on feedstock type, processing technology, and regional economic conditions. According to Zhang et al., (2023), bioethanol production from lignocellulosic biomass using conventional acid-based pretreatment methods had resulted in an estimated cost of $0.35 to $0.50 per litre. The economic benefit of the DES pretreatment method employed in this analysis was highlighted by another study by (Kotwal et al., 2024) which found that using agricultural residues resulted in a production cost of about $0.40 per litre. The current study's lower cost showed that employing DES for biomass pretreatment greatly lowered processing costs, making the production of bioethanol from corn stover a more affordable and competitive choice.

On the revenue side, ethanol sales were the main source of income, bringing in $22.5 million annually per plant, assuming that ethanol was sold for $1.50 per litre. Along with ethanol, the plant also produced valuable by-products like carbon dioxide, which is used in the beverage industry; residual biomass, which can be turned into animal feed or bio-based materials; and wastewater that has been treated for industrial reuse. An additional $900,000 was generated annually by the combined revenue from by-products, which included $150,000 from water reuse projects and $750,000 from sales of leftover biomass. As a result, each plant now generates $23.4 million a year.

The production of bioethanol is a very profitable endeavor, with an estimated net annual profit per plant of $20.23 million when revenue is compared to operating costs. With a payback period of 2.78 years—the amount of time needed for the initial investment to be recouped through operational profits—the investment was clearly both financially appealing and able to produce returns quickly. In contrast, a study by Lamichhane et al., (2021) on the production of bioethanol from lignocellulosic biomass found that the payback period varied from 4 to 7 years, contingent on feedstock availability, enzyme costs, plant efficiency, and government subsidies. In contrast to our situation, where a pretreatment based on Deep Eutectic Solvent (DES) greatly decreased chemical and operational expenses, the longer payback period in that study was mostly caused by higher enzyme costs and capital-intensive pretreatment techniques, which made bioethanol production less cost-effective. Our analysis's shorter payback period of 2.78 years showed that producing bioethanol from corn stover with DES pretreatment was not only possible but also cheaper compared to traditional lignocellulosic ethanol plants. Because of this, the investment was very alluring, particularly in light of Zambia's E10 policy and the growing global trend towards renewable fuels.

With 44.5 million litres of petrol replaced annually by ethanol, the Energy Regulation Board (ERB) and Zambia Bureau of Standards (ZABS)-mandated adoption of E10 blending would lead to a significant decrease in petrol imports(Chitandula et al., 2024). Zambia could produce ethanol locally instead of depending solely on imported petroleum products, which would improve fuel security and save foreign exchange. E10 blending provided consumers with immediate financial advantages because, at an estimated $1.0762 per litre, the blended fuel price was $0.0962 less than that of regular gasoline, which translates to an 8.2% fuel cost savings. Considering Zambia's high fuel consumption, these savings would add up to significant financial gains for businesses, public transportation systems, and drivers.

Despite testing and showing better engine performance, the E20 blend was not widely adopted due to issues with phase separation, fuel system compatibility, and storage stability. Due to its hygroscopic properties, ethanol was more likely to absorb moisture, increasing the possibility of fuel separation and possible injector clogging, particularly in humid conditions or when cars were left unattended for extended periods of time (Mousavi-Avval et al., 2023). Additionally, material deterioration in fuel system components was linked to elevated ethanol concentrations, especially in older cars with gaskets and rubber seals that were not made to withstand high ethanol exposure (Pedicini et al., 2023). A widespread switch to E20 would have required significant infrastructure adaptation, regulatory changes, and consumer education initiatives, even though modern cars were becoming more and more ethanol-compatible. Because it balanced engine performance, cost savings, and long-term vehicle reliability, E10 blending was determined to be the best option.

4.5.4 Effect Of E10 Gasoline/Bioethanol Blends On GHG Emissions

There is a great chance to lower greenhouse gas (GHG) emissions in the transportation sector with Zambia's planned introduction of E10 petrol blending. Zambia consumed 445,044.58 metric tons of gasoline in 2023. Based on an emission factor of 3.07 metric tons of CO_2e per metric ton of gasoline, the total CO_2 equivalent (CO_2e) emissions from pure gasoline are estimated to be 1,366,286.86 metric tons (Makondo, 2023). he projected emissions were estimated to be 1,229,658.17 metric tons of CO_2e, which would result in an annual reduction of 136,628.69 metric tons of CO_2e by switching to E10, where 10% of the fuel had been bioethanol. This is equivalent to a 10% reduction in emissions, which makes a substantial contribution to Zambia's efforts to mitigate climate change.

The main cause of the emission reduction will be bioethanol's renewable nature. Bioethanol was produced from biomass sources like corn or sugarcane, which absorbed CO_2 during their growth phase, in contrast to fossil-based gasoline. Because of this biological process, bioethanol is partially carbon-neutral throughout its lifecycle, offsetting the CO_2 released during ethanol combustion (Moreira & Goldemberg, 2023). According to studies like Mesarch et al., (2021) ethanol made from corn could reduce greenhouse gas emissions over its lifecycle by about 40% when compared to petrol. In a similar vein, Higgins et al., (2023) discovered that ethanol blends such as E10 will lower carbon intensity to 43.4 g/MJ or less, primarily because they will displace the high-carbon aromatics in petrol. The benefits of ethanol blends like E10 and E15 in lowering emissions are further supported by a 2023 study from the University of

California, which supports the idea that ethanol should be used more widely in fuel markets.

The environmental advantages of using ethanol in E10 blending are further supported by the fact that its contribution to emissions is not taken into account because it has no known emission factor. However, switching to E10 blending still has significant overall environmental benefits. Zambia must make investments to increase the capacity for producing bioethanol, upgrade the infrastructure for distributing fuel, and assist agricultural suppliers in producing feedstock to have the greatest possible impact. In order to ensure long-term sustainability, the transition will also need incentives and policy reinforcement to draw investment in ethanol production

In addition to lowering CO_2 emissions, research has demonstrated that ethanol blending significantly lowers other pollutants like particulate matter (PM), carbon monoxide (CO), and volatile organic compounds (VOCs), all of which improve urban air quality. The health benefits of using bioethanol are supported by lower rates of breathing problems associated with air pollution in countries that use ethanol-blended fuels (Tang et al., 2023). Therefore, Zambia's approval of E10 will align with more general environmental and public health regulations aimed at mitigating the negative consequences of burning fossil fuels.

The approval of E10 blending will establish Zambia as a regional leader in the adoption of cleaner fuels, as the sustainability benefits of ethanol are becoming increasingly recognised on a global scale. The nation will benefit not only from lower emissions but also from increased fuel security, economic growth, and environmental preservation as more research is conducted on the lifecycle advantages of ethanol. Increasing the production of bioethanol will also benefit local businesses by creating jobs in the processing and agricultural sectors, boosting rural economies, and lowering dependency on foreign fuels.

4.6 Summary of Research Findings

The characterisation of corn stover revealed that it contains a high amount of cellulose and has the right composition for producing second-generation bioethanol. The material consisted of approximately 46% cellulose, a moderate level of hemicellulose, and a low amount of lignin, indicating it could serve as a renewable biomass feedstock. These findings suggest that maize stover, a common agricultural waste in Zambia, could potentially be converted into fermentable sugars with the appropriate pretreatment.

The study optimized pretreatment by analyzing various process parameters, including temperature, reaction time, and the ratio of choline chloride to lactic acid. This was achieved using Response Surface Methodology (RSM) and Central Composite Design (CCD), with machine learning models (ANN and GBRT) further enhancing the results. The optimal pretreatment conditions were found to be 105°C, a reaction time of 10.5 hours, and a molar ratio of 1:6 ChCl:LA, yielding a cellulose recovery of 46.1%. The machine learning models, particularly GBRT, outperformed traditional quadratic models because they provided more accurate predictions ($R^2 = 0.91$). This demonstrates that computational models can effectively predict cellulose yield and improve biofuel production processes.

During enzymatic hydrolysis and fermentation, the optimal conditions—10 mg/g of enzyme, 50°C, and 72 hours of reaction—produced 78% fermentable sugar, confirmed by High-Performance Liquid Chromatography (HPLC). After fermentation with

Saccharomyces cerevisiae, Gas Chromatography–Mass Spectrometry (GC-MS) indicated that the bioethanol yield was 80%. At 78.5°C, distillation produced 3.57 liters of ethanol with a high alcohol content suitable for mixing. Testing engines with E10, E20, E30, and E40 blends showed that E20 performed best. It increased brake thermal efficiency by 7.4% and reduced carbon monoxide (CO) and hydrocarbon (HC) emissions by 21% and 26%, respectively.

The techno-economic analysis (TEA) demonstrated that constructing a DES-based bioethanol plant with a capacity of 50,000 liters is 27% cheaper than using traditional methods. The Life Cycle Assessment (LCA) also revealed that greenhouse gas emissions are 32% lower than those from petrol derived from fossil fuels. Key costs include enzymes, feedstock collection, and energy consumption, but the fact that DES can be recycled and decomposed in the environment significantly reduced operating expenses. Overall, Chapter Four shows that producing bioethanol from Zambian corn stover using DES is both feasible and profitable.

CHAPTER FIVE: CONCLUSIONS, RECOMMENDATIONS AND FUTURE WORKS

5.0 Chapter Introductions

5.1 Conclusions

Interest in bioethanol as a substitute for fossil fuels has grown globally due to the increasing need for sustainable energy solutions. Through improving pretreatment effectiveness, optimising enzymatic hydrolysis yields, evaluating engine performance with bioethanol-gasoline blends, and assessing the economic viability of large-scale production, this study aimed to maximise the production of bioethanol from Zambian corn stover. The following conclusions highlight the most important takeaways from each research goal based on the research's findings.

a. In order to determine the ideal cellulose yield, the application of deep eutectic solvents for corn stover pretreatment was assessed. Three modelling techniques: Central Composite Design (CCD), polynomial regression, and Boosted Regression Tree (BRT), were used for this. Using important statistical indicators, each of these models produced intriguing results regarding the performance of the DES pretreatment of corn stover under various circumstances.

b. The experimental results utilising the CCD optimisation process produced 0.4599 g of cellulose. However, the low coefficient of determination (R^2 = 0.4391) indicates that the model has limited statistical reliability. Additionally, the model was statistically insignificant, according to the ANOVA result, which had a p-value of 0.2189 and an F-value of 0.0372. Therefore, the findings indicated that the model fit was not strong and that additional modelling was necessary to increase the dependability of the outcomes.

c. Conversely, the polynomial regression model demonstrated a more robust predictive performance. It approximated a lower optimal cellulose yield of 0.4369g while the R^2 was high at 0.9098, exhibiting a strong correlation amid predicted and actual results. The ANOVA outcomes for the model were statistically significant, giving an F-value of 10.80 and a p-value that was less than 0.0001. The polynomial model produced precise predictions and demonstrated robustness and dependability in identifying the major variables affecting cellulose yield.

d. By predicting the highest cellulose yield of 0.4610g, the BRT model further enhanced the predictive modelling potential. Additionally, it demonstrated a good model fit with an R2 value of 0.80. The BRT approach proved to be effective in modelling the system, especially when modelling complex nonlinear relationships, even though its statistical strength was lower than that of the polynomial model.

e. The most dependable and statistically significant method for forecasting cellulose yield in DES pretreatment of corn stover was, in general, the polynomial regression model. However, when it came to complex data patterns, the BRT model continued to be a dependable alternative because it provided the highest yield prediction. In that situation, the actual experimental CCD model proved unsuitable for trustworthy optimisation due to its low predictive accuracy.

f. The second goal examined the ideal circumstances for enzymatic hydrolysis with the aim of increasing the yield of fermentable sugars and improving the production of bioethanol. The hydrolysis process was once more optimised using Central

Composite Design (CCD). The quadratic polynomial model confirmed a validated value of 78.22%, while the experimental results showed an optimal fermentable sugar yield of 78%. A coefficient of determination (R2 = 0.8707) that showed a strong correlation between experimental and predicted values supported the model's dependability.

With an F-value of 9.42 and a P-value of 0.0052, the ANOVA results validated the significance of the model and demonstrated its statistical significance. The model maintained its robustness for prediction and optimisation even though the ANOVA table showed variability across various model terms, making it significant both up and down.

g. Following enzymatic hydrolysis, the best fermentable sugars were fermented and distilled, yielding 3.57 mL of ethanol at 80% alcohol by volume (ABV). These results confirm that enzymatic hydrolysis optimisation increases sugar yield and enhances the efficiency of subsequent bioethanol production, bolstering the process's potential for the development of sustainable biofuels.

h. After enzymatic hydrolysis, the optimal fermentable sugars were fermented and distilled which resulted in ethanol yield of 3.57 mL at 80% alcohol by volume (ABV). These findings affirm that optimizing enzymatic hydrolysis does improve sugar yield as well as positively improve the efficiency of subsequent bioethanol production, strengthening the process’s prospects in sustainable biofuel development.

i. Evaluating engine performance and emission characteristics when petrol and bioethanol were blended in different amounts was the third goal. 10%, 20%, 30%, and 40% blending ratios, denoted by E10, E20, E30, and E40 for ethanol levels, respectively, were tested in a spark ignition engine with performance compared to G100, which represents 100% petrol. The primary engine performance metrics that were assessed were heat balance, brake power (BP), brake-specific fuel consumption (BSFC), brake thermal efficiency (BTE), and indicated power (IP). Also, engine emissions during testing were measured and they included: carbon monoxide (CO), hydrocarbons (HC), carbon dioxide (CO_2), and oxygen (O_2).

j. The overall outcomes demonstrated that the E20 blend showed the best engine performance. It had the highest brake power (34.03 kW), the least BSFC (0.2333 kg/kWh), and the optimal brake thermal efficiency (32.00%), implying enhanced combustion and reduced fuel consumption as compared to the rest of the blends and pure gasoline. E20 also provided the highest indicated power (37.5 kW), reflecting its superiority in converting chemical energy into mechanical energy. Further, heat balance at E20 had the minimum heat loss (51.97 MJ/h), augmenting its efficient thermal energy usage.

k. In terms of emissions, E20 also showed lower levels of carbon monoxide (0.08%) and hydrocarbons (2.01 ppm), meanwhile exhibiting a comparatively higher oxygen concentration in the exhaust (1.15%), a sign of complete combustion. Even though the CO_2 emissions were lower (2.36%) than those of G100 (3.46%), this decrease was incongruous with the full combustion of fuels containing ethanol. However, because of leaner air-fuel mixtures and incomplete combustion, higher ethanol blends, E30 and E40, resulted in lower engine performance, high BSFC, and increased emissions.

l. An economically feasible and ecologically responsible method was used to evaluate the techno-economic viability of a large-scale integrated DES-based bioethanol production process. For Zambia's E10 blending intentions, a production setup of a plant with a capacity of 50,000 litres per day and a target of operating for 300 days per year was evaluated. It was found that such plants would be necessary to meet the baseline petrol consumption of 589.1 million litres per year in 2023.

m. The cost of bioethanol, according to techno-economics, is US$0.21 per litre, which is substantially less than the US$1.1724 per litre price at the gas pump. Blending at E10 would result in a fuel pump price reduction of US$1.0762 per litre, saving US$0.0962 per litre, or 8.2%. The procedure yielded a favourable payback period of 2.78 years, making it an appealing investment opportunity.

n. The assessment also looked at the benefits of using E10 in terms of environmental pollution. It showed a big decrease in greenhouse gas emissions. With an emission factor of 3.07 metric tons of CO_2e for every metric ton of gasoline, Zambia's gasoline uses in 2023 resulted in 1,366,286.86 metric tons of CO_2e. If E10 blending is used, emissions would decrease to 1,229,658.12 metric tons of CO_2e, resulting in an annual reduction of 136,628.90 metric tons of CO_2e. This would create chances for earning carbon credits and help fight climate change.

o. This work has also resulted in the publication of three (3) peer-reviewed journal papers, as shown in APPENDIX 5.

5.2 Recommendations

The production of bioethanol using Zambian corn stover was the focus of this study. However, it was noted that to consider large-scale bioethanol production from Zambian corn stover, several issues must be addressed. For upscale innovations like these to be successful, the following important stakeholders should be taken into account. These comprise the agricultural sector, which includes farmers, industry, researchers, and policymakers.

a. Adopting and implementing national policies that encourage bioethanol blending, with a focus on levels between E10 and E20, is necessary to advance the acceptance of bioethanol as a resilient fuel. Financial incentives like tax breaks and subsidies should be a part of the policies. Investment in the bioethanol production industry would increase under such a strategy. In order to guarantee consistency in the blending quality, quality control standards should also be incorporated into the introduction and execution of the policy. In order to reduce waste, the policy should also guarantee that corn stover is collected safely at the time of collecting corn cobs.

b. Industry players should be encouraged to think about investing in the full bioethanol production process with integrated technology advancement, from DES pretreatment to enzymatic hydrolysis, fermentation, and distillation standards to fuel grade at 95–99% ABV. Storage, blending facilities, and a distribution network that keeps up with the country's petrol distribution pace should all be included.

c. To improve cellulose yield and optimise delignification and hemicellulose solubilisation, researchers should keep looking into alternative deep eutectic solvents (DES). The application of precise machine learning models that can forecast the performance of enzymatic hydrolysis on an industrial scale should be part of the research since it would greatly enhance process optimisation. Additional research into genetically modified yeast strains and fermentation enhancers may increase ethanol yield and reduce production time. Bioethanol-gasoline blending methods that improve blend homogenisation and storage life should also be the subject of research.

d. Research should concentrate on improving ignition timing and fuel system injectors to support higher blending levels, such as E30 and E40, in order to maximise the advantages of higher ethanol blends. Consideration on research should include modified engines specifically designed for gasoline/bioethanol blends to minimize the inherent challenges of vehicles specifically designed for G100 operations.

e. This proposed transition to bioethanol should in line with circular economy principles in the utilizing residual biomass for extra energy production, such as biogas and biochar. A detail of life cycle assessments (LCA) should be undertaken for industrial scale bioethanol plants and consider potential environmental impacts and possible sustainability improvements. Delving into carbon credit trading by bioethanol producers can be financial incentives for supporting eco-friendly production.

f. Encouragement should be given to farmers to form cooperatives in order to have efficient and economical collection centres of agricultural residues, ensuring a continuous bioethanol feedstock supply. Improved training initiatives should be taken into consideration in order to inform farmers and communities about the need for bioethanol production and sustainable residue management. Bioethanol production facilities should be located in rural areas to reduce costs and improve energy access, job creation, and economic growth.

Consider expanding bioethanol adoption with governments and industry and explore export opportunities to generate extra revenue streams for the country. Conducting cost-benefit analyses such as levelised cost of energy and developing an investment model attractive to investors in the biofuel industry.

g. Making it mandatory for oil marketing companies to distribute only blended gasoline can reduce the overall pump price of gasoline, which reduces the cost of doing business and the environmental pollution since bioethanol, when combusted with gasoline, absorbs carbon dioxide produced by gasoline.

h. By integrating policy measures, technological advancements, and industrial adoption strategies, bioethanol can become a viable solution to the world's growing energy demands while mitigating the effects of climate change. Additionally, regulatory measures such as blending mandates and emissions reduction targets will be instrumental in encouraging industries to transition toward bioethanol as a sustainable fuel source.

5.2.1 Future Works

After DES pretreatment and enzymatic hydrolysis, a substantial amount of solid biomass residue remains, which is typically discarded. Future studies should investigate the potential of converting this residue into biochar, a value-added product with environmental benefits. It has been demonstrated that biochar increases soil fertility, improves water retention, and—above all—sequesters carbon, which helps to mitigate climate change. Besides enhancing the bioethanol production process for the environment, exploring the possibility of producing biochar from these leftovers could offer a sustainable way to manage waste. This strategy would reduce the environmental impact of disposing of biomass while also aligning with the principles of the circular economy. This field offers a chance for creative and significant research because it is still largely unexplored, especially when it comes to the production of bioethanol from corn stover using DES.

5.3 Research Implication and Contribution

This research provides a substantial scientific and technical advancement by formulating and refining a sustainable technique for generating bioethanol from Zambian corn stover utilising Deep Eutectic Solvents (DES). The study demonstrated that DES pretreatment is a more environmentally friendly and cost-effective option than traditional acid or alkali methods. It can also improve the efficiency of cellulose recovery and enzymatic hydrolysis. The study achieved elevated predictive accuracy for cellulose and bioethanol yields by combining Response Surface Methodology (RSM) with Central Composite Design (CCD) and advanced machine learning models, including Artificial Neural Networks (ANN) and Gradient Boosted Regression Trees (GBRT). The experimental validation of bioethanol-gasoline blends showed that the E20 blend provided optimal engine performance and minimal emissions, confirming its technical feasibility and environmental benefits.

The results have important implications for Zambia's renewable energy sector from both an industrial and policy perspective. The study supports the idea of implementing a national bioethanol blending policy (E10–E20) to reduce dependence on fossil fuels, encourage investment in biofuel infrastructure, and improve the country's energy security. It also recommends establishing integrated production systems that include pretreatment, fermentation, and distillation, along with rural collection centers for agricultural waste. These measures would help improve energy access in rural areas, promote industrial growth there, and demonstrate Zambia's commitment to low-carbon development.

The research offers new insights into the application of DES in biomass pretreatment and creates a methodological framework for AI-assisted bioenergy process optimization. Using both real-world experiments and predictive modeling is a method that can be applied in future studies of sustainable fuel technologies. The study's results have been published in peer-reviewed journals, which has helped scientists understand how to produce lignocellulosic bioethanol more cheaply in developing countries.

The impacts on the environment and society are equally important. Using DES-based bioethanol production and blending ethanol with gasoline can reduce greenhouse gas emissions by over 30%, improve urban air quality, and decrease health risks associated with burning fossil fuels. Decentralized bioethanol facilities would also generate jobs in rural areas, help farmers earn more money by systematically collecting waste, and reduce Zambia's fuel import expenses. This work offers a comprehensive framework for a circular bioeconomy, combining scientific innovation with policy and sustainability goals to promote clean energy and economic resilience in Zambia.

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APPENDICES

Appendix 1: Equipment Used For The Processes

Figure 1A: The Edibon Computer Controlled Bioethanol Processing Unit

Figure 1B: Atico Engine Unit

Figure 1C: The Atico Tilting Furnace

Figure 1D: The Drying Oven

Figure 1E: The digital Scale

Figure 1F: The Magnetic Stirrer

Appendix 2: Deep Eutectic Solvents Synthesis

Lactic acid and choline chloride were bought for pretreatment of biomass. The two chemicals were acquired in two states with lactic acid in liquid state and choline chloride in solid state. The ratios of synthesizing the two chemicals choline chloride to lactic acid were 1:2, 1:6 and 1:10 (Procentese et al., 2018). Since choline chloride was in solid state, there was need to convert it to liquid state and the molecular masses used even in the synthesis of the two chemicals. This was according to labels on the containers of the chemicals. The calculations were as follows:

1. Choline Chloride=139.62g/mol
2. Lactic acid=90.08mols

88% Lactic Acid=88g/100m =0.88glmol

Number of moles of lactic Acid= $(0.88g/ml)/(90.08g/mol)$=0.009769moles/ml

3. Preparation of Choline Chloride Solution

80g of choline Chloride in 100ml of H_2O

$$80\text{g}/(139.62\text{g/mol}) = 0.5729\text{mols}/100\text{ml} = 0.005729 moles/ml$$

100ml of choline chloride =0.005729×100=0.5729moles/100mls

Since the ration of Choline Chloride: Lactic Acid=1:2

Therefore, Lactic Acid=0.5729×2=1.1458mols

Since lactic Acid=0.009769

Then,

0.009769mol=1ml

1.1458mols=X

X=1.1458/0.009769 = 117.28ml

4. Therefore, volume of Choline Chloride to volume of lactic acid will be as follows:

Ratio	Choline Chloride	Lactic Acid
1:2	100ml	117.2ml
1:6	100ml	351.8ml
1:10	100ml	586.0ml

Appendix 3: Unpretreated & Pretreated Corn Stover Biomass

Figure 4A: Untreated Corn Stover

Figure 4B: Pretreated Corn Stover at 150^0C

Figure 4C: Pretreated Corn Stover at 60^oC

Figure 4D: Pretreated biomass at 110ºC

Appendix 4: Gasoline/Bioethanol Blends Engine Performance

Table 4. 11: Brake power engine test results

Fuel Blend	Brake Power (kW)
G100	31.42
E10	32.72
E20	34.03
E30	30.11
E40	28.80

Table 4. 12: Engine BSFC test results

Fuel Blend	BSFC (kg/kWh)
G100	0.2706
E10	0.2516
E20	0.2333
E30	0.2765
E40	0.3194

Table 4. 13: Brake Thermal Efficiency (BTE)

Fuel Blend	BTE (%)
G100	29.00
E10	30.50
E20	32.00
E30	28.00
E40	26.50

Table 4. 14: Engine Test Indicated Power (IP)

Fuel Blend	Indicated Power (kW)
G100	34.5
E10	35.8
E20	37.5
E30	32.5
E40	30.0

Table 4. 15: Heat Balance during engine tests

Fuel Blend	Heat Input (MJ/h)	Useful Work (MJ/h)	Heat Loss (MJ/h)
G100	92.5	32.1	60.0
E10	94.0	34.0	60.0
E20	95.2	31.8	51.97
E30	96.0	30.1	65.9
E40	98.1	28.8	66.77

Table 4. 16: Engine Tests Emissions

Fuel Blend	CO (%)	HC (ppm)	CO_2 (%)	O_2 (%)
G100	0.13	2.55	3.46	0.25
E10	0.09	2.44	2.98	0.58
E20	0.08	2.01	2.36	1.15
E30	0.11	2.76	2.75	1.45
E40	0.14	2.95	2.10	1.85

Appendix 5: Journal Papers Published

1. International Journal of Research and Innovation in Social Science (IJRISS)

Title: **Enhanced Bioethanol Production from Corn Stover Using Choline Chloride and Lactic Acid Pretreatment: A Gravimetric analysis Approach**

Cosmas S. Mwanakaba Zachary Siagi Paul Maina and Mwansa Kaoma

Publishers: RSIS International

DOI: https://dx.doi.org/10.47772/IJRISS.2024.803211S

Date of Publication: 28th August, 2024

2. Journal of Clean Energy and Energy Storage (JOCEES)

Title: Optimization of Deep Eutectic Solvents (DES) Pretreatment for Enhanced Cellulose Yield from Lignocellulosic Biomass: A Case Study in Zambia

Cosmas S. Mwanakaba Zachary Siagi Paul Maina and Mwansa Kaoma

DOI: https://doi.org/10.1142/S2811034X25500017

Publishers: World Scientific Publishing

Date of Publication: 15th January, 2025

3. Journal of Sustainable Energy Systems

Title: Techno-Economic Stepwise Analysis Approach for Optimization of Bioethanol Production from Zambian Corn Stover: Environmental and Economic Implications

Cosmas S. Mwanakaba Zachary Siagi Paul Maina and Mwansa Kaoma

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Publishers: Scientific Research-An Academic Publisher

Date of publication: 3rd September, 2025

Appendix 6: Plagiarism Awareness Certificate

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