Pretreatment of Industrial Potato Peel Waste to Produce Enhanced Bio-ethanol under Optimal Conditions

Mastewal Andualem Sewnet

A thesis Submitted to the Department of Chemical Engineering

School of Mechanical, Chemical and Materials Engineering

Office of Graduate Studies

Adama Science and Technology University

June, 2024

Adama, Ethiopia

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Advisor: Dr. Zelalem Tumsa

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Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Chemical Engineering (Specialization in process engineering)

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DECLARATION

I hereby declare that this master's thesis entitled "**Pretreatment of Industrial Potato Peel Waste to Produce Enhanced Bio-ethanol under Optimal Conditions"** is my original work and has not been submitted to any other university for similar purpose. The reference used in this proposal is duly recognized by proper citations.

Mastewal Andualem _____ _____

Name of student Signature Date

RECOMMENDATION

We, the major advisor/supervisor and the co-advisor of this thesis, hereby certify that we have closely advised/supervised the student while developing this proposal and read the draft thesis entitled "**Pretreatment of Industrial Potato Peel Waste to Produce Enhanced Bio-ethanol under Optimal Conditions"** prepared under our guidance by Mastewal Andualem submitted in partial fulfillment of the requirements for the degree of Mater's of Science in Chemical Engineering (Specialization Process Engineering). Therefore, we recommend the submission of revised version of the thesis to the department following the applicable procedures.

Dr. Zelalem Tumsa (Ph.D.) _____ _____

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Dr. Selvakumar Periyasamy (Ph.D.) _____ _____

Co-advisor Signature Date

APPROVAL PAGE

We, the advisors of the thesis entitled "**Pretreatment of Industrial Potato Peel Waste to Produce Enhanced Bio-ethanol under Optimal Conditions**" and developed by Mastewal Andualem Sewnet, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Dr. Zelalem Tumsa	_____	_____
Major advisor	Signature	Date
Dr. Selvakumar Periyasamy	_____	_____
Co-adviser	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Mastewal Andualem Sewnet have read and evaluated the thesis entitled **"Pretreatment of Industrial Potato Peel Waste to Produce Enhanced Bio-ethanol under Optimal Conditions"** and examined the candidate during the open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Chemical Engineering (Specialization in Process Engineering).

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Finally, approval and acceptance of this thesis is contingent upon submission of its final copy to the office of postgraduate studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

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LIST OF ACRONYMS

AASTU	Addis Ababa Science and Technology University
ANOVA	Analysis of Variance
CCD	Central Composite Design
CrI	Crystalinity Index
DNS	Dinitrosalicylic acid
FAO	Food and Agricultural Organization
FC	Fixed carbon
FTIR	Fourier Transform Infrared Spectroscopy
GHG	Green House Gas
HMF	Hydroxymethyfurfural
ILF	Liquid Based Fraction
LCB	Lignocellulosic biomass
NREL	National Renewable Energy Laboratory
PPW	Potato Peel Waste
RSM	Response Surface Methodology
SEM	Scanning Electro Microscope
SHF	Separated Hydrolysis and Fermentation
XRD	X-ray Defraction
%DL	Delignification Efficiency
UV	Ultra violate

ABSTRACT

The need for sustainable and safe alternative energy sources has arisen due to environmental issues, the growing demand for energy, increasing fossil fuel prices, and the depletion of fossil fuel reserves. Biofuels are considered promising alternatives to fossil fuels. Especially the second- and third-generation biofuels, which are generated from agro-industrial waste, can minimize the total disposal of residues and contribute to the total renewable energy supply. This study focuses on the sustainable utilization of potato peel waste (PPW) as a viable raw material in a bio-refinery, aiming to produce bioethanol. The Potato peel used in this study consists of 35.2% starch, non-starch polysaccharide (21.65%), lignin (20.4%), extractives (14%) and ash (5.3%) on an air-dry basis. The conversion of these potato waste materials into bioethanol involves several stages. In this particular study, Alkali pretreatment of potato peel waste was conducted and optimization of different pretreatment conditions such as alkali concentration, pretreatment temperature, and time were performed using design expert software. Maximum delignification efficiency was 77.82 ±2.1%, with enhanced cellulose recovery of 89.7±1.5%, and starch recovery of 80.3±1.2% were attained at the optimum conditions of alkali concentration (3.3% v/v), pretreatment temperature (70^{o}C) and time (3.5 hr). The data obtained from pretreatment were statistically analyzed using a one-way ANOVA and validated experimentally. SEM, FTIR and XRD analyses were used to characterize the raw PPW and alkali pretreated PPW. The SEM and FTIR spectra revealed the removal of lignin from the PPW biomass by alkali pretreatment. The XRD results also confirmed a change in the crystallinity index of PPW. Subsequently, the dilute sulfuric acid hydrolysis of pretreated PPW hydrolset was investigated under various acid concentrations, temperatures and hydrolysis times. The maximum glucose concentration attained was 46.5 mg/mL at 65 ^{o}C with an acid concentration of 15% (v/v) for 60 min of hydrolysis time. Ethanol fermentation was carried out at 30 ^{o}C with 150 rpm for 3 days with the recovered glucose using Saccharomyces cerevisiae. The maximum bioethanol concentration of 21.04 mg/mL was attained.

Key words: potato peel waste pretreatment, delignification efficiency, hydrolysis, glucose concentration, fermentation

1. INTRODUCTION

1.1. Background of the Study

According to the United Nations, the global population, currently standing at 8.04 billion as of December 2023, is projected to reach 8.6 billion in 2030 and 9.7 billion in 2050. This rapid population growth has led to a significant increase in worldwide food demand, which is expected to rise by up to 50% by 2050, as estimated by the Food and Agriculture Organization of the United Nations (FAO) (Felekis et al., 2023). The continuous expansion of agricultural production to meet this demand is anticipated to result in the degradation of land and natural resources, as well as increased greenhouse gas (GHG) emissions. Consequently, it is imperative to prioritize the production of food with low carbon footprints, minimize food waste, and find alternative uses for the waste generated from food consumption (Awogbemi et al., 2022). These measures are crucial in addressing the environmental challenges associated with food production and consumption in order to achieve sustainable food systems. Over the past century, energy consumption has risen significantly due to global population growth and development. This escalating demand for energy has led to a heavy reliance on fossil fuels, resulting in increased anthropogenic greenhouse gas emissions. Furthermore, as the world's energy reserves are inevitably depleted, there has been a growing global interest in exploring alternative energy sources that are not reliant on petroleum. In recent years, substantial research and development efforts have been focused on the commercial production of ethanol, which is considered the most promising biofuel derived from renewable resources. The aim is to generate biofuels and bio-based products through the valorization of waste and biomass, providing a more environmentally friendly alternative to fossil fuels (Arapoglou et al., 2010; Sanusi et al., 2022). This approach aims to utilize renewable resources and reduce dependence on finite fossil fuel reserves while minimizing the impact on the environment. Biomass is a natural resource widely utilized worldwide and serves various purposes, including energy production. Particularly in developing countries like Ethiopia, biomass is considered a crucial component of their energy sources. Biomass resources include municipal solid waste, agricultural waste, and woody biomass. The desire to reduce dependency on fossil fuels and their negative effects on global warming has prompted a global push to use biomass energy, which is believed to be comparatively cleaner (Gabisa & Gheewala, 2018).

Biofuels are fuels derived from biomass, including organic waste materials, and they offer a significantly reduced ecological footprint compared to conventional fossil fuels. One notable biofuel is bioethanol, whose production is projected to exceed 130 billion liters per year worldwide. The United States and Brazil are the primary contributors to the global supply of ethanol (Tse et al., 2021). Biomass-derived fuels are gaining significant global attention as valuable forms of renewable energy. The production of biofuels from renewable resources offers a viable solution to meet rising energy demands while simultaneously mitigating greenhouse gas emissions. Agricultural biomass, consisting primarily of cellulose, hemicelluloses, and lignin, represents an abundant and sustainable energy source (Roy et al., 2023). In various industries, households, and commercial areas, substantial quantities of fruit and vegetable peels are generated as waste. Often, these peels are mixed with other types of waste, making them unsuitable for further use. However, these discarded peels can be efficiently utilized for sugar extraction, enabling the production of biofuels (Soni et al., 2023). This approach not only reduces waste but also harnesses the potential of these peels to contribute to the production of sustainable biofuels. Potatoes, scientifically known as Solanum tuberosum, are a widely consumed staple food worldwide. They can be consumed raw or processed into a variety of well-known goods, including mashed potatoes, chips, fries, and dehydrated goods. However, a large portion of the raw potato material between 20% and 50% is wasted during the processing of potatoes, mainly as peels. Finding beneficial uses for potato waste, such as leftover starch, leftover mash, and leftover peels, has gained recognition recently, especially for the synthesis of bioethanol. The conversion of these potato waste materials into bioethanol involves several stages. Firstly, there is a pretreatment process, followed by hydrolysis to extract fermentable sugars. Subsequently, fermentation takes place to convert these sugars into bioethanol, and finally, the bioethanol is recovered as the end product (Soni et al., 2023). By utilizing potato waste as feedstocks for bioethanol production, researchers aim to reduce waste generation and transform it into a useful and sustainable resource. This approach contributes to the development of a more environmentally friendly and economically viable bioethanol production process.

1.2. Statement of the Problem

In recent decades, the issue of cleaner, sustainable energy sources has gained prominence. The majority of nations rely mostly, or even entirely, on fossil fuels. Therefore, in order for Ethiopia to diversify its energy mix, the security of the petroleum supply or other energy sources that can replace petroleum are essential. However, the future of the petroleum products reserve is uncertain with the increase in price, which makes the foreign currency expenditure intolerably high and affects the transport tariff and price of other commodities negatively.

Ethiopia is considered one of the countries in the world facing energy scarcity, which makes the development of alternative energy resources reasonable in the sense that energy shortage has a direct contribution to poverty. The introduction of alternative energy will improve lifestyles and make economic sustainability attainable. More studies have been conducted regarding sustainable and affordable alternative fuels for a range of uses as a result of environmental concerns about air pollution caused by the burning of fossil fuels. It is believed that biofuels can be a good alternative to fossil fuels in order to reduce reliance on fossil fuels, slow down climate change, improve fuel security, and reduce harmful emissions. Food insecurity and price inflation could result from the production of biofuel from staple crops like corn, maize, and wheat. Waste from food processors, lodging facilities, dining establishments, cafeterias, chip manufacturers, and juice bars can be converted into alternative energy to help with these issues.

Ethiopia produces about 1,294,300 tons of potato in 2022 and the peels are discarded. The amount of waste and by-products in the potato industry is estimated to be around 15–40% of their total production volume (Arapoglou et al., 2010). During the processing of these crops, potato companies produce enormous volumes of waste; for every ton of processed potatoes, 0.16 tons of solid waste are generated (Abebaw, 2020; Felekis et al., 2023). Typically, this food waste is disposed of in landfills, leading to environmental concerns such as groundwater pollution, unpleasant odors, and significant greenhouse gas emissions. As a result, the potato industry is very concerned about managing potato peel waste in an environmentally friendly way. Potato peels have the potential to be used as a feedstock for the production of bioethanol because of their chemical composition. One potential approach to improve the biofuel industry and address the problem of waste disposal is to turn this waste into value-added products like ethanol.

Alkali pretreatment has been carried out in an attempt to maximize the energy value from potato peel waste; however, the ideal conditions for this process have not yet been found. This study focuses on treating potato peel waste by alkali pretreatment and optimizing process parameters (temperature, alkali concentration, and time) in order to enhance the delignification efficiency for improved bioethanol production.

1.3. Objective of the Study

1.3.1. General Objective

The general objective of the study was pretreatment of industrial potato peel waste for improved bioethanol production under optimal conditions.

1.3.2. Specific Objectives

- To investigate the proximate analysis (moisture content, volatile constitutes, and ash content) and chemical composition (lignin, cellulose, hemicellulose, starch and extractives) of potato peel waste.
- To characterize raw and treated potato peel waste using FTIR, XRD, and SEM.
- To optimize pretreatment parameters, including, alkaline concentration, time and temperature, for potato peel using response surface methodology.
- To investigate the effects of hydrolyzing parameters (time, acid concentration and temperature) on glucose concentration by using one factor at a time.
- To produce bioethanol and measure its concentration.

1.4. Significance of the study

The widespread and ongoing overuse of fossil fuels is causing depletion. This rate of use will soon lead to the entire depletion of the fossil fuel reserve. Ethiopia does not have oil deposits and relies entirely on imported petroleum products. Due to the high expense of importing petroleum and increased pollution from CO_2 emissions, Ethiopia's government gives attention to environmentally friendly renewable energy sources, such as bioethanol. Therefore, this study has substantial implications for addressing environmental concerns, energy security, and the economy. Also, this research gives information about the use of potato peel as a potential feedstock for bioethanol production.

Potato peel waste is preferred as a renewable and non-food-competitive feedstock for the synthesis of alternative fuel oils like bioethanol. By generating neutral CO_2 and lowering the cost of imported petroleum, bioethanol made from leftover potato peels could support the economy. Additionally, it boosts the economy by giving the transportation and agriculture sectors access to value-added markets.

This study explores the production of bioethanol from industrial potato peel wastes and advances knowledge regarding Ethiopia's long-term bioethanol production and changes in the country's transportation fuel substitution patterns. Thus, by offering in-depth research and analysis on the effectiveness and optimization of bioethanol production from potato peel waste, it significantly adds value to academic researchers, business societies, industrialists, policymakers, and all interested groups. This study introduces a new approach for potato peel waste biomass pretreatment optimization that enhances delignification efficiency. This will improve the overall production of bioethanol.

1.5. Scope of the study

Within the given time and resources, the scope of this thesis generally covers bioethanol production from potato peel waste using alkali (NaOH) pretreatment and dilute acid hydrolysis. It includes analysis of the proximate and chemical composition of potato peel, optimization of pretreatment parameters for enhanced delignification efficiency using response surface methodology, instrumental characterization of raw and alkali treated potato peel (FTIR, XRD, SEM), acid hydrolysis of treated potato peel to produce fermentable sugar and studying the effect of the hydrolysis conditions on the sugar concentration and fermentation of glucose to bioethanol.

2. LITERATURE REVIEW

2.1. Bioethanol

The ever increasing energy demand is met primarily (≈88%) by fossil fuels. However, this energy demand is likely to intensify by 26% over the next 20 years due to the rapid growth of industries, automobiles and the world population (Suresh et al., 2020). Hence, there is an alarming concern about future vast energy demands with limited and non-renewable resources. Furthermore, there is an increased awareness of environmental pollution, global warming, high usage of fossil fuels and increasing crude oil prices. All of these disturbing factors highlighted the necessity for alternate, viable, proficient, economical, and eco-friendly energy sources to achieve present and future demands. Given that Ethiopia is the nation most affected by energy scarcity worldwide, the development of alternative energy sources makes sense because energy scarcity has a direct contribution to poverty (Gabisa & Gheewala, 2018). The adoption of alternative energy will enhance living standards and enable economic sustainability. The search for alternative and renewable energy sources is prompted by the world's fuel prices rising, the world's energy demand increasing, and concerns about global warming. At the moment, there exist three categories of renewable resources: wind, hydropower, and biomass. While the transportation sector primarily depends on liquid fuels, which can only be produced from biomass, the electricity industry may be able to meet its energy needs by using these renewable resources. Presently, biofuels (biodiesel, biogas, and ethanol) are considered the most promising alternatives in energy generation that can compete with fossil fuels and reduce the world„s dependence on fossil fuels. The two most widely used types of biofuel are biodiesel and bioethanol (Birhanu & Ayalew, 2017).

Recently, biofuels have proven to be the best fuel source for achieving these energy goals in a sustainable way. More precisely, as the transportation sector is currently responsible for the bulk of CO_2 emissions, bioethanol may be used as a substitute for petroleum sources and has grown to become one of the most widely utilized biofuels in industry. Furthermore, ethanol is easily stored, renewable, and has a higher energy and oxygen content (Chohan et al., 2020).

Bioethanol is one of the most promising alternatives to fossil fuels that can ensure energy security and address environmental pollution problems. Bioethanol is produced from agricultural

residues, ligno-cellulosic biomass, and starchy biomass. The ligno-cellulosic biomass is cheaper and available in plenty, but its conversion to bioethanol involves many steps and is expensive. The primary motive for producing fuel ethanol is to reduce the foreign exchange burden of oil imports (Malik et al., 2018).

Ethanol (C_2H_6O) is a simple liquid alcohol that is formed from the fermentation of sugars in their natural occurrences or from starch-rich grains or lignocellulose feed stocks. Ethanol is also called ethyl alcohol, grain alcohol, or simply alcohol, and is used as a disinfectant, an organic solvent, a chemical feedstock, and a transportation liquid fuel. Ethanol is produced in various countries in the world and its global quantity has changed from 110 billion liters in 2019 to 98.6 billion liters in 2020 due to the pandemic (Hoang & Nghiem, 2021). One of the main uses of bioethanol is as a fuel additive in gasoline. It can be blended with gasoline in different concentrations, such as E10 (10% ethanol and 90% gasoline) or E85 (85% ethanol and 15% gasoline). A blend of ethanol and 95% gasoline can lower CO_2 and SO_2 by about 90% and 60–80%, respectively. This preserves environmental security, lowers greenhouse gas levels that contribute to climate change, and helps the world address some of its air pollution issues. Currently, ethanol is produced commercially from a variety of feed stocks via fermentation.

2.2. Ethanol Production in Global

The Renewable Fuels Association's (RFA) 2023 report states that global fuel ethanol production reached 28 billion gallons in 2022. The United States of America contributed the most, with 15.4 billion gallons, followed by Brazil with 26.9 billion gallons, and the rest of the world Europe, Asia, Canada, Latin America, and other regions with the remaining 19% (RFA, 2023). Africa makes a negligible contribution to the global ethanol market. According to RFA (2023), many ethanol producers advance toward net-zero carbon emissions in 2022 by encouraging the production and use of ethanol by 2050 or earlier.

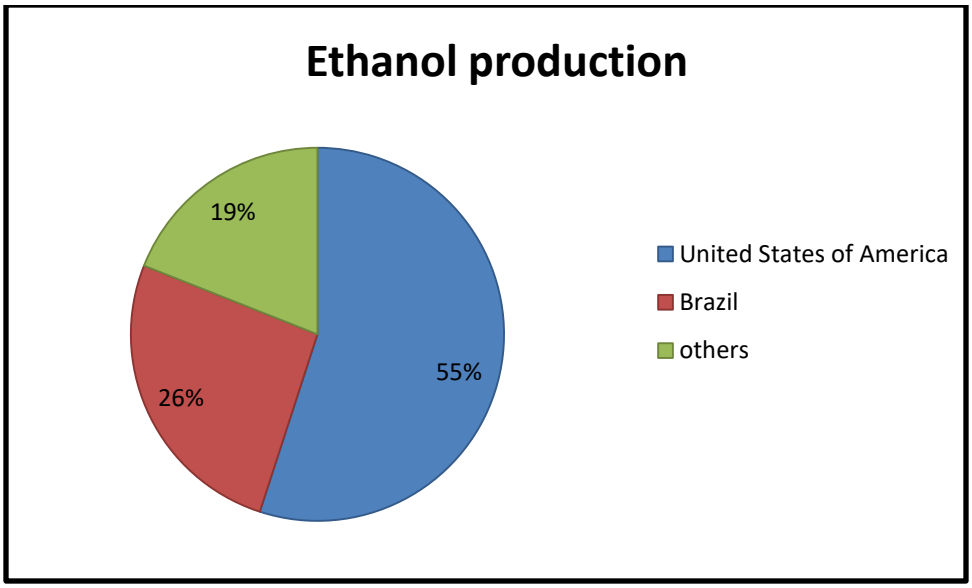


Figure2. 1: Ethanol production in globe

Since Brazil produces ethanol from sugar cane and the United States produces more than 94% of it from corn starch, there is competition for food as a result of the global food insecurity that primarily affects Asia and Africa. One way to mitigate these negative effects is to use wastes like cheese whey molasses and sugar-rich potato peel waste to produce ethanol (Robak & Balcerek, 2020).

2.3. Ethiopia's Status of Bio-Ethanol Production

Ethiopian ethanol production is associated with sugar factories and aims to improve agricultural development and agro-processing, create jobs, and increase export revenue, in addition to serving as an import replacement for petroleum products. There are four sugar factories in Ethiopia: Tendaho, Fincha, Wonji-Shoa, and Metahara. However, Ethiopia only has two sugar factories, the Fincha and Metehara, that are engaged in the fermentation and distillation of molasses to produce bioethanol. In 2014/15 about 20.5 million liters of ethanol were supplied to the energy system of the country (8 million liters from Fincha sugar factory, whereas about 12.5 million liters per year were from the Metahara sugar factory) and all used in the transport sector. There are currently 13 planned, operational sugar factories in Ethiopia. They have the capacity to produce 3.6 million MT of sugar, 339 million liters of ethanol, and 250 MW of excess power for cutting-edge production, or roughly 400,000 MT of sugar and 21 million liters of ethanol (Birhanu & Ayalew, 2017). Since 2009, Ethiopia has been blending 5% ethanol with gasoline; in

2010 the ratio was raised to 10%. One sugar factory and one Blender Company (Fincha and Nile Petroleum) were the starting points for these blends. In addition, the nation began 10% blending in 2011 with three blender camps (Fincha, Metehara and Nile petroleum, Oil Libya, and NOC) and two sugar factories. Ethiopia has combined over 44 million liters of ethanol with gasoline so far, saving over 35 million USD (Birhanu & Ayalew, 2017).

2.4. Feed Stocks for Bioethanol Production

Bioethanol can be produced from a large variety of potential feedstock all around the world. These consist of starch-based feedstocks (corn, barley, wheat, and other grains), sugar-based feedstocks (sugar cane, sugar beet), and lignocellulosic feedstocks (agricultural residues, forest residues, dedicated energy crops, and municipal solid wastes). First-generation ethanol is made from feedstocks that are based on sugar or starch, while second-generation ethanol is made from feedstocks that are based on lignocellulose (Hoang & Nghiem, 2021; Guarango, 2022).

2.4.1. Sugar Based Feed Stocks

The production of bioethanol from sugar-based feedstock‟s has the greatest potential to replace fossil fuels as a fuel for transportation since it is less expensive, emits less carbon dioxide, and produces more bioethanol per hectare compared to starch-based crops (Ayodele et al., 2020). Sugar-based feedstocks are commonly used for ethanol production due to their high sugar content, which can be easily fermented into ethanol. Sweet sorghum, whey, sugar cane, and sugar beet are a few of the primary sugar-based feedstocks used in the production of ethanol. The two most significant sugar-producing plants in the world are sugar cane and beet. Sugar cane accounts for two thirds of global sugar production, while sugar beet accounts for one third. The majority of Saccharomyces species synthesize the enzyme in-vertase, which can readily hydrolyze them. Because bioethanol can be produced from feedstocks containing sugar (sucrose) without the need for pretreatment, this bioprocess is more feasible than one involving feedstocks containing starch. All sugar crops need to do is grind their sugars into a fermentation medium, where juice or molasses can be used to make ethanol right away. A primary byproduct of the sugar industry, molasses is primarily used as a substrate for the biochemical and bioethanol production of yeast, but it can also be used to make feed materials. Molasses has a total residual sugar content of 50–60% (m/V), of which about 60% is sucrose. This makes the substrate ideal for producing bioethanol on a large scale (Bušić et al., 2018;Hoang & Nghiem, 2021)

2.4.2. Starch based feedstock

Starch is found in high concentrations in root and tubular crops such as cassava, sweet potato, Jerusalem artichoke, cactus, or arrowroot, as well as grain crops like corn, barley, wheat, and grain sorghum. It is possible to produce bioethanol or further convert isolated native starch from various sources into bio-based products. Proteins and fiber found in the residue left over after starch is isolated have a lot of potential uses in the manufacturing of food and feed. The United States produces the most corn starch, accounting for over 80% of the global market. Over 95% of the bioethanol produced in the United States comes from corn; the remaining 5% is made from barley, wheat, whey, and beverage residues. The grain sorghum cultivating regions in the USA show an increasing interest in bioethanol production from this crop (Bušić et al., 2018). Starch is a mixture of linear (amylose) and branched (amylopectin) polyglucans. α-amylase is an essential enzyme for the hydrolysis of starch; it is active on α-1,4 linkages in amylopectin but inactive on α-1,6 linkages. To produce bioethanol from feedstocks containing starch, starch must be hydrolyzed (mostly by α-amylase and glucoamylase) into glucose syrup, which the yeast Saccharomyces cerevisiae can then ferment into ethanol. When compared to the process of producing bioethanol from feedstocks containing sugar, this step faces additional costs (Li et al., 2022).

2.4.3. Lignocellulose based feedstock

Second-generation ethanol production is derived from non-food crops or crop residues with the main component that is lignocellulose biomass. The main chemical components of lignocellulose biomass are lignin, cellulose, and hemicellulose; small amounts of water, protein, minerals, and other elements that are also present in the raw material can be converted into biofuel through thermochemical or biological processes. Every biomass has a different chemical makeup from the others (Ayodele et al., 2020). The overall composition of lingocellulosic material reveals that hydrogen bonds and van der Waals forces bind the cellulose microfibrils (at least 1000–5000 glucose units joined by β-1,4-glucosidic linkages). These grouped or united microfibrils are referred to as cellulose fibrils coated with hemicellulose. Hemicellulose, which is found at the primary cell wall, is an amorphous, heterogeneously branched polymer of pentoses and hexoses (at least 500–3000 sugar monomers). Lignin is a hetero polymer composed of phenyl propionic alcohol units connected by ether (C-O) and carbon-carbon (C-C) bonds. Plant cell walls are 11 made stable and resistant to pathogen invasion by this kind of linkage. Because of its strong bond

with the cellulose fibrils, it functions as a physical barrier against microbial and enzymatic hydrolysis. Unlike cellulose and hemicellulose, lignin is characterized by the presence of aromatic rings as opposed to lengthy molecular chains with a variety of chemical structures and monomer compositions. Lignin is used to bind cellulose and hemicellulose together; it is located between them (Sharma et al., 2020; Rezania et al., 2020).

Domestic wastes (lignocellulose garbage and sewage), food industry residues, forest residues, energy crops (such as herbaceous or woody plants), and agricultural residues (sweet sorghum bagasse, sugarcane bagasse and straw, corn Stover) are all examples of lignocellulose biomass. Using lignocellulose-containing raw materials to produce bioethanol is appealing and sustainable because lignocellulose biomass is non-competitive with food crops and renewable. Additionally, there is a significant correlation between the utilization of bioethanol derived from lignocellulose biomass and a decrease in greenhouse gas emissions. Compared to fossil resources, lignocellulose biomass is almost equally distributed throughout the planet, ensuring supply security through the use of domestic energy sources. It can be obtained from different residues or directly harvested from forest and its price is usually lower than of sugar- or starch-containing feedstocks, which require full agricultural breeding approach(Bušić et al., 2018).

2.4.3.1 Agro- industrial wastes feedstock

As long as food security is maintained, the production of bioethanol using an agro-industrial basis has gained priority. Agro-industrial substrates with high availability, biodegradability, and nutrient richness include wastes derived from starch. It also solves waste disposal issues when used in biofuel production processes. A wide range of agricultural and industrial feedstocks can be utilized as ethanol's bioconversion substrates. Waste products from tuber crops, like potatoes, sweet potatoes, and cassava, make good substrates because they have enough starch to be hydrolyzed into sugars and then fermented to produce ethanol. Because potatoes have a high yield of fermentable carbohydrates, they are particularly suitable. Moreover, potatoes are the third most important food crops in the world after rice and wheat, all of which are considered staple crops. Potato peel wastes (PPW) are produced in large quantities as a result of its widespread use in industry. 20 to 50% of the raw product is wasted in the potato processing industries. These wastes are typically disposed of in landfills, where microbial deterioration results in environmental issues. The valorization of PPW reduce this waste stream, greenhouse

gas emissions and the carbon footprint while generating value-added products such as biofuels and bio products (Chohan et al., 2020).

Wastes from agro-industrial processes, which are mainly made up of cellulose, hemicelluloses, lignin, and starch, are currently the most plentiful source of renewable energy. Fruit and vegetable peels are produced in large quantities in commercial areas, homes, and industries. They are frequently combined with other waste, making them unfit for use in other contexts. But these leftover peels can be used efficiently to recover sugar and make biofuels (Soni et al., 2023).

Table2. 1: Summary of literatures on bioethanol production from different feedstocks

Feed stock	Microorganism	Fermenter condition	Fermentation time (hr)	Ethanol concentration (g/l)	Ethanol yield (%)	Reference
Sugarcane juice	S. cerevisiae	PH- 3.86 Temp. 30^{o}C	32	89.73	92	Liang et al. (2008)
Sugar beet molasses	S. cerevisiae	PH-5.5, Temp.30^{o} 100rpm	48	83.2	96.56	Razmovski and Vučurović (2012)
Sweet sorghum juice	S. cerevisiae	pH-4.9, Temperature 30^{o}C,	40-72	120.68	99.8	Laopaiboon et al. (2009)
Wheat straw	S. cerevisiae	pH-5.0 Temp.34^{o}C	48	38.1	71	Olofsson et al. (2008
Corn Stover	S.cerevisiae	PH 5.5 Tem.30^{o}C	72	47.5	93	Lau & Dale (2010)
Potato peel	Saccharomyces cerevisiae	PH 5.5 Tem.35^{o}C	48	9.5	87.8	(Felekis et al., 2023)

2.5. Potato Peel Waste as a Feedstock for Bioethanol Production

Potato (Solanum tuberosum L.) is an important food crop worldwide and an extremely popular vegetable crop. It is the first crop among root and tuber crops, followed by cassava, sweet potato, and yams in production. Potatoes are a staple crop globally since it is the world's fourth-largest food crop. This rise in production and processing has led to increased generation of large volumes of residues in the form of peels, usually making up between 20 and 50% of the whole tuber (Sanusi et al., 2022). Potato is consumed almost daily by more than a billion people. Hundreds of millions of people in developing countries depend on potatoes for their survival. It

is an extremely important crop for countries such as Ethiopia, where inadequate protein and supplies of calories are apparent nutritional problems (Gelaye et al., 2022).

Figure2. 2: Potato peel waste

Potato is an economical food, and it provides a source of low cost energy to human diet. It has become one of the most popular crops in Ethiopia. Potatoes are starchy crops, which do not require complex pre-treatments (Bekele et al., 2015). Due to the widespread use of potatoes in food industries, such as the production of commercial products including food items, a significant quantity of PPW is produced during unavoidable potato processing such as peeling, cutting, and trimming approximately 18% of the potatoes are generated as waste. The current PPW management method of landfilling has a negative environmental impact by emitting greenhouse gases and poisonous leachates. It is estimated that around 8000 kilotons of PPW could be generated in 2030, with greenhouse gas emissions of 5 million tons of CO2 equivalent associated with its disposal(Khanal et al., 2023). The most common source of environmental contamination in food industries is the decomposition of organic wastes, which takes place when bacteria and other biological systems consume chemicals as food sources. However, a significant amount of starch and lignocelluloses are present, making them a source for the production of biobased products. The current trend towards the valorization of wastes demands the transformation of industrial and municipal waste into value-added products.

2.6. Chemical composition of different feed stocks

Table2. 2: The average composition of a few raw lignocellulosic materials (% dry weight basis)

Raw material	Cellulose (%)	Starch (%)	Hemicellulose (%)	Lignin (%)	Ash (%)	Moisture (%)	Reference (%)
Potato mesh	2.31	49.78					(Chintagunta et al., 2016)
Sugarcane bagasse	39.72		26.96	40.98	0.12		(Wirawan et al., 2020)
Wheat straw	46.63		27.47	15.20			(Prasad et al., 2018)
Corn stove	37.72		20.62	30.5	5.03	6.69	(Mensah et al., 2021)
Potato peel	20.2	19.4	15.2	20	6.5	6	(Soni et al., 2023)
Cassava peel	14	29.84	24	9.16	4.66		(Aruwajoye et al., 2017)
Barley straw	44		27	7			(Iroba et al., 2013)

2.6.1. Chemical composition of potato peel waste

PPW is a lignocellulose substrate that mainly consists of lignin, hemicellulose, cellulose, and starch. As Barampouti et al. (2023) states the chemical composition ranges of potato peel waste are listed in the Table below.

Table2. 3: chemical composition range of potato peel waste

Composition	**Literature range**
Cellulose	5.7- 33.5%
Starch	16.8- 42.0%
Hemicellulose	5.5-7.4%
Lignin	5.7-22.9%
Ash	4-12%

According to Abebaw (2020) potato peel waste contains starch 25%, non-starch polysaccharide 30%, protein 18%, acid-soluble and acid insoluble lignin 20%, lipids 1% and ash 6% on dry basis. And also as of Kumar Soni et al. (2023a) potato peel waste contains 20.2% cellulose, 19.4% starch, 15.2% hemicellulose, 20% lignin and 6% ash. Due to its composition, this waste can be considered a potential feedstock for bioethanol production (Malik et al., 2018)

2.7. Bioethanol Production Process

Generated bioethanol through the microbial fermentation of sugar-based, starchy and lignocellulose feedstock has been considered as a potential renewable fuel. The feedstock's chemical and structural properties determine the difficulty of the production process. The processes involved in the production of bioethanol from different feedstocks include pretreatment, hydrolysis, fermentation and ethanol recovery.

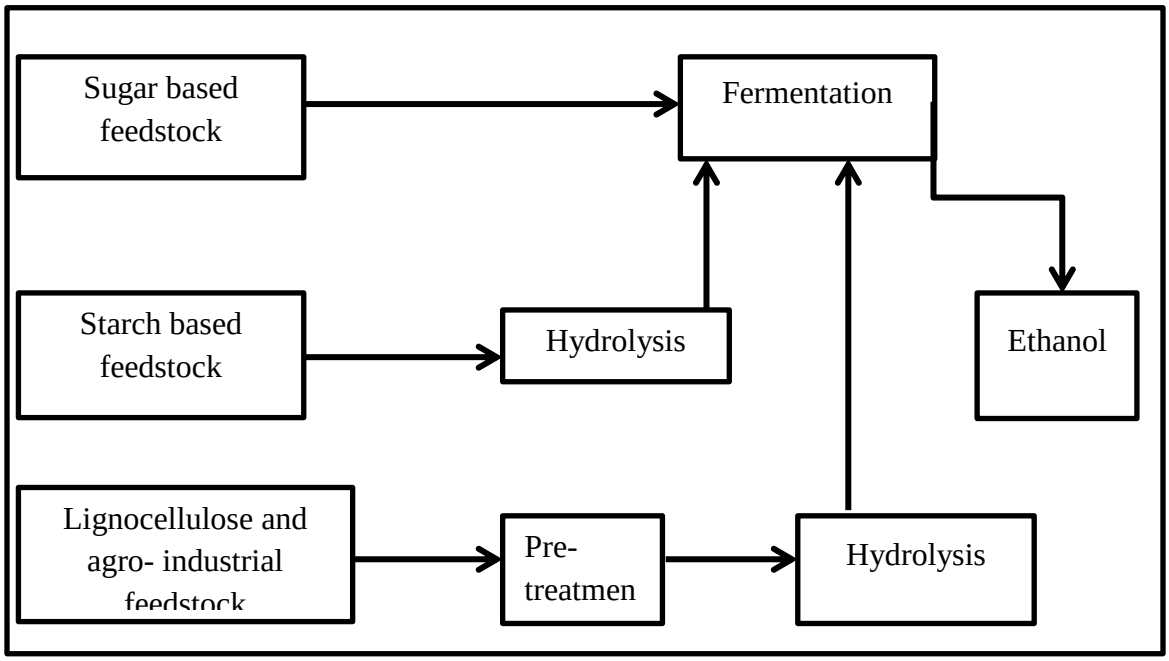


Figure2. 3: Bioethanol production process

2.7.1. Pretreatment

The first, most important, difficult, and expensive step in producing bioethanol from lignocellulose biomass is pre-treatment. De-lignification, which modifies cellulose's structure to facilitate hydrolysis and boost the amount of sugars from cellulose and hemicellulose that can be fermented into ethanol, is the aim of all pre-treatment technologies (Sharma et al., 2020). It should be mentioned that in order to achieve a higher fuel yield, pretreatment efficiency is a crucial factor. The main goals of the pretreatment stage are to increase the surface area of the biomass, dissolve hemicellulose and/or lignin, and decrease the biomass's particle sizes (Rezania et al., 2020). Agricultural feedstocks and byproducts like PPW are composed of polysaccharide polymers that are stubborn to microbial degradation. In addition, these polysaccharides are associated with lignin, which acts as a physical barrier that protects polysaccharides from enzyme action. Therefore, prior to hydrolysis, a pretreatment step is mandatory to modify the structure of the lignocellulosic matrix and make it more accessible to hydrolysis. Pretreatments aim to depolymerize lignin, increase the porosity of the matrix, solubilize hemicellulose and

starch, decrease cellulose crystallinity, and consequently increase its digestibility (Kalafat et al., 2018).

Pretreatment processes can improve the digestibility of lignocellulosic biomass as pretreatment removes lignin and hemicelluloses from lignocellulosic materials. It can also lead to a higher conversion of cellulose and enhance the hydrolysis efficiency of different lignocellulosic materials. Pretreatment frequently results in higher sugar levels and stops the development of inhibitory products during the fermentation and hydrolysis processes that follow.

Several pretreatment techniques are available with the goal of raising cellulose's reactivity and the possible yield of the fermentable sugars. These pretreatments can be advanced or conventional. Conventional pretreatment techniques fall into four groups: chemical, physical, physicochemical, and biological techniques. Advanced pretreatment techniques can be Ionic Liquid-Based Fractionation (ILF) or Acid-Based Fractionation. Chemical categories are the most effective among the conventional pretreatment techniques, which is why they are most frequently utilize (Edeh, 2021). The type and conditions of pretreatment affect the structural modifications of various lignocellulosic raw materials.

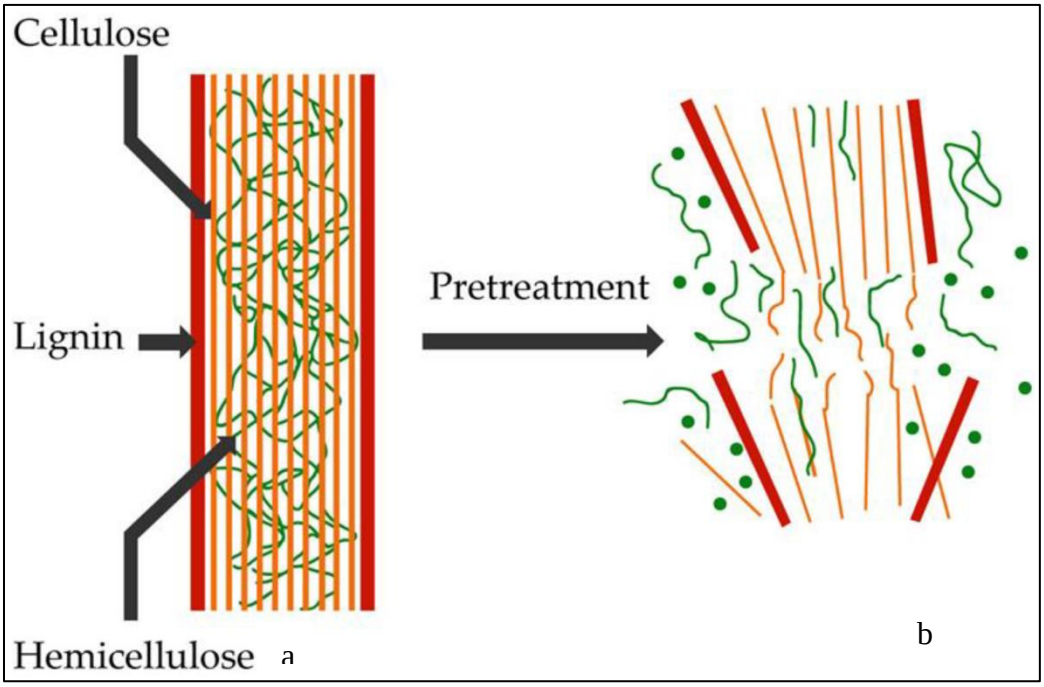


Figure2. 4: (a) Lignocellulosic biomass before pretreatment, and (b) Lignocellulosic biomass after pretreatment (Edeh, 2021)

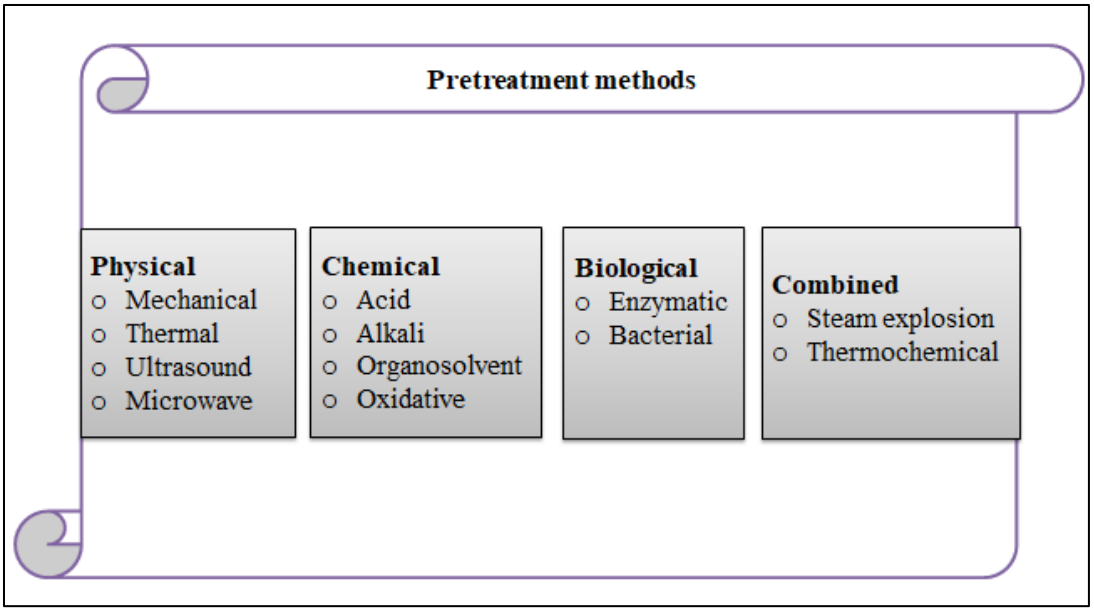


Figure2.5: Classification of pretreatment methods

2.7.1.1. Physical pretreatment methods

Physical pretreatments, such as pyrolysis and mechanical commination in the form of dry, wet, and vibratory ball mills, are used to alter the physical properties of materials in an effort to reduce the cellulose crystal and increase biomass digestibility. In LCB pretreatment, a significant portion of the overall running costs are attributed to the grinding process. The two most important variables for physical pretreatment are the final particle size and the characteristics of the biomass. Meanwhile, this method's primary shortcomings are its high power consumption and generally low efficiency. Numerous physical pretreatments exist, but they are rarely employed. These include milling, high pressure homogenization, electron beam irradiation, hot compression, and photo catalysis. Thermal pretreatment is considered as a type of physical pretreatment which is based on heating LCB at a certain temperature and pressure in the range of 50-240^{o}C (Bušić et al., 2018; Sharma et al., 2020). The most common physical pretreatment includes milling, freezing, microwave and extrusion.

2.7.1.2. Chemical pretreatment methods

Using chemicals for pretreatment is the basis of chemical pre-treatment. The main chemical pretreatments that enhance enzymatic digestibility are ozonolysis, acid, alkaline, oxidative, ionic liquid, and organosolv. According to Ye Sun (2002), chemical pretreatments help to specifically remove undesirable compounds like lignin or hemicellulose compounds; as a result, the application of these treatments varies depending on the type of biomass.The primary disadvantage of chemical pre-treatment is the formation of inhibitors for processes downstream and the need to adjust pH before hydrolysis when using acid treatment.

Acid pretreatment is a well-known method for producing bioethanol from LCBs. It has been applied in various investigations to break the structure of lignin and solubilize hemicellulose, increasing the accessibility of cellulose chains prior to enzymatic hydrolysis (Wirawan et al., 2020). Temperature, residence time, acid concentration, and solids loading are the primary effective parameters for acid pretreatment. Additionally, acid can dissolve all polysaccharidelignin bonds, facilitating the recovery of most monomeric sugars. By altering the structure of LCB, hemicelluloses were transformed into soluble sugar using this method. Raising the temperature during the diluted acid pretreatment can accelerate the hemicelluloses' conversion. Diluted acid hydrolysis has an advantage over concentrated acid hydrolysis in that it uses less acid, but a higher temperature is needed to produce a reasonable amount of glucose from crystalline cellulose.

Alkali pretreatment is a widely studied chemical pretreatment method which is based on the solublization of lignin in the alkali solution. The various alkaline reagents used commonly for alkali pretreatment are the hydroxides of sodium, potassium, calcium and ammonium. Alkali pretreatment typically takes place at low temperatures, although it can also be carried out with a high base concentration and a relatively long time. Compared to acid pretreatment, alkali pretreatment effectively dissolves lignin and mildly breaks down polysaccharides. Furthermore, alkaline agents are said to be more efficient on wood materials and have the ability to significantly remove the acetyl group from hemicellulose (Wirawan et al., 2020).

In terms of technology, mild alkaline pretreatment can be carried out under reduced pressure and temperature. When compared to acid pretreatment, this method produces fewer inhibitors (Fernández-Delgado et al., 2019) .

Compared to diluted acid, alkaline pretreatment is more important for delignification. Additionally, alkaline reagents can be recovered and recycled, which could lower pretreatment costs. In addition to increasing corrosion and being unsuitable for scaling up, acid pretreatment also forms large amounts of fermentation inhibitors like furfural and HMF, which decreases the effectiveness of subsequent steps.

Organosolv pretreatment: Organosolv is a chemical method that uses pure or diluted organic solvents to extract lignin from lignocellulosic biomass and purify cellulose. It is thought to be an interesting and environmentally friendly process. Additionally, this method may reduce the synthesis of harmful inhibitors while concurrently producing highly pure cellulose and lignin (Robak & Balcerek, 2020).

2.7.1.3 physic-chemical pretreatment

Physical-chemical pretreatment techniques integrate chemical and physical procedures to facilitate the synthesis of ethanol. These are more successful and efficient techniques for breaking down the crystalline complicated structure of cellulose. The breakdown of cellulose crystalline structure improves the enzymatic hydrolyzing efficiency of cellulose and hemicellulose and boosts its yield of recovery following pretreatment. Steam explosion, liquid hot water, moist oxidation, CO_2 explosion, ammonia fiber explosion, and ultra-sonication are examples of physical-chemical pretreatments.

2.7.1.4. Biological pretreatment

Plant cell wall material has been investigated and broken down using microorganisms. Because they produce a large number of saccharifing enzymes, fungi are used in the degradation of lignocellulosic materials; different chemicals have been targeted by a variety of fungi, including brown, white, and soft rot fungi (Nathan et al., 2005). Whereas, white and soft rots can break down both cellulose and lignin, brown rot fungus primarily break down cellulose (Hiben, 2013). Due to the absence of gut microbes, the wood-boring marine crustacean limnoria quadripunctata has discovered a novel method for biologically degrading lignocellulosic biomass.

This method involves the crustacean's ability to digest crystalline cellulose directly into fermentable glucose, suggesting that it may possess all the enzymes needed for lignocellulose digestion. Although biological pretreatment requires low energy at moderate conditions, the extent of decomposition is less.

2.8. Hydrolysis

After the lignocellulosic biomass has been pretreated, sugar monomers are created by the hydrolysis of polymeric carbohydrates such as cellulose, starch, and hemicellulose. Since the enzymes needed for the next step (fermentation) can only break down sugar monomers, this step is necessary. Enzymes or acids can act as catalysts for the process.

2.8.1. Acid-catalyzed hydrolysis

It is the most commonly used method and it involves either the use of concentrated or dilute acid. Dilute acid hydrolysis is a process that uses inorganic acids such as sulfuric acid and hydrochloric acid mostly at a concentration of 1–3% for a short period of 3 min-10min and temperatures from 180 to 240 °C. The concentrated acid-catalyzed hydrolysis is used at lower temperature (below 70 ^{o}C) and high acid concentration. The disadvantage of this method is the high cost of production due to difficulty in acid recovery, disposal, concentration control and recycling. Another problem with the concentrated acid-catalyzed hydrolysis treatment is its capability to degrade sugar monomers due to the prevailing acidic environment. The dilute acid-catalyzed hydrolysis requires high temperature (100-240 ^{o}C) and low acid concentration. The most predominantly used acid is dilute acid (Edeh, 2021).

2.8.2. Enzymatic hydrolysis

Fermentable sugars can be obtained by enzymatic saccharification of lignocellulosic material that has been pretreated with acid, alkali, or fungus (Suhas et al., 2013). The best sources of celluloses and hemicelluloses that can be utilized to hydrolyze pretreated lignocellulose are bacteria and fungi. Enzymatic cocktails are typically composed of various hydrolytic enzymes, including hemicellulose, cellulose, and xylanases. It can function in mild circumstances (pH 4.8–5.0 and 50–60 °C) with good sugar recovery and no inhibitor formation. Temperature, pH, enzyme loading, and time are a few of the variables that impact the efficiency of enzymatic saccharification.

However, the cost of the enzymes for the enzymatic process remains high. It has been estimated that the cost of the enzymes used in the processes accounts for 10–20% of the total cost of producing ethanol. Acid hydrolysis is used for alcohol production from potato industry starchy waste (Sharma et al., 2020) Acid hydrolysis has lower cost than enzymatic hydrolysis.

2.9. Fermentation

Fermentation is the biological process in which microorganisms, mainly yeast and bacteria, convert monomeric sugars into acids, gases, and ethanol. Saccharomyces cerevisiae (baker's yeast) is the commonly used organism in the alcohol production process because of its high productivity and ethanol yield from different feedstock. Other than Saccharomyces cerevisiae, Pichia stipites, Kluyveromyces fagilis, and Candida shehatae were also reported yeast strains for the production of ethanol from different sugars. There are some fermentation technologies, i.e. separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF) and consolidated bioprocessing (CBP) used in bio refineries (Sharma et al., 2020).

In ethanol fermentation, derivation of energy from sugars by either yeast or bacteria, produce carbon dioxide and ethanol are produced. Because yeasts produce their energy without the need for oxygen, ethanol fermentation is a facultative anaerobic process. In general, the conversion of lignocellulosic material to sugar and then ethanol is governed by equation below:

$$(C_6H_{10}O_5)\,n + nH_2O \longrightarrow nC_6H_{12}O_6 + \text{yeast} \longrightarrow 2nC_2H_5OH + 2nCO_2$$

The fermentation process can be performed as a continuous, batch, or fed-batch process, which depends on lignocellulose hydrolyzate and on the kinetic properties of microorganisms used. In the continuous process, substrate containing feed and culture medium are continuously pumped into reactor, which holds active microorganisms. The batch process is a simple method and considered a closed culture system that contains an initial nutrient amount that is limited. Such culture is inoculated with microorganisms that perform fermentation. Fed-batch reactors are frequently used in industrial applications due to many advantages from both continuous and batch processes. The fed-batch process can increase the concentration of maximum viable cells, can extend the lifetime of culture used, and can accumulate product with higher concentration (Vasić et al., 2021).

Table2. 4: Recent studies of alkaline and acid pretreatment, hydrolysis and fermentation of LCB for bioethanol production.

Alkali pretreatment					
Feed stock	**Pretreatment condition**	**Hydrolysis condition**	**Fermentation condition**	**Bioethanol yield (g/L)**	**Reference**
wheat straw	Na_2CO3 (11%) 75^oC;10-85 min	Enzymatic 72hr	30^oC; 96 hr	65	(Yuan et al., 2018)
sugarcane bagasse	NaOH (15%) 175^oC; 1.5hr	H_3PO_4 (6%) 100^oC 5hrs	37^oC; 10 hr	8.8	(Lenihan et al., 2010).
Rice straw	NaOH (1%) 50^oC; 72 hr	Enzymatic 121^oC 20min	35^oC; 144 hr	13.8	(Zhu et al., 2015)
Potato peel	NaOH (3%) 50^oC; 6 hr	Enzymatic Amylose (65^oC)and cellulose (50^oC)	30^oC; 24 hr	15.47	(Barampouti et al., 2023)
Potato peel	NaOH (1%) 50^oC; 6 hr		30^oC; 24 hr	23.75	(Barampouti et al., 2023)

Acid pretreatment					
Feed stock	**Pretreatment condition**	**Hydrolysis condition**	**Fermentation condition**	**Bioethanol yield (g/L)**	**Reference**
Rice straw	H_2SO_4 100 ^oC; 2 hr	Enzymatic 121^oC 20min	30 ^oC; 72 hr	40.6	(Zhu et al., 2015)
Corn and corn Stover	H_2SO_4 60 ^oC;10 min	Alpha amylase (85 ^oC, 4 hr) Cellulose (50 ^oC, 48hr)	30 ^oC; 96 hr	99.3	(Yu et al., 2019)
Potato peel	HCl (3.68%) 69.6 ^oC; 3 hr	Alpha amylase enzyme, 90 ^oC, 100 rpm, 60 min	41.7 ^oC; 24hr	29.86	(Chohan et al., 2020)
Potato peel		HCl (0.5M) 121^oC ,15min	pH 5.0, 32 ^oC, 100 rpm, 48hr	19.37	(Arapoglou et al., 2010)

2.9.1. Factors affecting fermentation process

Plant cell wall material has been investigated and broken down using microorganisms. Fungi are used in the selection of the microbe, the raw material, the pretreatment technique, the hydrolysis technique, and environmental variables like pH, temperature, and substrate concentration all affect how effective the fermenting process is. Saccharomyces cerevisiae fermentation typically occurs at a pH of 5.0 and a maximum temperature of 37°C (Badger, 2002). Several process inhibitors produced by the upstream steps have an impact on the process's performance. Inhibitors are present in hydrolyzers along with fermentable sugars, which limit the growth of fermenting microorganisms and reduce the amount of ethanol produced. Process water recirculation raises these chemicals even further (Marutt, 2010). Depending on the fermentation medium, Saccharomyces cerevisiae prefer a pH range of 4 to 6.

Through affecting plasma protein activity, pH has an impact on how well ethanol ferments. The yeast cannot grow and produce ethanol efficiently if the pH is less than 4.0. However, when the pH is raised to 5.0, both the amount of ethanol produced and the efficiency of fermentation

increase. It was discovered that pH 5 is the ideal pH for Saccharomyces cerevisiae species (Rocha et al., 2017). Temperature influences yeast activity, which in turn impacts how efficiently ethanol is fermented. The optimal temperature for Saccharomyces cerevisiae species was found to be approximately 37 °C. If the temperature is higher than 37 °C, the yeast will not be able to grow and produce ethanol efficiently. This was demonstrated by decrease in ethanol production and fermentation efficiency. The rate of agitation is another element to take into account. The amount of ethanol created increases with the pace of agitation. The standard agitation rate for yeast cell fermentation is 150–200 rpm. A high pace of agitation could restrict the cells' ability to metabolically function (Edeh, 2021).

2.10. Distillation

In order to remove the water and obtain anhydrous ethanol, dehydration is required to prepare dry alcohol which called anhydrous ethanol. Anhydrous ethanol holds minimum of 99.5% of ethanol by volume (Taghizadeh-Alisaraei et al., 2019). A traditional method of removing of water content can be performed by the principle of distillation, which is done by utilizing the difference of boiling points of the mixtures in a solution. By heating the mixture to the ethanol boiling point (78.2 ^{o}C), ethanol will be vaporized and separated from the water.

3. MATERIALS AND METHODS

3.1. Study Sites Description

Valorization of industrial potato peel waste into bioethanol under optimal conditions carried out in Chemical Engineering laboratory of Adama Science and Technology University (ASTU). Some characterizations performed in different place due to the lack of some instruments. SEM characterization performed in the biology laboratory in ASTU, FTIR and UV characterization carried out in AASTU, XRD performed in material science laboratory of Adama science and Technology University. The raw material potato peel waste collected from sun chips factory located in Sheno around 65 km northwest of Addis Ababa.

3.2. Chemicals and Materials

For this research chemicals and the sample potato peel waste, Sodium Hydroxide (98%), Ammonium hydroxide(99.5%), Toluene (99%), Ethanol (97%) , Acetone and Distilled water ,nitric acid, Acetic acid, Sulfuric acid (98%), Phenol solution, Dextrose sugar, Yeast extract, Urea, $MgSO_4.7H_2O$, Yeast (Saccharomyces cerevisiae), Potassium dichromate. Potassium iodide and iodine were used.

3.3. Equipment's and Instruments

For this research Equipment's such as Mortar and Ball mill, Sieves analyzer, Oven , weighing balances, Vacuum Filter, microwave oven ,Shaking Incubator , Autoclave, Desiccator & Centrifuge , Plastic bag, Refrigerator & Water bath, Muffle furnace, Flasks & Measuring cylinders, Petri dish & Centrifuge tubes, Cotton & Aluminum foil, Micropipettes, and instruments such as Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy (SEM), UV spectrophotometer (UVD-3200), and pH- Meter were used.

3.4. Methods

3.4.1. Sample collection and preparation

Potato peel was collected from sun chips factory, which is located in Sheno around 65km northwest of Addis Ababa. First PPW was washed with distilled water to remove dusts and impurities, Then washed peels was oven dried at 50–55 °C and pulverized afterwards to a particle size in the range of 1–2 mm using a ball mill, and sieved to a particle size of < 0.5 mm as

physical pretreatment measures. The pulverized potato peel waste (PPW) was placed in sealed container and stored in cool, dry place for further use (Sanusi et al., 2022). Grinding of potato peel into fine powder gives increased surface area and which enhances the contact between hemicellulose and cellulose.

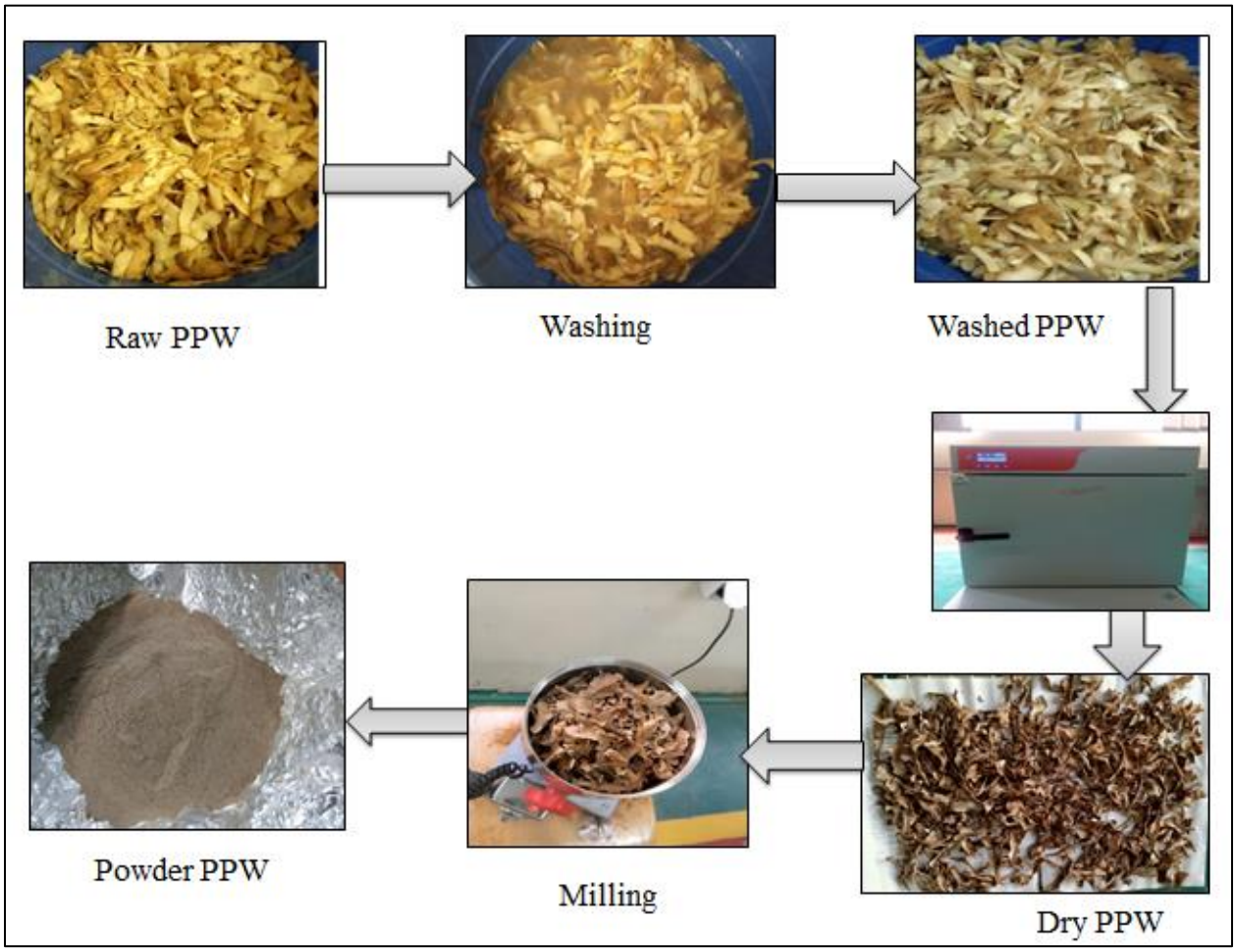


Figure3.1: pretreatment of potato peel

3.4.2. Proximate and chemical composition analysis of potato peel

3.4.2.1. Proximate analysis

The proximate analysis gives moisture content, fixed carbon content, ash content and volatile matter content was characterized based on the procedure of the national renewable energy laboratory (NREL), United State of America (Ayeni et al., 2015a). Knowing these parameters can help to determine the potential of potato peel as a feedstock for ethanol production

Moisture Content

To determine the moisture content, 5 g mass of the potato peel was weighed in clean preheated moisture crucible of known weight by using sensitive balance. The sample and crucible was kept in an oven 105 ℃ for an hour. The crucible was covered and transferred to desiccators, and weigh after reaching room temperature. The crucible was heated in the oven for another 2hours and was reweighed. It was repeated until constant weight is obtained. The loss of weight was calculated as percent of weight and expressed as moisture content.

$$Moisturecontent(\%) = \frac{W_1 - W_2}{W_1} \times 100\% \quad \text{.....Eq 3.1}$$

Where, W_1 = Initial weight; W_2 = Weight after oven-dried

Ash content

The empty Crucibles were weighed, and then the oven-dried samples put on it. The sample in the crucible placed in a muffle furnace and heated for 2 hours at 550 ℃. Then the crucible taken from the furnace and placed in a desiccator to cool the sample. Then it was re-weighed. High ash content can negatively impact the fermentation process.

$$Ashecontent(\%) = \frac{W_2}{W_1} \times 100\% \quad \text{.....Eq 3.2}$$

Where, W_1 = Original sample weight; W_2 = Weight of ash

Volatile matter

Oven-dried samples were put in a known weight of crucibles. The sample on the crucible placed in a muffle furnace and heated for 30 min at 600 ℃. The crucible was taken from the furnace and placed in a desiccator for cooling, then reweighted. The same procedure was repeated until a constant weight is obtained. The volatile content of the sample calculated as follows:

$$Volatilecontent(\%) = \frac{W_1 - W_2}{W_1} \times 100\% \quad \text{.....Eq 3.3}$$

Where, W_1 = Original weight of oven-dried sample; W_2 = Weight after heating in the furnace

High volatile matter content may lead to higher water content in the feedstock, which can affect the fermentation process by diluting the fermentable sugars and requiring additional steps for moisture control.

Fixed Carbon Content

This is the residue left after the moisture, volatile, and ash content is given up from the sample. It is deduced by subtracting from 100, the percentage of moisture, volatile matter, and ash content. The fixed carbon estimated using the formula,

$$Fixed carbon = 100 - (\%moisture + \%ash + \%volatile matter) \quad \text{.....Eq3.4}$$

3.4.2.2. Chemical composition analysis

Chemical composition of potato peel was analyzed using gravimetric method (Ayeni et al., 2015)

Extractives

5 g of dried potato peel was loaded into the thimble with the Soxhlet extractor set up, 300 ml of acetone used as solvent for extraction. Extraction temperature and time was carefully adjusted to 70 ^{o}C and 4 hr run period.

Figure3.2: Soxhlet extraction set up

After extraction, the sample was air dried at room temperature for few minutes and it was placed in the oven at 105 ^{o}C to remove the moisture content completely. The % (w/w) of the extractives content evaluated as the difference in weight between the raw extractive-laden biomass and extractive-free biomass.

$$Extractive content(\%) = \frac{W_1 - W_2}{W_1} \times 100 \quad \ldots Eq 3.5$$

Where, W_1 = weight of dry biomass before extraction in gram, W_2 = weight of dry biomass after extraction in grams

Hemicellulose

2g of extracted dried potato peel transferred into a 250 ml flask and 300 ml of 0.5 mole/l NaOH was added. The mixture was boiled for 3.5 hr with distilled water. It was filtered after cooling through vacuum filtration and washed until neutral pH is achieved. The residue was dried at 105^{o}C in oven until constant weight is obtained. The difference between the sample weight before and after this treatment is the hemicellulose content (%w/w) of dry biomass.

$$Hemicellulose content(\%) = \frac{W_1 - W_2}{W_1} \times 100 \quad \ldots Eq 3.6$$

Where, W_1 = Weight of dry biomass before treatment; W_2 = Weight of dry biomass after treatment

Lignin

To determine lignin content of potato peel waste 3g of dried extracted potato peel weighed in glass test tubes and 30 ml of 72% H_2SO_4 was added. The sample was kept at room temperature for 2 hr with carefully shaking at 30 min intervals to allow for complete hydrolysis. After the initial hydrolysis, distilled water was added to the sample to dilute to 4% .Then, for the second step of hydrolysis the sample placed in an autoclave for 1 hr at 121°C. The slurry was then cooled at room temperature. Hydrolyzates were filtered through vacuum filtration. The acid insoluble lignin was determined by drying the residues at 105^{o}C and weighted after drying. The lignin content was calculated using Eq 3.7.

$$Legnin content(\%) = \frac{W_2}{W_1} \times 100 \quad \ldots Eq 3.7$$

Where, W_1 = was the initial sample mass; W_2 = was the obtained lignin mass after pretreatment

Starch

To calculate the starch composition in potato peel, iodine colorimetric assay method was used. Specific amount (0.3 g) of the dried and ground peel sample was weighed and Place in a beaker. 1 ml of ethanol and 2.0 ml of distilled water was added to wet sample. Then 10 ml of hot ethanol was added to the sample and mixed by shaking then it was incubated for specified period (1 hr) at room temperature to allow sugar extraction, after that supernatant was separated from the solid residue. To the sediment 10 ml of water were added and boil at 80 ^{o}C leave for 20 min (for starch hydrolysis). The sediment was filtered using what man filter paper. Then, 1ml of extract was pipetted and iodine colorimetric assay method was performed by adding few drops of iodine solution and allowing the mixture to react for a specific time (10 min) to form a blue color complex with starch. Finally the absorbance of the mixture was measured at 610 nm wavelength using a spectrophotometer. Calibration curve was prepared using standard solutions of 1 g/ml starch concentrations as shown in the Table 3.1.

Table3.1: Criteria for preparation of starch solution

Tube	**1**	**2**	**3**	**4**	**5**
stock starch solution (ml)	0	2.5	5	7.5	10
Distilled water(ml)	10	7.5	5	2.5	0

Their absorbance was measured and graph of absorbance versus starch concentration were plotted and the starch concentration in the sample was determined from the calibration curve based on the absorbance value obtained. The starch composition in the potato peel sample was calculated by using the equation below:

$$starch concentration(g/ml) = \frac{unknown sample absorbance - y\_\text{int}}{slope} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq3.8$$

Cellulose

First, a reagent of 150 ml of acetic acid and 15 ml of nitric acid was prepared in order to measure the amount of cellulose present in potato peels. Subsequently, a 250 ml flask holding 100 ml of acetic and nitric acid reagent was filled with 5 g of potato peel and thoroughly mixed. After that, it spent 30 min at 100 °C on a water bath. To cool and dilute the sample, 100 ml of distilled water was added to the flask. After centrifuging the diluted sample, the supernatant was disposed of. To determine the amount of cellulose present in the residue, it was oven dried and weighed after being cleaned with distilled water until a neutral pH was reached.

$$cellulose\,content(\%) = \frac{W_2}{W_1} \times 100 \quad \text{Eq 3.9}$$

3.5. Sample Pretreatment

Dried and milled Potato peel waste was pretreated using alkali pretreatment technique. Some of the factors that affect the pretreatment are alkali concentration, treatment time, biomass loading and temperature. To determine the optimum conditions central composite design was used. PPW solid loadings were 1:10 ratio in all the pretreatment experiments. Alkali pretreatment was performed using NaOH by varying its concentration from 1-5% (v/v), pretreatment time (1-6 hr) and temperature (30-100 ^{o}C), using 250 ml auto-clavable bottles. These parameter ranges were chosen following an extensive literature (Barampouti et al., 2023). After each pretreatment, the slurry was filtered and the solid material was washed by deionized water to adjust the pH , After that, it was dried at 105 ^{o}C for 24 hr (Felekis et al., 2023).

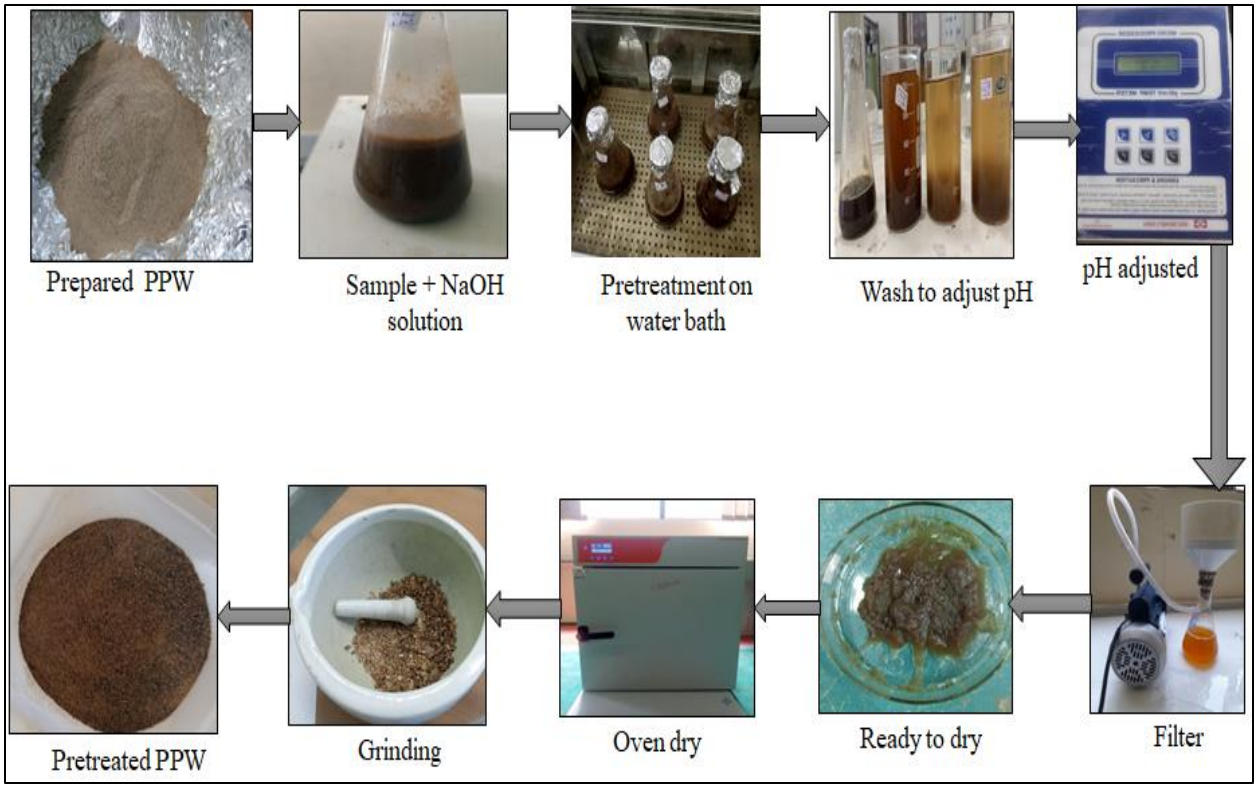


Figure3.3: Experimental procedure of pretreatment

3.6. Response Surface Methodology and Experimental Design

Response surface methodology (RSM) is a mathematical and statistical technique to develop an empirical model, which overcome the drawbacks of conventional methods. RSM is applied to find the most favorable operating condition or to explore a region that fulfills the operational specification. In this study the effect of various affecting parameters, like alkali concentration, pretreatment time and temperature on delignification efficiency was studied by central composite design (CCD) in RSM. Response of the study was delignification efficiency (%). For optimization and model development, all the experiment was performed on a batch scale. The delignification efficiency was calculated using Equations below:

$$Delignificationefficiency(\%) = \frac{L_1 - L_2}{L_1} \times 100 \quad \text{.....Eq3.10}$$

Where L_1 = Lignin before pretreatment; L_2= lignin after pretreatment

$$Starchrecovery(\%) = \frac{S_1}{S_0} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq3.11$$

Where S_o= Starch before pretreatment; $S_{1=}$ Starch after pretreatment

$$Celluloserecovery(\%) = \frac{C_1}{C_0} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq3.12$$

Where C_o = Cellulose before pretreatment; C_1 = Cellulose after pretreatment

A three-level factorial design was established using Design Expert®. The factor levels coded as −1 (low), and 1 (high). The experimental ranges and levels of the mentioned independent variables are shown in Table below

Table3.2: Values for each variables of the central composite design (CCD)

Variables	**Symbol**	**Levels and range**		
		Low(-1)	**Center(0)**	**High(1)**
Alkali concentration (%v/v)	X_1	1	3	5
Temperature (^oC)	X_2	30	65	100
Pretreatment time (hr)	X_3	1	3.5	6

Central composite design (CCD) is used to clarify the relationship between the independent variables and the response function. In this work, a three-level factorial CCD experimental design with 20 experimental runs was applied.

3.7. Instrumental Characterization of Potato Peel Waste

Raw and pretreated potato peel samples were characterized by Powdered X-ray Diffractometer (XRD), Furrier Transform Infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM).

3.7.1. FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The spectra produce a profile of the sample, a distinctive molecular fingerprint that can be used to screen and scan samples for many different

components. The FTIR spectrum of the sample was obtained at the wave number in the range of 4000-400 cm^{-1}(Rahman et al., 2017). In this study FTIR analysis was done to examine the changes that occur in the chemical structure of raw and alkali treated potato peel. FTIR spectroscopy discusses the functional group of the change of raw potato peel and alkali -treated potato peel.

3.7.2. XRD Analysis

The crystallinity of raw potato peel and alkali -treated potato peel was detected by XRD. The operating system (measurement Condition) was set at the voltage of 30 kV and current at room temperature using a sampling pitch of 0.02 (deg) and preset time 0.40 (sec) within a 2θ of 40 mA by applying the irradiation of Cu Kalfa (1.54) ranging from 10 to 50^{o} and a scan rate of 3^{o} min^{-1} in continuous scan mode. The crystallinity index (CrI) was calculated by Scherer equation (Eq. (3.13) (Awogbemi et al., 2022).

$$Crystallinity = \frac{Areaofcrystallinepeaks}{Areaofallpeaks} \times 100 \quad \text{.....Eq 3.13}$$

3.7.3. SEM Analysis

Scanning electron microscopy (FEIINSPECT-F50) was performed to observe the morphology and surface structure by scanning the ruptured surface of raw potato peel and alkali -treated potato peel. The effectiveness of the alkali treatment was evaluated through the surface morphology and structure (Rahman et al., 2017).

3.7. Hydrolysis

After the pretreatment of PPW hydrolysis was performed, in which polymeric carbohydrates are converted into sugar monomers. In this study, dilute sulfuric acid (acid hydrolysis) was used. Acid hydrolysis is an important chemical modification that can significantly change the structural and functional properties of starch without disrupting its granular morphology. A deep understanding of the effect of acid hydrolysis on starch structure and functionality is of great importance for starch scientific research and its industrial applications. During acid hydrolysis, amorphous regions are hydrolyzed preferentially. Starch is a polymer of glucose and contains amylase and amylopectin as building blocks. To release the free glucose, one has to open the

building blocks by different approaches. Glucose is slowly destroyed when heated in neutral solutions and the rate is accelerated by sulfuric acid.

In this research Pretreated potato peel (1:10, w/v ratio) was taken in 250 ml Erlenmeyer flasks and it was subjected to dilute acid scarification in the water bath at various acid concentration (%v/v) (5%, 10%, 15% and 20%), various time (30, 45, 60.and 75 min) and various temperature (35, 50, 65 and 80 ^{o}C) for maximum hydrolysis of starch to glucose (reducing sugar). After hydrolysis, the solid part was separated from the liquid by vacuum filtration unit to remove the non-fermentable component. One factor at a time was used to study the effect of these process parameters on glucose concentration.

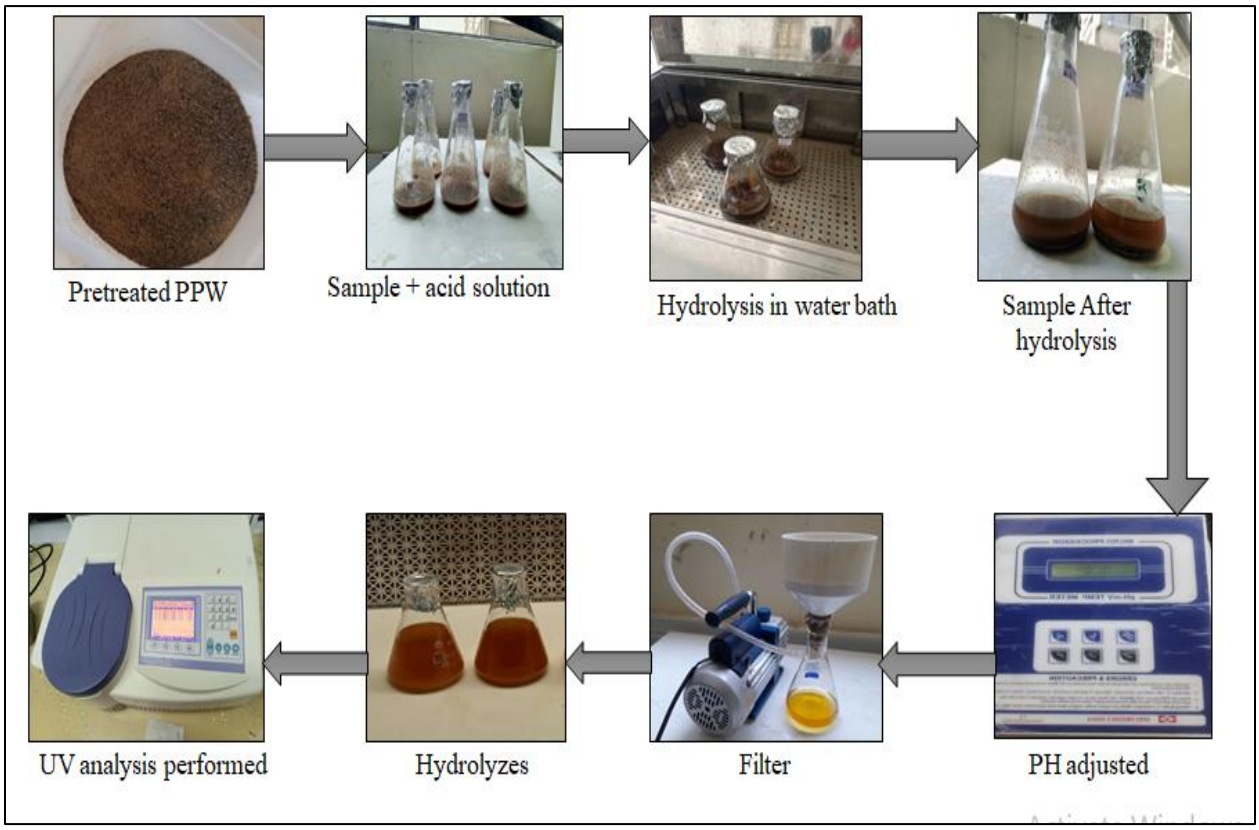


Figure3.4: Experimental procedure of hydrolysis process

3.8. Determination of glucose concentration

The concentration of glucose content of hydrolyzed, obtained from dilute acid hydrolysis was determined using a UV-VIS spectrophotometer by measuring absorbance vs. glucose concentration. The liquid fractions were analyzed using a UV-VIS spectrophotometer to

establish the glucose (reducing sugar) content by phenol sulfuric acid method. The total sugar concentration was determined by using UV-visible spectrophotometer at 490 nm wavelength of glucose absorbance and the quantification was made from calibration curve using glucose as standard and calculation was performed by equation of the linear regression obtained from calibration curve.

The basic principle of phenol sulfuric acid method is that carbohydrates when dehydrated by reaction with concentrated sulfuric acid produce furfural derivatives. Further reaction between furfural derivatives and phenol develops detectible color (yellow-gold or yellow-orange) compounds. The absorbance of these yellow to gold or yellow to orange compounds is measured with a spectrophotometer at a wavelength of 490 nm (absorption peaks of glucose, fructose and galactose is at 490 nm). This method is non-stoichiometric; therefore, it is necessary to prepare a Standard calibration curve using glucose as a standard.

Standard stock solution was prepared by dissolving 10 g of D-glucose in 100 ml of distilled water. Various dilutions of the stock glucose solutions were made separately by pipetting a known volume of the stock solution (0, 20, 40, 60, 80, and 100 ml) in to test tubes and add distilled water to make up to 100 ml as shown in the (Appendix B). Then 1 ml of the sample containing glucose standard was pipetted to each tube, and 1 ml of 5% phenol was added to all tubes and mixed. Then 5 ml of 96% concentrated sulfuric acid was added, simultaneously the tubes were shaken to effect fast and for complete mixing. For color development, the tubes were allowed to stand for 10 min and then placed in water bath (25 °C – 30 °C) to cool and display color. Then, light absorption at 490 nm was recorded on a spectrophotometer.

The total amount of reducing sugar found in our samples was determined from the prepared standard graph of glucose concentration and its absorbance.

$$y = mx + b \quad \text{Eq 3.14}$$

Where; Y = absorbance of unknown sample; m = is the slope; x = concentration and b is y-intercept

$$Con.of unknown sample = \frac{absorbance of unknown sample - y.\text{int}ercept}{slope} \ldots\ldots\ldots\ldots\ldots Eq 3.15$$

3.9. Detoxification of Acid Hydrolyses Of Potato Peel

Before the addition of any microorganism to the above prepared samples, pH of these samples has to be adjusted to avoid the decease of micro-organism in hyper acidic or basic state. The acid hydrolysate of potato peel was detoxified by adjusting sample pH to 4-6, using 0.5 N NaOH to remove the inhibitors generated during the acid hydrolysis. After the pH adjustment, the hydrolysate was vacuum filtered to remove the residue. Then, the filtrate, i.e., acid hydrolysate of potato peel, was used for fermentation as a carbon source.

3.10. Fermentation

The fermentation process was carried out in a shaker incubator, at 30 °C, with stirring at 150 rpm, for a 72 hrs. because this fermentation conditions are good for the production of bioethanol using Saccharomyces cerevisiae (Sumphanwanich et al., 2008).The prepared hydrolysate were adjusted to pH of 5.0, optimum for Saccharomyces cerevisiae using 1N sodium hydroxide solution.

Before conducting fermentation, the media for the preparation of yeast was prepared. In order to prepare the media the favorable condition for yeast growth must be established to supply the required amount of nutrients. 200 ml of production medium was prepared according to the requirements of Saccharomyces cerevisiae, containing 4 g dextrose, 2 g malt yeast extract, 4 g peptone, 1 g Urea; 1 g $MgSO_4.7\ H_2O$ and distilled water was added to make up to 200 ml and adjusts the pH to 5. This was autoclaved at 121 °C, for 15 min. After that, 1 g of yeast Saccharomyces cerevisiae was added to the above 200 ml media in a 500 ml conical flask. Then the conical flasks were properly covered with an aluminum foil (Bekele et al., 2015). Finally, the conical flask was placed in a shaker incubator for 24 hr, at a temperature of 30 °C and 180 rpm.

To eliminate the contaminant microorganisms, all the equipment's that were used for fermentation purposes were sterilized (autoclaved). The sterilization was carried out at a temperature of 121 °C for 15 min & at 1 bar.

The next step is fermentation to perform the fermentation, the prepared sterilized sample and media were mixed in the 500mL flasks with the ratio of 10 % (1% media with 10% sample) (Sumphanwanich et al., 2008). Then, it was placed on shaker incubator at a temperature of 30 °C and at 150 rpm for 72 hrs. In addition, after 72 hr of fermentation, the samples were take-out and distilled.

3.11. Distillation

Distillation is the method used to separate two liquid based on their different boiling points. In this study rotary evaporator set up was used at a temperature of 70 ºC for 1 hour on water bath. Water bath is used in order to control the temperature at set point. Then the concentration of ethanol was measure by UV-VIS spectroscopy. The determination of alcohol content found in the sample was carried out by spectrophotometer UV-visible quantitative analysis of alcohols using potassium dichromate reagent.

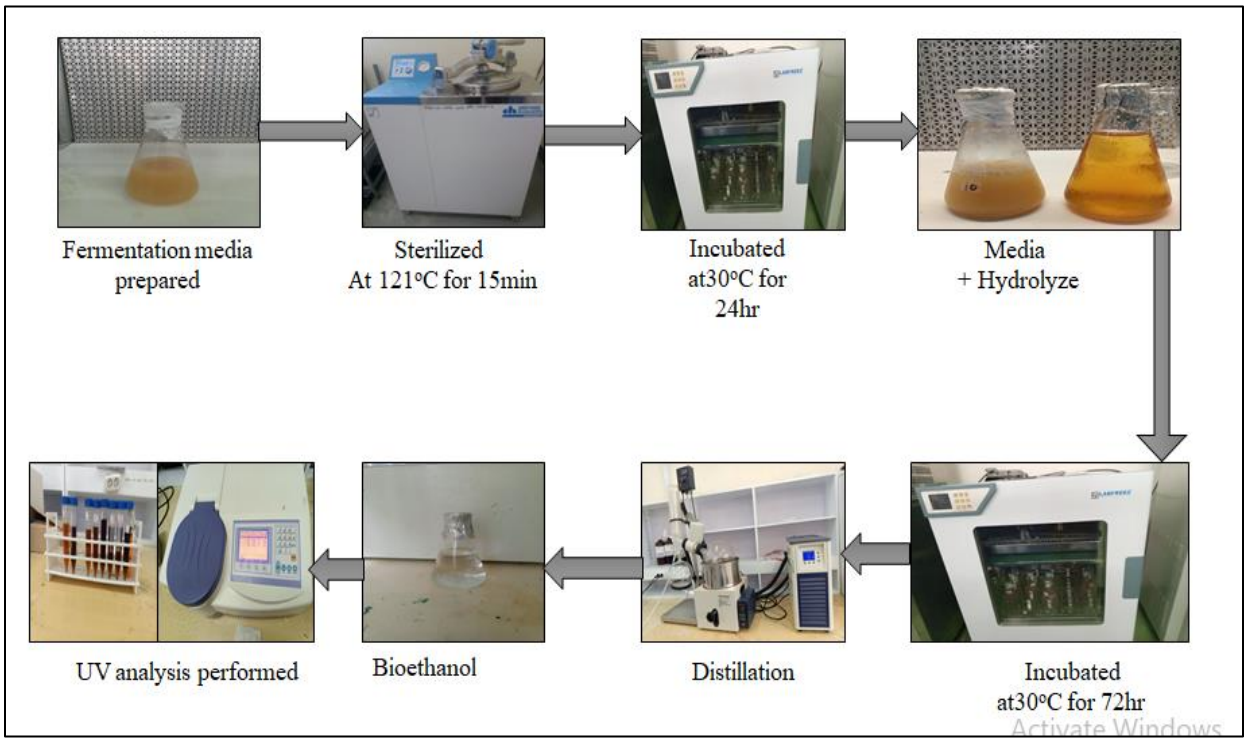


Figure3.5: Experimental procedure of fermentation process

3.12. Determination of bioethanol concentration

In this study, the determination of alcohol content found in our sample was carried out by spectrophotometer UV-visible quantitative analysis of alcohols using potassium dichromate reagent. First potassium dichromate reagent was prepared by dissolving 7 grams of potassium dichromate dissolved in 100 ml of distilled water in a 250 ml volumetric flask. Then 65 ml of concentrated H_2SO_4 was added drop wise (Tupe et al., 2018). Prepare standard ethanol solution by taking concentration from 1 to 3% (v/v) ethanol which is equal to 10,15,20,25 and 30 mg/ml. then linearity curve was plotted by taking the absorbance of the standards. To measure the absorbance 1 ml of the standard solution and 10 ml of dichromate reagent was mixed. Then the flask was incubated on a water bath at 60 ^{o}C for 20 min. The blank solution was prepared with distilled water. The absorbance of each concentration was measured at 580 nm using a UV-visible spectrophotometer (Pourkarim et al., 2021). Then 1 ml of each produced bioethanol sample collected from distillation was poured in test tubes. Then 10 ml of dichromate reagent was added. Then the mixture was allowed to stand for 20 min in water bath at 60 ^{o}C. Then the absorbance was measured at 580 nm using the UV-VIS spectrophotometer. Then the concentration of ethanol in the test sample was determined by UV from the linearity Curve plotted at 580 nm and the ethanol conversion yield was calculated as,

$$Ethanolyield(\%) = \frac{\max imumethandconcentration(mg/ml)}{utilisedglucose(mg/ml) \times 0.511mg/ml} \times 100\% \quad \text{.....Equation 3.16}$$

4. RESULTS AND DISCUSSIONS

In this section raw material and product analysis results are presented. Proximate and chemical composition of potato peel waste, amount of starch concentration in the raw material and treated sample, glucose concentration after hydrolysis and the amount of ethanol found in the samples was measured and the results are shown as follows.

4.1 Characterization of potato peel

4.1.1 Proximate analysis

Wet PPW had 78.5% moisture content. The proximate analysis (moisture content, ash content, volatile matter, and fixed carbon content) of potato peel waste on an air-dry basis is shown in Table 4.1 below.

Table4.1: Results of proximate analysis of potato peel

Proximate analysis	**%wt. dry basis This study**	**Previous works %wt. dry basis**	**Reference**
Moisture content	5.3 ±0.3	6.78	(Khawla et al., 2014)
Ash content	8.25 ±0.17	6	(Abebaw, 2020)
Volatile matter	78.08 ±0.7	60.85	(Aman et al., 2008)
Fixed carbon	8.35 ±0.15	15	(Aman et al., 2008)

Although there is a significant difference between the weight percentage of the sample and the standard weight, the results of this study are comparable to the values reported by the previously mentioned researchers. This difference may be due to variations in the source of the potato peel, handling techniques, or process equipment efficiency. The moisture content of a potato peel is a measurement of its water content. According to Abdul et al. (2014), moisture content analysis is used to determine the proportionality of the solid-to-liquid ratio in the pretreatment and hydrolysis techniques. As the moisture content increases, the product quality is impacted.

The moisture content of potato peel in this work was 5.3%, slightly lower than the previous work conducted by Khawla et al. (2014) which was 6.78 ± 0.22%, w/w. The ash content is the residue after the complete combustion process of carbon-free compounds, like minerals and inorganic salts in the fresh tuber, fertilizer use and can also come from soil and air contamination during processing. The low ash content of potato peel constituents could decrease sludge formation during bioethanol production. In this work, potato peel had an ash content of 8.25%, which is slightly higher than previously conducted work by Khawla et al. (2014), which is 7.2 ± 0.2%, w/w, Abebaw (2020) and Soni et al. (2023) which is 6% and lower than previous work conducted by Ben et al. (2019) which is 11%. Also, it contains a high amount of volatile matter (78.08%) and fixed carbon (FC) (8.35%). The carbon found in the material, which is left after volatile materials are driven off, is used for the determination of carbon in the potato peel. Having a high amount of carbon is important for the production of sugar because if the raw material has a high amount of carbon, it will produce a high amount of sugar.

4.1.2 Chemical composition analysis

The results of the chemical composition of raw potato peel are listed in the table below. The results from this study are in a comparable range with the values reported by the former researchers.

Table4.2: Chemical compositions of potato peel waste

Chemical composition analysis	**%wt. dry basis This study**	**Previous works %wt. dry basis**	**Reference**
Extractives	14.5 ±0.25		
Cellulose	9.03 ±0.15	5.69	(Chintagunta et al., 2016)
Starch	35.2 ±0.5	28.52	(Chintagunta et al., 2016)
Hemicellulose	12.62 ±0.1	15.2	(Soni et al., 2023)
Lignin	20.4 ±0.25	20	(Abebaw, 2020)

4.2. Pretreatment of potato peel

In this section, dried and milled potato peel waste was pretreated using the alkali pretreatment technique. Alkali pretreatment was chosen because of the high lignin content of the raw material used and because it is the best choice to remove the lignin content by rupturing the ester bonds that form cross links and distort the structure of the biomass. And also, alkaline pretreatment shows less sugar degradation and furan derivative formation (Subhedar & Gogate, 2014). As reported by Subhedar and Gogate (2014), 44.4% of delignification efficiency was obtained when pretreating newspaper containing 23.3% lignin using 1.5N NaOH. In another study conducted by Kininge and Gogate (2022), 48.09% of delignification efficiency was obtained when treating sugarcane bagasse using 1M NaOH.

4.3. Optimization of alkali pretreatment of potato peel

Alkali pretreatment can be affected by many factors, and for this study, alkali concentration, temperature, and hydrolysis time were chosen. In order to study the combined effect of these factors, experiments were performed for different combinations of these physical parameters using statistically designed experiments. The range and level of these factors are shown in Table 3.1. To accomplish this task, statistical analysis using Design Expert was carried out. The main tasks done here were selecting the model based on literature and then making an ANOVA analysis. After that, the effects of the variables were checked and plotted to see the interaction of the factors and effects by using a one-factor plot, a two-factor plot, a contour plot and a 3D surface plot. In addition to this, the optimal value of the factors was found in the optimization part of the software.

4.3.1. Statistical Analysis of the Experimental Results

4.3.1.1 Analysis of variance

The concentration of NaOH (%v/v), pretreatment temperature (^{o}C), and pretreatment time (hr) were the inputs for design experts, while delignification efficiency (%) is a dependent variable. The design expert program was used to develop the build information for the experimental analysis of the response delignification efficiency (%) process using the previously indicated parameters.

Table4.3: Optimization conditions for alkali pretreatment

Study type	Response surface
Initial point	Central composite design
Central point	6
Design model	Quadratic polynomial
Run	20
Block	No

Table4.4: CCD arrangement and response for delignification efficiency

Run	Factor1 A:Alkali concentration (%v/v)	Factor 2 B:Temperature (^{o}C)	Factor 3 C: Pretreatment time (hr)	Response Delignification efficiency (%)
1	3	100	3.5	76.2
2	5	30	1	33.8
3	3	65	3.5	75
4	3	65	3.5	76.1
5	1	30	6	31
6	3	65	3.5	72.3
7	5	100	6	76.87
8	3	30	3.5	37
9	3	65	3.5	75.4
10	1	100	1	46.25
11	3	65	1	73.03
12	1	65	3.5	57.31
13	3	65	3.5	75.5
14	3	65	6	76.5
15	1	30	1	21.5
16	5	65	3.5	75.98
17	3	65	3.5	75.54
18	1	100	6	60.78
19	5	30	6	41
20	5	100	1	71.2

The maximum delignification efficiency obtained in this study was at run number 7.

4.3.1.2 ANOVA analysis

The model chosen for this analysis was a quadratic model. In order to check whether the quadratic model is the right model or not, it was crucial to perform an analysis of variance (ANOVA). The probability and p-value were used to check the significance of each coefficient of the regression model equation. The p-value of the corresponding coefficient should be less than or equal to 0.05. This is the benchmark for checking the significance of the proposed model.

Table4.5: ANOVA for Quadratic model for alkali pretreatment

Source	Sum of Squares	df	Mean Square	F-value	P-value	
Model	6724.17	9	747.13	142.44	<0.0001	Significant
A-Alkali concentration	670.27	1	670.27	127.78	<0.0001	
B-Temperature	2784.23	1	2784.23	530.80	<0.0001	
C-pretreatment	161.85	1	161.85	30.85	0.0002	
AB	43.24	1	43.24	8.24	0.0166	
AC	15.96	1	15.96	3.04	0.1117	
BC	1.41	1	1.41	0.2690	0.6153	
A^2	195.45	1	195.45	37.26	0.0001	
B^2	938.69	1	938.69	178.96	<0.0001	
C^2	0.2651	1	0.2651	0.0505	0.8267	
Residual	52.45	10	5.25			
Lack of Fit	43.26	5	8.65	4.70	0.0573	Not significant
Pure Error	9.20	5	1.84			
Cor Total	6776.63	19				

The F value is calculated by dividing the mean square for a specific factor or interaction by the mean square error. It represents the ratio of the variance between groups (or factors) to the variance within groups (or errors). The F-value is used to test the null hypothesis that there are no significant differences between the group means. The F-value is often used in the context of factorial experiments or designed experiments where multiple factors are manipulated simultaneously to evaluate their effects on a response variable. The F-value helps assess the significance of each factor and determine if it has a significant impact on the response. A higher F-value indicates a higher likelihood of significant effects. Here, the model F-value of 142.44

implies the model is significant. There is only a 0.01% chance that a "model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case, A, B, C, AB, A^2 and B^2 are significant model terms. Values greater than 0.1 indicate the model terms are not significant. The Lack of Fit F-value of 4.7 implies that the Lack of Fit is insignificant relative to the pure error. There is a 5.89% chance that a "Lack of Fit F-value" this large could occur due to noise. A non-significant lack of fit is good.

Table4.6: Model adequacy measures

Std. Dev	2.29	R^2	0.9923
Mean	61.41	Adjusted R^2	0.9853
C.V %	3.73	Predict R^2	0.9358
		Adeq precision	36.1183

The predicted R^2 of 0.9358 is in reasonable agreement with the adjusted R^2 of 0.9853. i.e., the difference is less than 0.2. Adeq Precision measures the signal to disturbance ratio due to random error. A ratio greater than 4 is desirable. Here, a ratio of 36.12 indicates an adequate signal. Therefore, this model can be used to navigate the design space.

4.3.1.3. Development of the RSM models

The application of RSM gives an empirical relationship between the response function and the independent variables. The mathematical relationships between the response, delignification efficiency and the independent variables alkaline concentration (A), temperature (B) and time (C) in terms of coded and actual factors can be determined by Design Expert software.

Table4.7: Regression coefficients in terms of coded factors

Factor	Coefficient Estimate	Df	Standard Error	95% Cl Low	95% Cl High	VIF
Intercept	75.01	1	0.7873	73.26	76.77	
A-Alkali concentration	8.19	1	0.7242	6.57	9.80	1.0000
B-Temperature	16.69	1	0.7242	15.07	18.30	1.0000
C-pretreatment	4.02	1	0.7242	2.41	5.64	1.0000
AB	2.32	1	0.8097	0.5208	4.13	1.0000
AC	-1.41	1	0.8097	-3.22	0.3917	1.0000
BC	0.4200	1	0.8097	-1.38	2.22	1.0000
A^2	-8.43	1	1.38	-11.51	-5.35	1.82
B^2	-18.48	1	1.38	-21.55	-15.40	1.82
C^2	-0.3105	1	1.38	-3.39	2.77	1.82

Final equation in terms of coded factors:

$$\text{Delignification efficiency} = 75.01 + 8.19A + 16.69B + 4.02C + 2.32AB - 1.41AC + 0.42BC - 8.43A^2 - 18.48B^2 - 0.3105C^2$$

Where A is alkali concentration, B is temperature and C is pretreatment time

4.3.2. Graphical Analysis

4.3.2.1. Model Adequacy Check for delignification efficiency

It is necessary to assess the suitability of the underlying model before drawing any conclusions from the analysis of variance that was used. And by creating a normal probability plot of the residuals, the normality assumption may be verified. Creating a normal probability residuals plot is a very helpful process. To verify that the raw data is normal, a normal probability plot is created. Using the residuals makes the analysis of variance process often more efficient and simple. This figure will have a linear appearance if the underlying error distribution is normal. When visualizing the straight line, give the plot's central values more emphasis than its extremities. The normal probability plot in figure 4.1 indicates the residuals following a normal

distribution, in which case the points follow a straight line. This shows that the quadratic polynomial model satisfies the assumption of ANOVA.

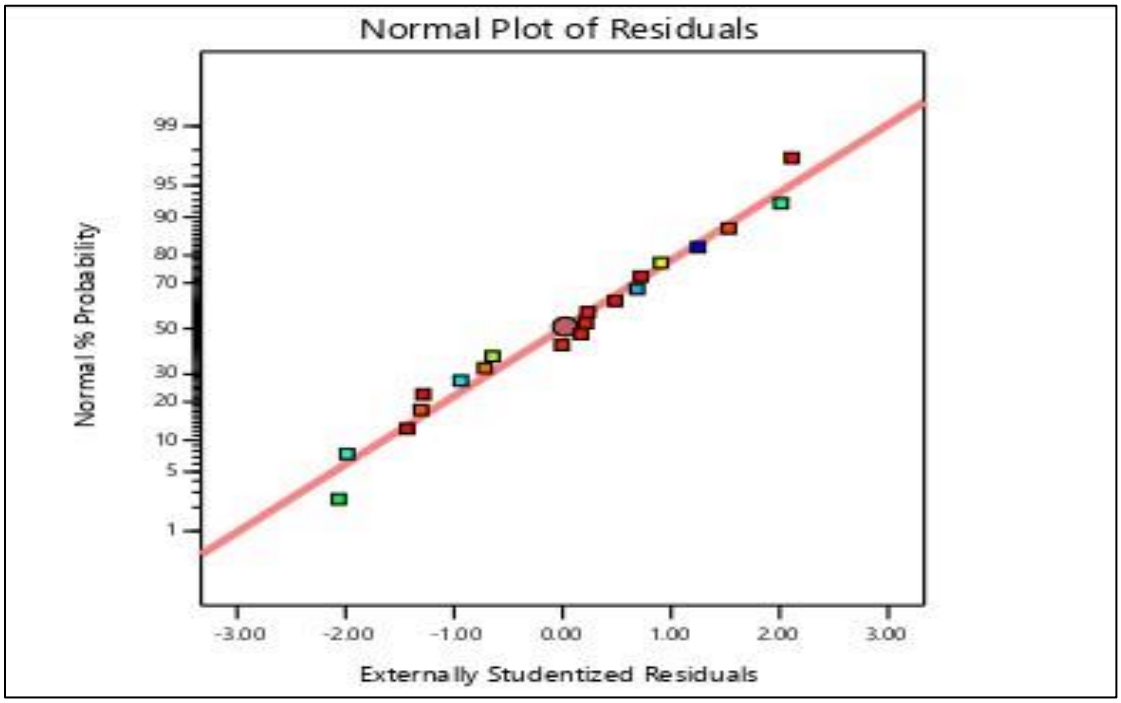


Figure4. 1: Normal plot of residuals for delignification efficiency

In addition to Figure 4.1, the predicted versus actual value of delignification efficiency was plotted in Figure 4.2. The plot shows how precisely the model is modeled, and the points show how the predicted value and actual value of each run approach the straight line. The straight line shows how the predicted and actual values are closer to each other. When the point is above the straight line, the predicted value is greater than the actual value, and the reverse is also true.

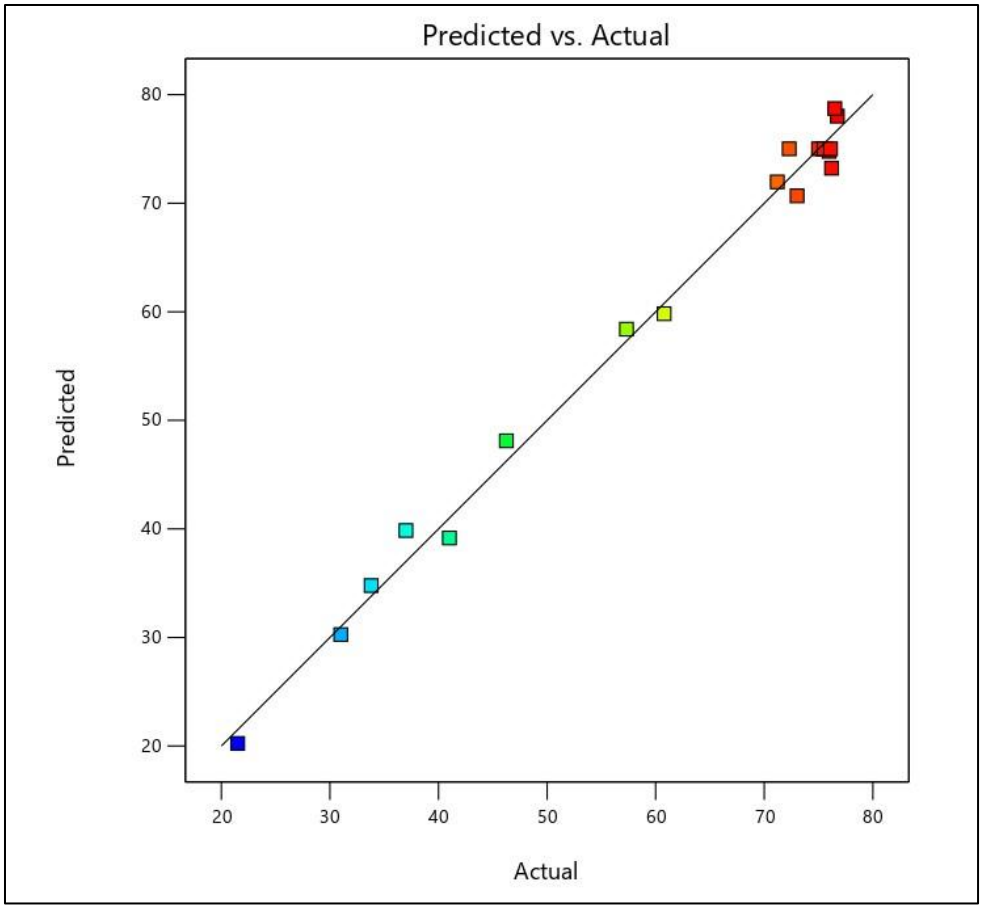


Figure4. 2: Actual vs. predicted graph

4.4. Individual effect of experimental variables on delignification efficiency

4.4.1. Effect of alkali concentration on delignification efficiency of potato peel

The effect of different alkali concentration on delignification of potato peel waste has been shown in Figure 4.3. The results from the present study showed that as alkali concentration increased, extent of delignification also increased. It has been observed that the extent of delignification increased with an increase in the alkali concentration indicating that higher alkalinity is favorable for the delignification.

As shown in the figure below significant increase in delignification was observed as alkali concentration was increased from 1-3%. In this range the delignification efficiency increased from 58.3967 to 75.4%. For alkali concentration change from 3 to 5% (v/v) delignification efficiency increased from 75.4-75.98%. The change in the extent of delignification was marginal for this range. This implies increasing of alkali concentration above 3% has negligible effect on the removal of lignin. Lignin degradation during alkaline pretreatment occurs due to the

breakage of aryl ether linkages which constitute approximately 50–70% of total linkages found in the lignin. Higher rate of delignification with an increase in alkali concentration can be attributed to the fact that hydroxyl ions catalyze the cleavage of ether linkages in the lignin and thus liberate the soluble sodium phenolates in the liquid. The breakage of these bonds increases the hydrophobicity of lignin in the solution. High alkalinity of the NaOH reagent causes solvation of hydroxyl groups in carbohydrates and creates the swelling effect in sugar residue (Subhedar & Gogate, 2014).

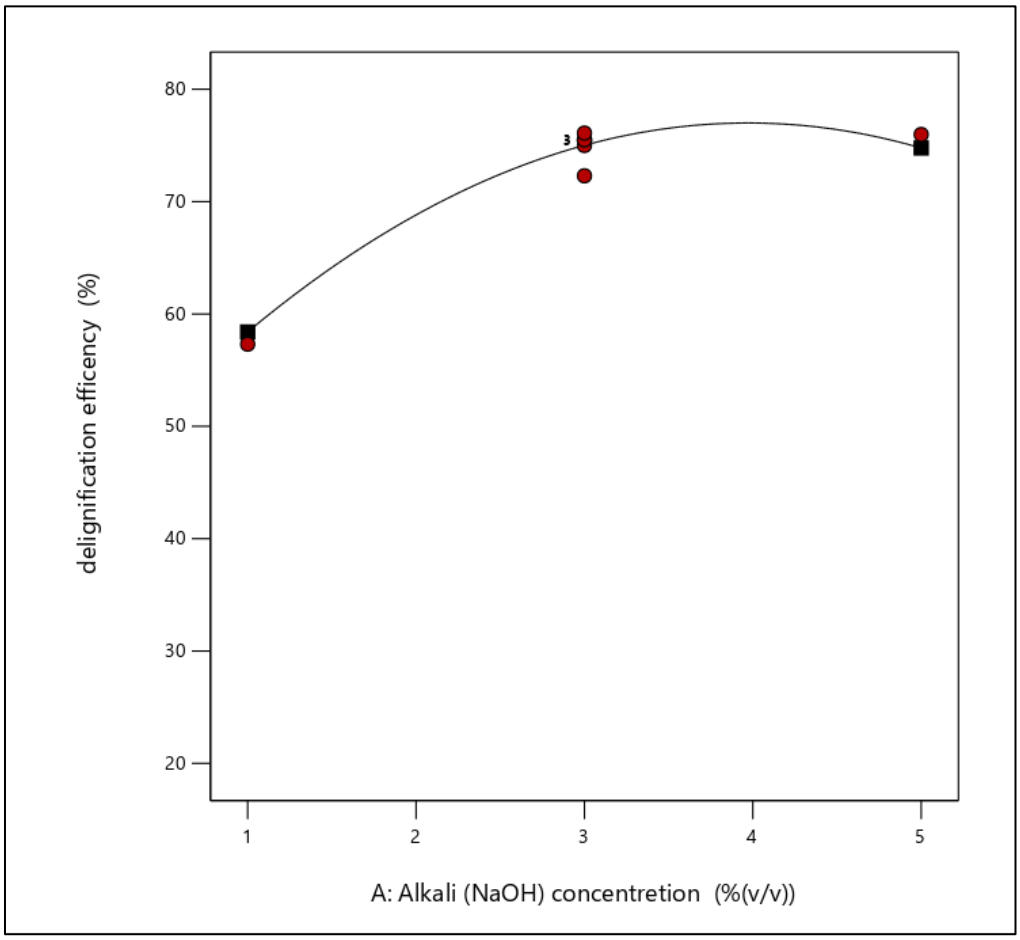


Figure4. 3: Effect of alkali concentration on the delignification efficiency

4.4.2. Effect of Temperature on delignification efficiency of potato peel

As shown in the figure below, the delignification efficiency increases from 37% to 76.1% at temperature values of 30–65 ^{o}C. However, in the range of 65-100 ^{o}C it increases from 74.54% to 76.2%. The change in the extent of delignification was marginal in this range. From this, it can be concluded that a further increase in temperature beyond 65 ^{o}C is not recommended since it

has an insignificant effect on lignin removal, and an increase in temperature also leads to the production of inhibitory chemicals and the degradation of carbohydrates, which ultimately affects the sugar yield.

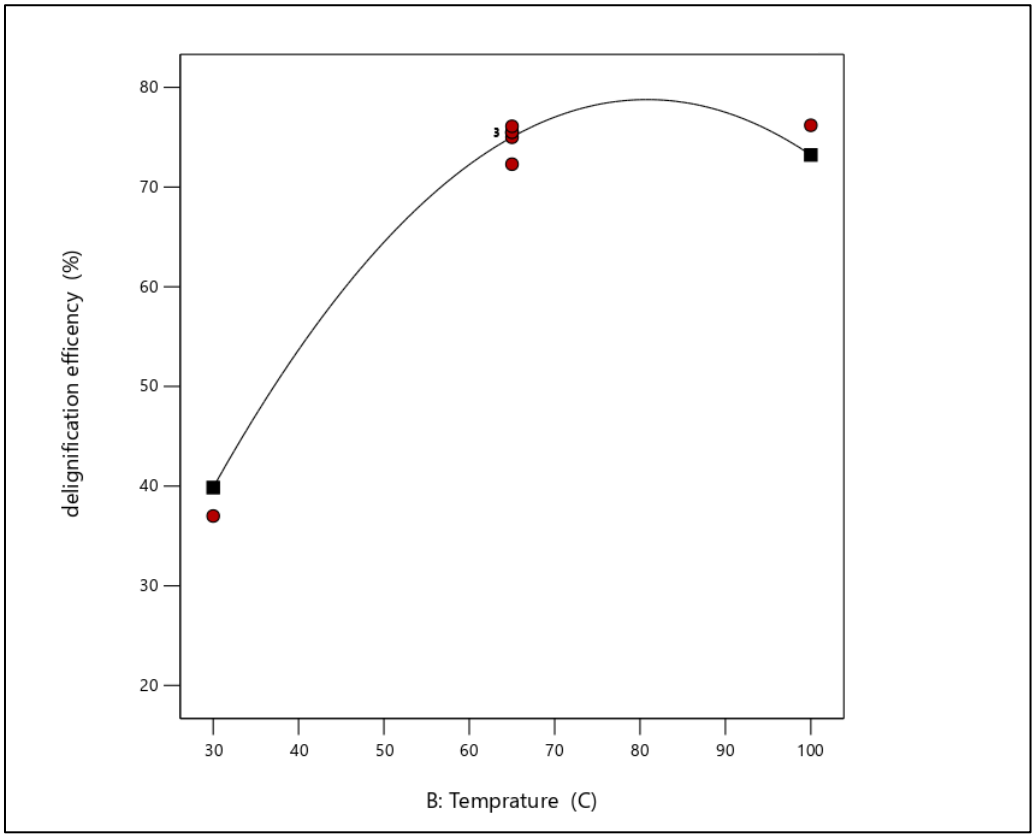


Figure4. 4: Effect of temperature on the delignification efficiency

4.4.3. Effect of pretreatment time on delignification efficiency of potato peel

The resulting plot of time versus lignin removal efficiency, when alkali concentration and temperature were actual factors, is depicted in Figure below. As shown in the plot, increasing time from 1 hour to 6 hours increases lignin removal efficiency from 73.03% to 76.5%, which indicates that time, has an insignificant effect on lignin removal from potato peel.

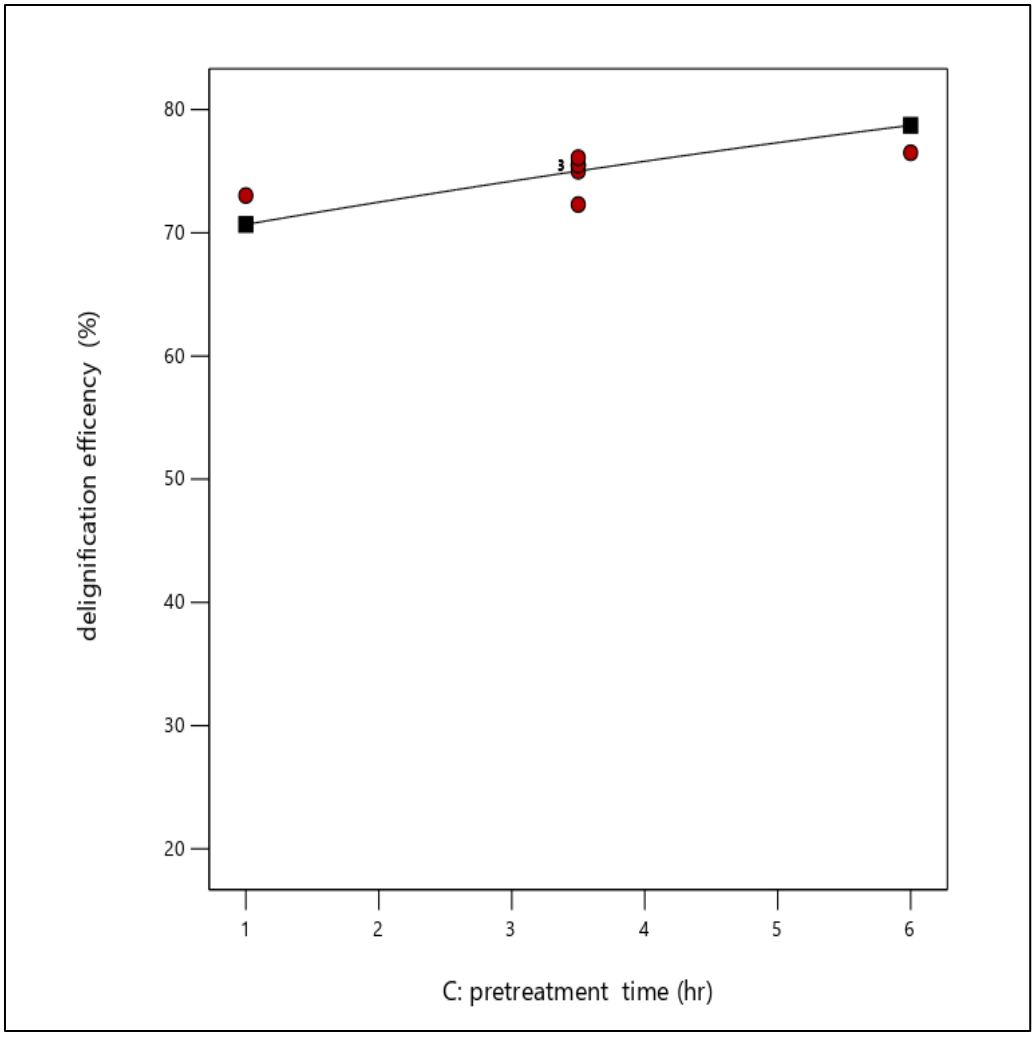


Figure4.5: Effect of time on the delignification efficiency

4.5. Interactive effects of process parameters on delignification efficiency

Interaction effects are effects that independent variables impose on one another. All controllable factors are obvious variables that affect the output of the response variable. The delignification efficiency from the various experimental runs is shown in Table 4.4. Delignification efficiency ranged from 21.5% to 76.73%.

4.5.1. The effect of alkali concentration and temperature on delignification efficiency of PPW

Figure 4.6 shows the effect of alkali concentration and temperature on the delignification efficiency when time was at the center point (3.5 hr). As observed from the model equation 4.1, the alkali concentration and temperature had a positive interaction effect on the delignification

efficiency. As temperature and alkali concentration increase from their lowest values to medium, the lignin removal efficiency increases sharply. After reaching their center point, the %lignin removal efficiency shows insignificant change. Maximum lignin removal efficiency (75.98%) was obtained at their maxima values of 100 ^{o}C and 5%, respectively.

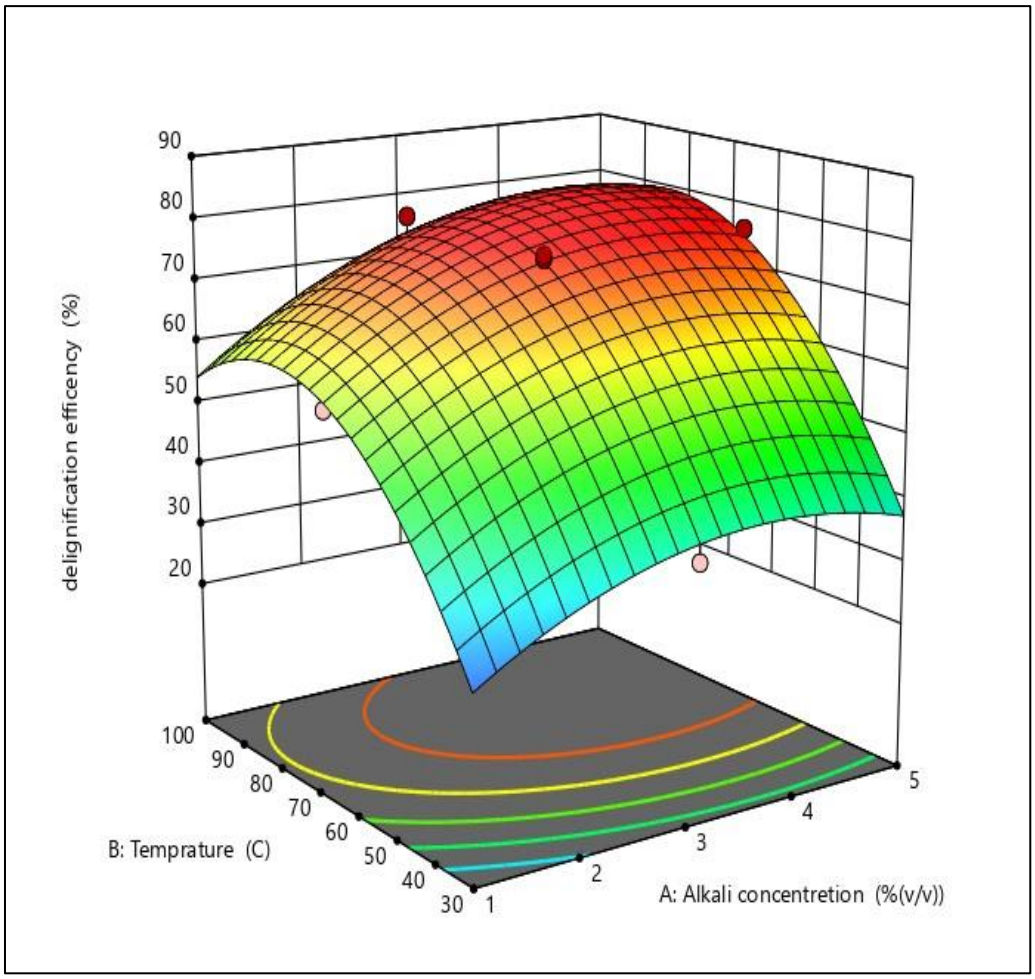


Figure4. 6: 3D plot of alkali concentration and temperature effect on %DL

4.5.2. Effect of alkali concentration and time on delignification efficiency of PPW

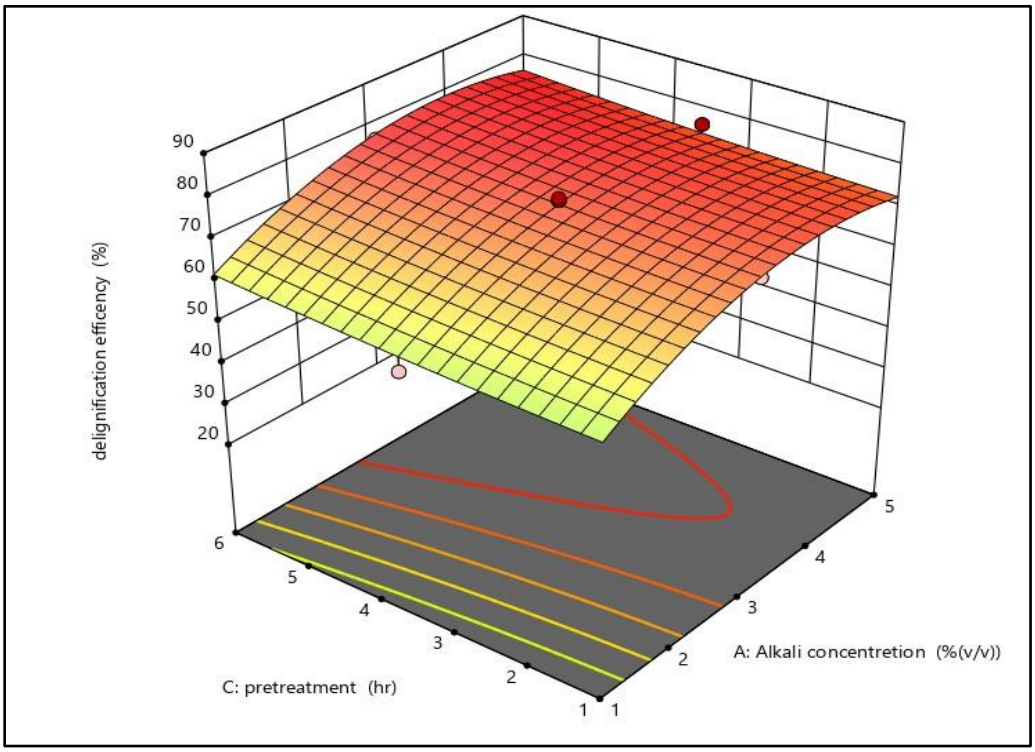


Figure4. 7: 3D plot of alkali concentration and pretreatment time effect on %DL

The graph showed above shows the effect of alkali concentration and pretreatment time on delignification when the temperature kept at 65 °C. When the alkali concentration increases for both high level and low level time the % lignin removal increases. When alkali concentration was at low (5%) and, time was also low (1hr), the amount of % lignin removal was 52.65%. as it can be seen from the graph the rate of increment beyond the center point is very low.

4.5.3. Effect of temperature and pretreatment time on delignification efficiency of PPW

The effect of pretreatment temperature and time on lignin removal at a constant alkali concentration (3%) is shown in Figure (4.8) below. The figure depicts that maximum lignin removal was obtained at a higher temperature (100 ^{o}C) and at the center point of time (3.5 hr). The % lignin removal change from the midpoint of temperature and time to their higher limit is marginal; there is no significant change as it can be observed from the 3D plot of temperature and time interaction effects on delignification efficiency.

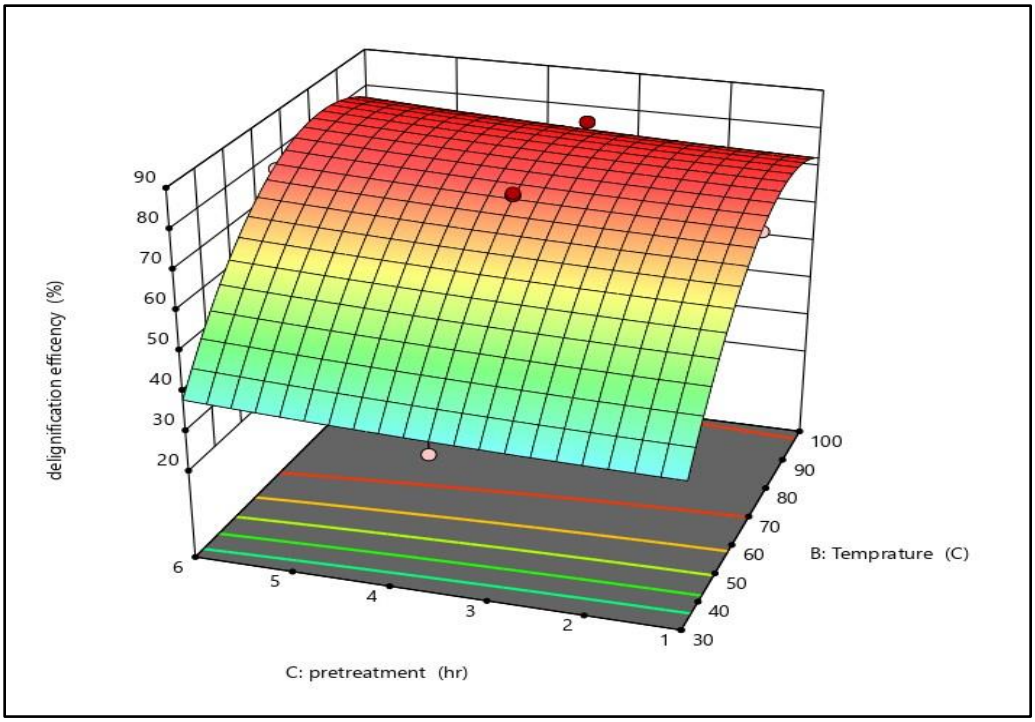


Figure4.8: 3D plot of temperature and pretreatment time effect on %DL

4.6. Optimization of operating process variables in pretreatment of potato peel

One of the primary objectives of the present study was to find the optimum process parameters for maximizing delignification efficiency. The process variables alkali concentration, time and temperature have been optimized using central composite design and their output value is executed using Design-Expert software 13. In the optimizing process, alkali concentration, time and temperature are a set of process parameters that should be "in range," while the delignification efficiency needs to be "maximized." Numerical optimization was used to identify the optimum level of pretreatment process factors. The criteria for %lignin removal from potato peels are summarized as follows in the table below.

Table4. 8: Constraints applied for optimization

Name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
A: alkali concentration	Is in range	1	5	1	1	3
B:temperature	Is in range	30	100	1	1	3
C: time	Is in range	1	6	1	1	3
Delignification efficiency	Maximize	21.5	76.73	1	1	3

According to the experimental work in Table (4.4), the maximum delignification efficiency 76.87%, was obtained at the interaction parameters of alkali concentration (5%), pretreatment temperature (100 ℃), and time (6 hr). From the response optimization technique used in this study, the delignification efficiency was optimized to 78.4%, at a desirable parameter interaction of alkali concentration (3.3% v/v), pretreatment temperature (70 °C), and reaction time (3.5 hr). From the 45 possible solutions of optimization, the table below shows the optimized solution at 1desirability.

Table4.9: Optimized solution of % lignin removal from potato peel waste

No	Alkali (NaOH) concentration %V/V	Temperature (^{o}C)	Time (hr)	Delignification efficiency (%)	Desirability	
1	3.3	70	3.5	78.4	1.00	**Selected**

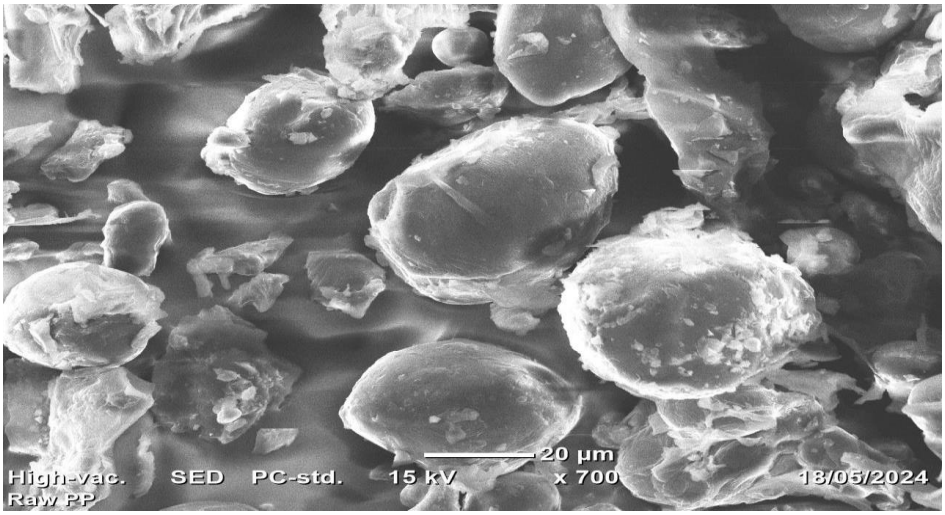
4.6.1. Model validation

In order to validate the adequacy of the model, experiments were carried out at the predicted optimum conditions, and an average delignification efficiency of 77.82 ±2.1% was obtained; this was in good agreement with the predicted one. Therefore, the model is considered to be accurate and reliable. At these optimum parameters, starch recovery and cellulose recovery were calculated and found to be 80.3 ±1.2% and 89.7± 1.5% recovery, respectively.

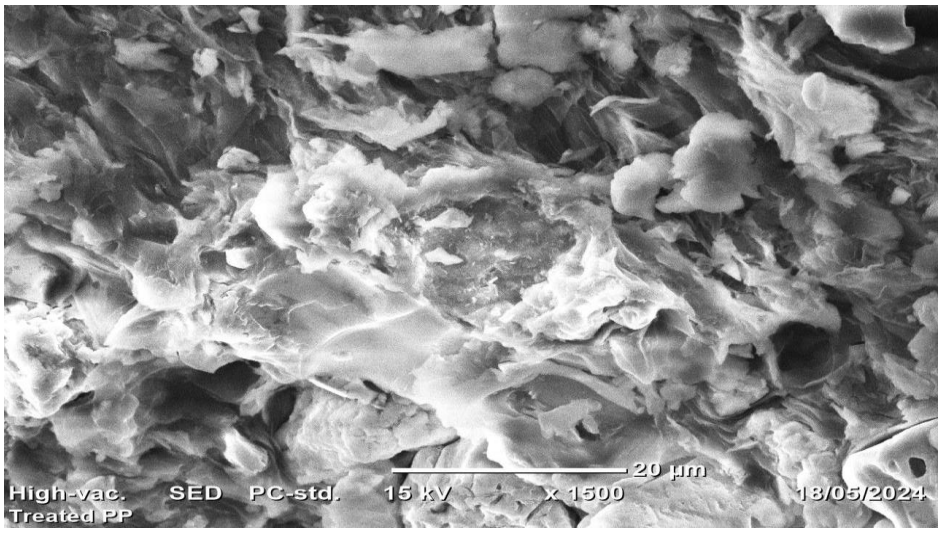
4.7 Characterization of Raw and Alkali Pretreated Potato Peel at Optimum Parameters

4.7.1. SEM

The morphological variations and deformations in PPW after alkali treatment were clearly revealed by SEM, as shown in Figure 4.9. The alkali reaction destroyed the structural bonding by creating some irregular pores and cracks. Electron micrographs depicted a significant difference between the surface structures of the raw and treated samples. In Figure 4.9A, the surface of raw PPW was rough, regular, and crusted with some contaminants, such as extractives, pectin, and waxy elements. However, after the alkaline process, the surface morphology of PPW appears relatively clean, porous, with cavity formation, amorphous and cracked, as shown in Figure 4.9B. The surface of alkaline-treated PPW appeared cleaner than raw PPW; this may be because the impurities and extractives from the fibers were partially diminished. Hemicellulose is disintegrated and becomes water-soluble due to the alkaline treatment process. Similar findings and observations have been reported in the study by Osman et al. (2019). They have confirmed the production of deformations, porosity, cracks and crevices in the SEM images of nanoparticle-pretreated (NAAP) PPW. Ghazanfar et al. (2022) have also reported a disrupted and perforated biomass structure after NaOH treatment using SEM images.



A



B

Figure4.9: Scanning electron micrographs of PPW (A) Raw PP (B) Alkali treated PPW

4.7.2 FTIR

In this study, as mentioned in the materials and methods part, the Fourier transform infrared (FTIR) spectroscopy technique was carried out to identify the functional groups of raw potato peel and to test chemical changes that occurred during the chemical treatments on raw potato peel. The FTIR spectra of alkali treated PPW ranged from 4000 to 500 cm^{-1}.

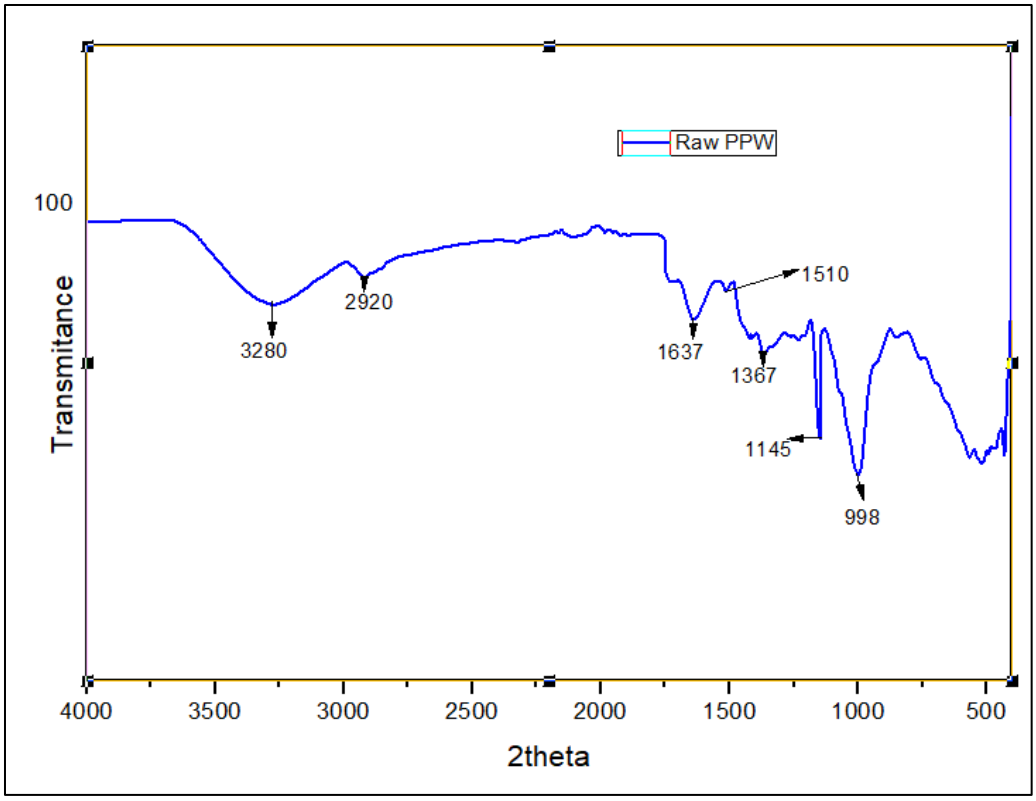


Figure4.10: FTIR of raw and alkali treated PPW

In the region 3500–3000 cm^{-1}, spectra of raw PPW show a strong band which can be attributed to the OH group from the intermolecular hydrogen bonds of phenols and alcohols in lignin and cellulose. The bands around 2920cm^{-1} represent C–H stretching vibrations consist in cellulose components, interrelating to the lignin molecules (Zhang et al., 2018).

The band at 1637 cm^{-1} represents the C = C stretching vibration of alkene. The vibration band at 1510 cm^{-1} corresponds to C=C of aromatic rings of lignin (Silva et al., 2020). The peaks at 1367 cm^{-1} revealed C–H deformation in various polymers such as starch, lignin and cellulose. Additionally, the vibration bands observed at 1145 and 998cm^{-1} attributed to C–O–C elongation and C–O elongation respectively it can be related to the glycosidic linkages of cellulose and hemicellulose (Ellerbrock & Gerke, 2021).

FTIR of raw and treated PPW

Both raw and pretreated PP spectra revealed a band between 3000 and 3300 cm^{-1} , which confirmed the presence of a free and bounded -OH stretching vibration derived from degraded polymers such starch, cellulose and lignin. Other researchers Mushtaq et al. (2023) have also reported the same results.

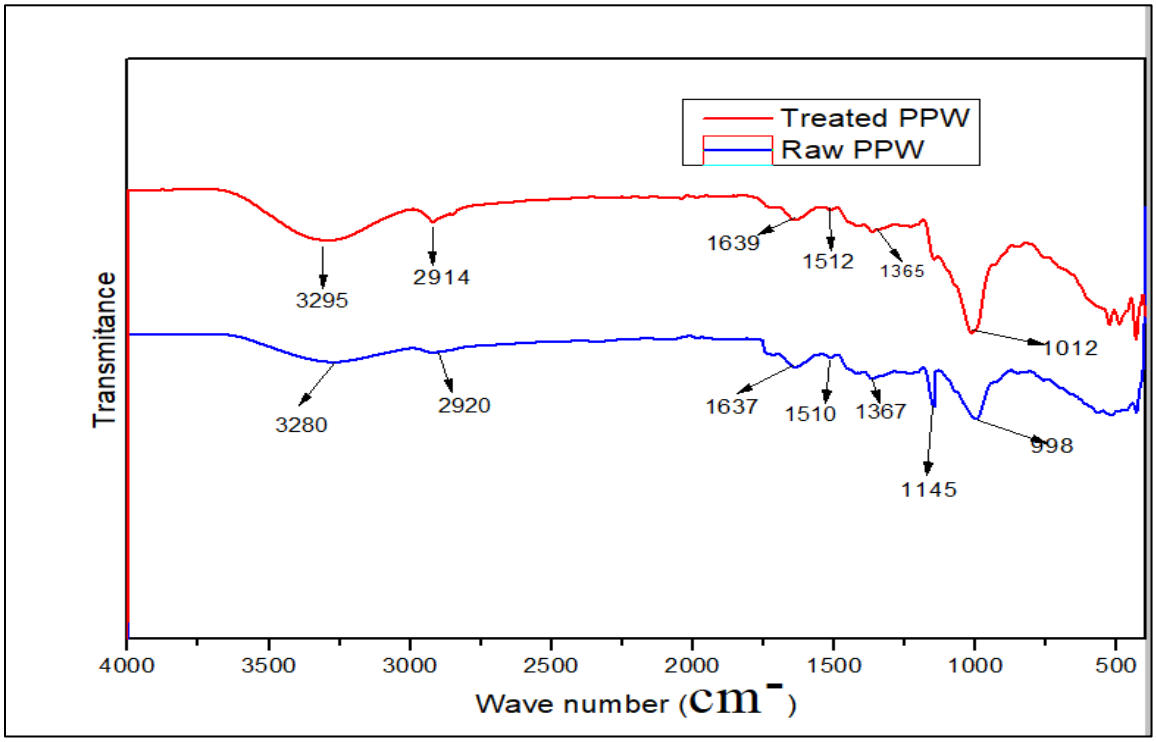


Figure4. 11: FTIR of raw and treated PPW

It was observed that the peak at 2920 cm^{-1}, which is found on the raw PPW is assigned to the indication of C – H stretching vibrations consist in cellulose components, interrelating to the lignin molecules whereas the shifting of this peak to (2914 cm^{-1}) in alkali treated PPW implies that the structure of the side chain in lignin was changed (Mushtaq et al., 2023) .

The shifting of the peak 1510 in raw PPW to 1512 cm^{-1} in the FTIR spectrum of alkali-treated potato peel waste (PPW) indicates that lignin was partly destroyed. This could be indicative of changes in the aromatic skeletal vibrations of lignin due to the alkali treatment. Zhang et al.(2018) also observe the same thing.

The band at 1145 cm^{-1} present in raw PPW was completely removed after alkali pretreatment which indicates the removal of hemicellulose .The band at 1012cm^{-1} in treated PPW depicted the deformation of various compounds such as polysaccharides and aromatic and halogen compo.

4.7.3. XRD

A wide peak around 2θ=17.06° was observed for raw PPW indicating the presence of cellulose in crystalline form(Kumar Soni et al., 2023b). A drop at this peak has been noticed at alkaline treated PPW indicating that cellulose transformed into a more amorphous form or it was broken down into its oligomeric form. A peak around 2θ =22^{o} was observed for all both samples indicating the presence of cellulose in crystalline form. A drop and slight shift at this peak observed after treatment indicating cellulose transformed into amorphous form as it was broken down into oligomers. The peak at around 31.86 probably corresponds to crystalline salts related to chemical neutralization of the sample.

The influence of pretreatment methods on crystallinity of potato peel biomass was calculated from XRD data. The crystallinity index (CrI) of biomass is the quantitative indication of its crystallinity which hinders the action of enzyme and acid in hydrolysis. The CrI of untreated potato peel was found out to be 19.9%, while the CrI of NaOH (3%) treated potato peel was found out to be 11.9%. This decrease in CrI may be due the breakdown of inter and intra hydrogen bonding in the crystalline. This result is compatible with previse works conducted by (Barampouti et al., 2023) and (Kumar Soni et al., 2023b)

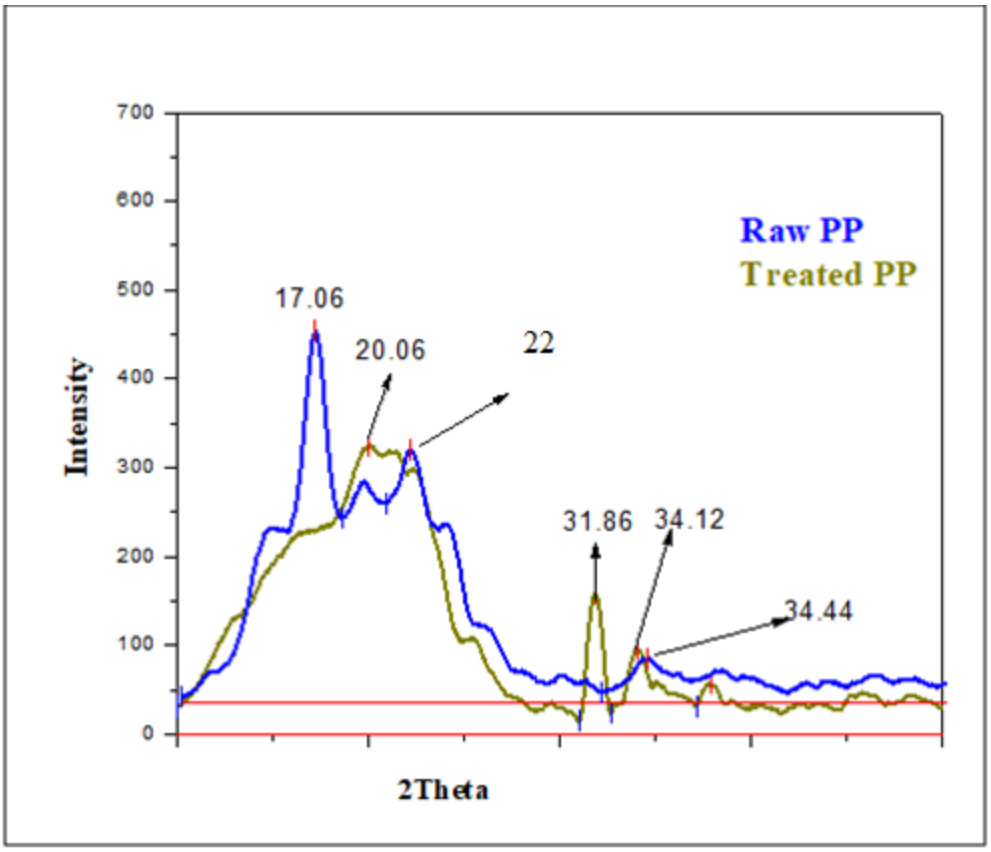


Figure4. 12: XRD of raw and alkali treated PPW

4.8. Acidic hydrolysis of pretreated potato peel

Acid hydrolysis is an important chemical modification that can significantly change the structure and functional properties of pretreated potato peel without disrupting its granular morphology. A deep understanding of the effect of acid hydrolysis on starch structure and functionality is important. During acidic hydrolysis amorphous regions are hydrolyzed preferentially. Sulfuric acid was used to hydrolyze the potato peel obtained after alkaline pretreatment. In this study, the total reduced sugar content after the dilute acid hydrolysis process was investigated. To determine the total amount of glucose concentration obtained from alkali treated potato peel, standard glucose solution was prepared and phenol sulfuric acid method was used. The results of glucose concentration and its absorbance, and the result of the glucose calibration curve are shown below.

Table4.10: Glucose concentration and its absorbance

Glucose concentration(mg/ml)	Absorbance
20	1.256
40	1.613
60	2.067
80	2.872
100	3.56

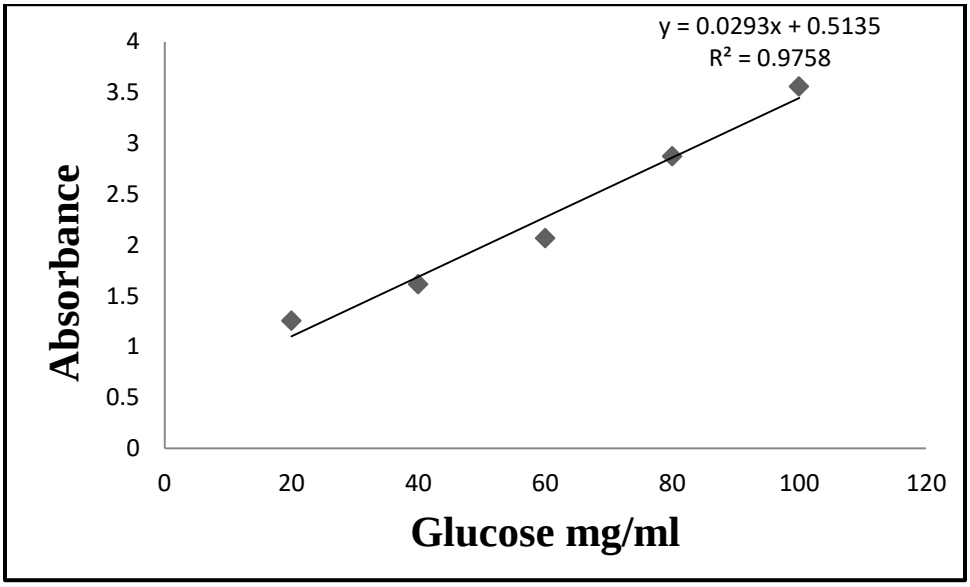


Figure4. 13: Glucose standard curve

The concentrations of unknown samples were determined from a standard curve of glucose

$(y = 0.0293x + 0.5135)$ …….Eq4.1

$R^2 = 0.9758$

Effect of each parameter on glucose concentration was studied using the above mentioned ranges.

4.8.1. The effect of acid concentration on glucose concentration

The effect of acid concentration on glucose concentration can vary depending on the specific experimental conditions and the type of acid used. Generally, when an acid is used to hydrolyze a polysaccharide (such as starch) into its constituent glucose molecules, the acid concentration can influence the rate and extent of the hydrolysis reaction.

In the context of hydrolyzing starch to obtain glucose, an increase in acid concentration typically leads to an increase in the rate of hydrolysis. Higher acid concentrations provide more protons (H^+) in the solution, which can catalyze the hydrolysis reaction by breaking the glycoside bonds in the starch molecules. As a result, a higher concentration of glucose is produced over a given period of time. However, it's important to note that excessively high acid concentrations can have adverse effects. Very high acid concentrations can promote side reactions or degradation of glucose, leading to lower overall glucose concentrations. In this study effect of H_2SO_4 concentration (5%, 10%, 15%, and 20%) were studied by performing the hydrolysis reaction at 65 ^oC for 60 min in a water bath. The results obtained are listed below unknown glucose concentration was calculated using the standard curve equation 4.1.

Table4.11: Effect of H_2SO_4 concentration on glucose concentration

No	**H_2SO_4 concentration**		**Absorbance @ 490 nm**	**Concentration mg/ml**
1	5%	**Boiling at 65 ^oC for 60 min**	1.481	33.02
2	10%		1.856	45.8
3	15%		1.875	46.46
4	20%		1.743	41.96

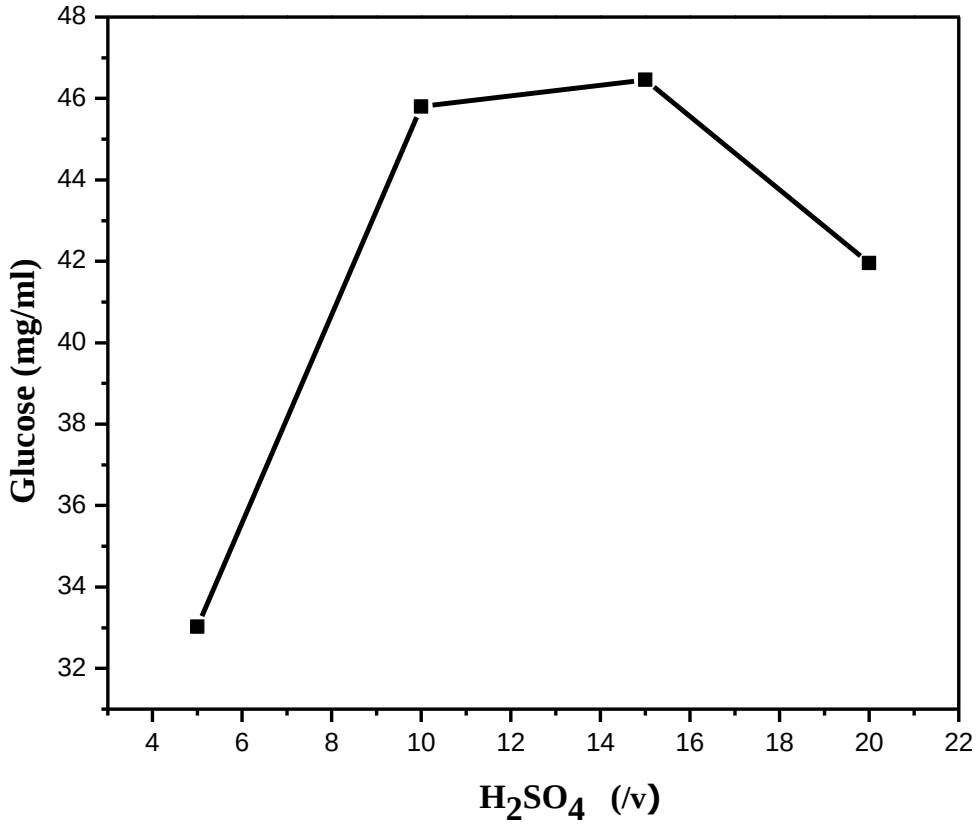


Figure4.14: Effect of H_2SO_4 on glucose concentration

As it can be observed from the above graph of acid concentration VS glucose concentration graph. The glucose concentration of the sample increased from 33.02mg/ml to 45.8mg/ml when the acid concentration increased from 5% to 10%. And when the acid concentration is increased to 15% the glucose concentration was 46.46mg/ml, however, further increased in acid concentration from 15% to 20% results in decreasing of glucose concentration to 41.96mg/ml. These might be due to the possible formation of other molecules or due to the degradation of glucose molecules to byproducts such as 5-hydroxymethylfurfural (5- 61 HMF).therefore the chosen optimum sulfuric acid for hydrolysis was 15%.

4.8.2. Effect of hydrolysis time on glucose concentration

Hydrolysis involves breaking down a complex substrate (e.g., polysaccharides) into its constituent glucose molecules. The duration of hydrolysis can influence the extent of substrate conversion to glucose and, consequently, the glucose concentration. Generally, longer hydrolysis times provide more opportunity for the acid to break down the substrate into glucose molecules, resulting in higher glucose concentrations. However, excessively long hydrolysis times can lead to side reactions or the degradation of glucose, causing a decrease in glucose concentration. In this study, the effects of hydrolysis time (30, 45, 60, and 75%) were studied by performing the hydrolysis reaction at 65 ^{o}C using 15% sulfuric acid in a water bath. The results obtained are listed below, and the unknown glucose concentration was calculated using the standard curve equation 4.1.

Table4.12: Effect of time on glucose concentration

N0	**Time (min)**		**Absorbance @ 490 nm**	**Concentration mg/ml**
1	30	**15% H_2SO_4**	1.268	25.75
2	45	**Boiling at 65^{o}C**	1.585	36.569
3	60		1.856	46.46
4	75		1.773	42.896

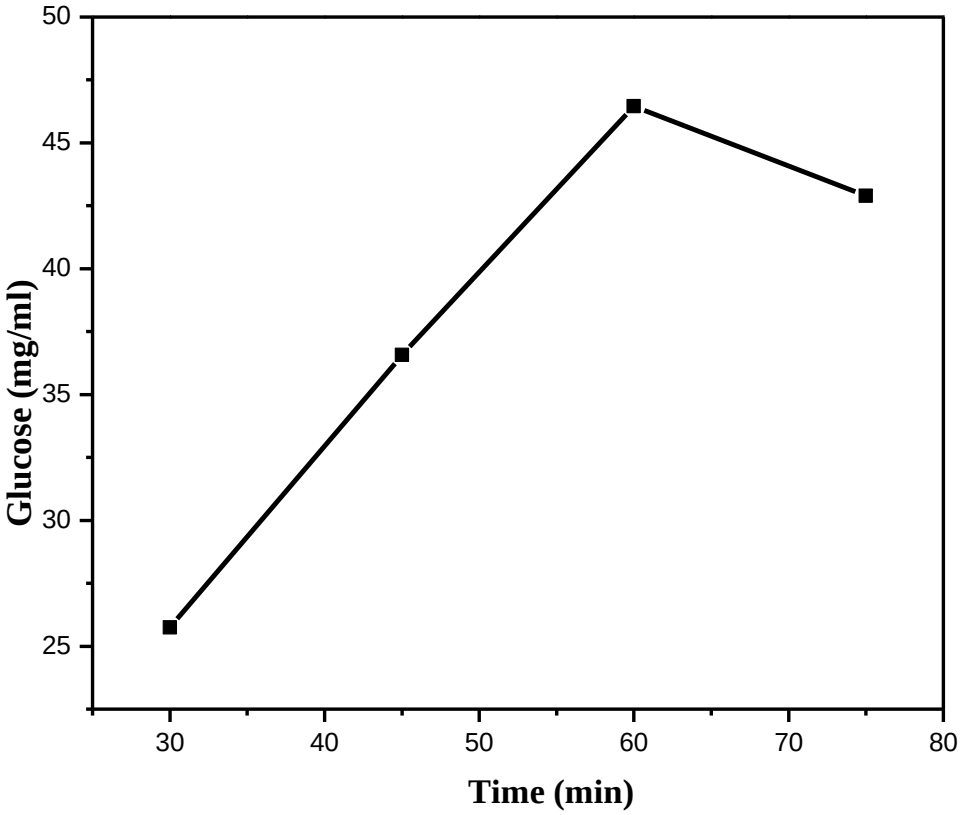


Figure4.15: Effect of pretreatment time on glucose concentrate

The resulting plot of hydrolysis time versus the glucose concentration, when acid concentration and hydrolysis temperature were constant at 15% and 65 ^{o}C was depicted in Figure 4.15 above. As revealed from the plot increasing hydrolysis time from 30 to 75 min, glucose concentration was increased from (25.75 to 46.46 mg/mL). However, further increase in hydrolysis time from 60 to 75 min can decrease the glucose concentration to (42.896mg/mL). This was due to the degradation of obtained glucose molecules and produce byproduct 5-hydroxymethylfurfural, furfural and formic acid due to long reaction time.

4.8.3. Effect of hydrolysis temperature on glucose concentration

The pretreated PPW was further treated with a local optimum of acid concentration (15%) and local optimum hydrolysis time (60 min) at various heating temperatures (35, 50, 65 and 80^{o}C). The resultant glucose concentration is determined from the glucose standard curve and shown in the Table (4.13) below:

Table4.13: Effect of temperature on glucose concentration

No	Temperature		Absorbance @490nm	Glucose concentration (mg/ml)
1	35	**15% H_2SO_4 For 60min**	1.669	39.436
2	50		1.786	43.43
3	65		1.856	46.5
4	80		1.71	40.83

From the figure below it can be observed that the concentration of glucose increases from 39.436 mg/ml to 46.5 mg/ml when the temperature increases from 35 ^{o}C to 65 ^{o}C. Further increase in temperature to 80 ^{o}C results lower glucose concentration.

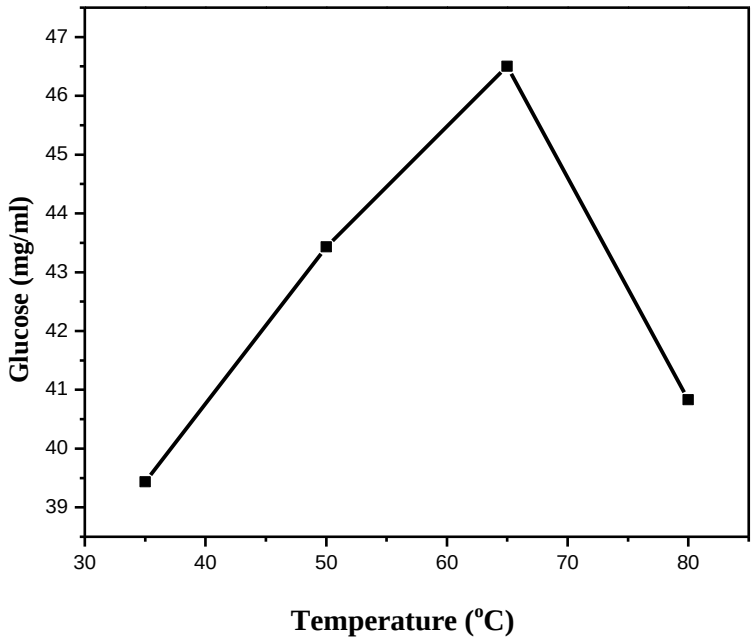


Figure 4. 16: Effect of temperature on glucose concentration

4.8.4 Glucose concentration of raw PPW

Absorbance of raw PPW hydrolysate obtained by hydrolyzing raw PPW using 15% H_2SO_4, at 65^{o}C for 60 min was measured and found to be 1.39 and using the above equation, its glucose concentration was 29.9 mg/ml.

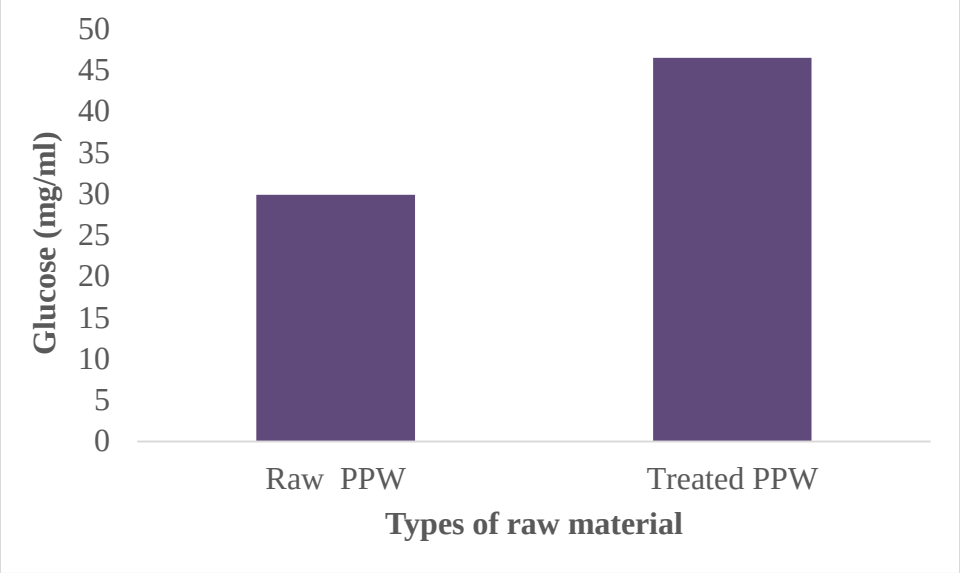


Figure4.17: Glucose concentration of raw and NaOH treated PPW

4.9. Fermentation of glucose to bioethanol

4.9.1. Determination of bioethanol concentration

Hydrolysates obtained by acidic hydrolysis, were subjected to ethanol fermentation by a 24 hr old culture of Saccharomyces cereviciae var. anaerobic agitated conditions (pH 5, 30 ^{o}C, 150 rpm) in a 250 ml Erlenmeyer flask. Fermentation was carried out for 3 days. To determine the total amount of bioethanol concentration found in the fermented sample standard bioethanol stock solution was prepared and acidified potassium dichromate method were used. The results of ethanol concentration and its absorbance, and the result of the ethanol calibration curve are shown in Appendix D. The ethanol concentration obtained from this study was 21.04mg/ml. compering with the former researchers (Sivasakthivelan et al., 2014) obtained 11.46 g/l of bioethanol concentration when performing the fermentation of Sunflower head waste at pH 5.5, temperature of 30 ^{o}C and 48hrs. (Barampouti et al., 2023) obtained 23.75±2.49 g/l of bioethanol concentration when PPW was pretreated with 1% w/v NaOH for 6 hr at 50 °C and fermented for 24hrs at 30 ^{o}C using Saccharomyces cereviciae and also (Arapoglou et al., 2010) performed

fermentation of acidic hydrolyzed PPW on conditions (pH 5, 32 ^{o}C, 100 rpm, 48 hr) and obtained 6.97 g/l bioethanol concentration. Compared to the mentioned literature, the ethanol concentration in this study was comparable. Ethanol yield of this study was calculated using Equation 3.14 and 88.5% of ethanol was obtained.

4.9.2. Characterization of produced bioethanol using FTIR

Alcohols have characteristic IR absorptions associated with the O-H, C-O and the C-H stretching vibrations. When run like a liquid film, the region 3500-3200 cm^{-1} with a very intense and broadband indicated the O-H stretch of alcohols, while the region 1260-1050 cm^{-1} confirms the C–O stretch. The bands at around 2880 and 2930 cm^{-1} were assigned as the symmetric stretching modes of the $–CH_2$ and$–CH_3$ groups, respectively. This assures that the product obtained from alkali pretreated PPW was ethanol due to the confirmation of these regions, as shown below :

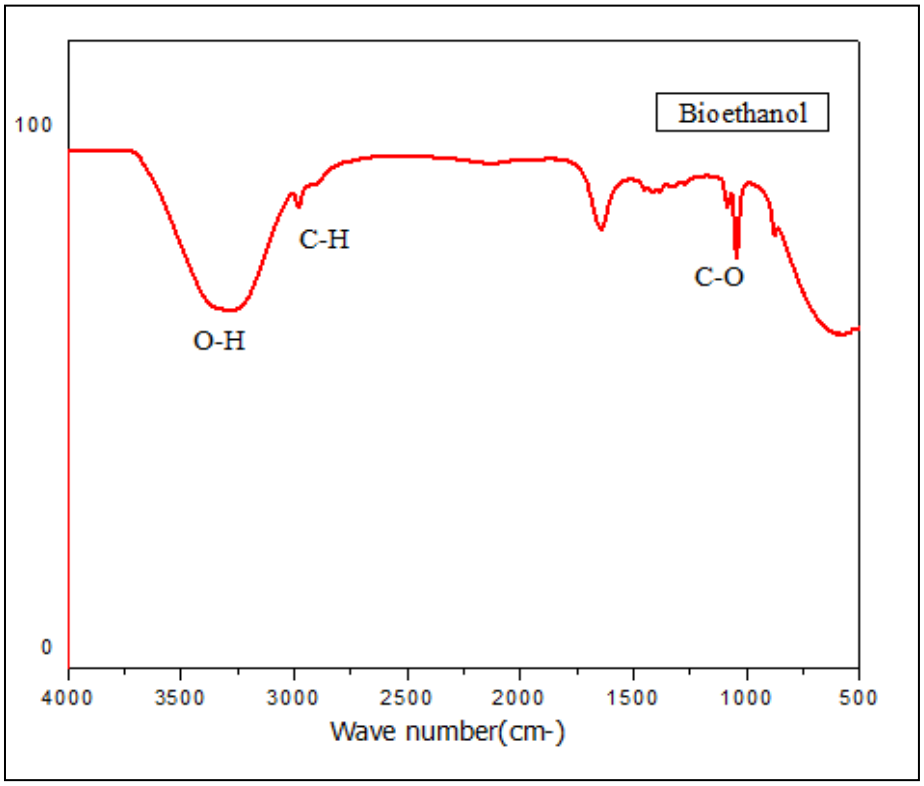


Figure4.18: Result of FTIR analysis of bioethanol produced from alkali treated PPW

5. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

The process of producing biofuels from lignocellulosic biomass involves a significant challenge in selecting an affordable and suitable raw material. This study investigates the potential of potato peel for the synthesis of ethanol. The fundamental challenge scientists facing are to develop pretreatment methods that are both economically viable and provide high levels of bioethanol.

In order to optimize the production of bioethanol, this research focuses on finding optimum pretreatment conditions that significantly remove lignin components of potato peel. Alkali pretreatment method was used in this investigation. The experimental design was conducted by central composite design to determine optimum pretreatment parameters (NaOH concentration, pretreatment time and temperature) in order to maximize the delignification efficiency. The optimum operating conditions were found to be at alkali concentration of 3.3%v/v, pretreatment time 3.5 hr and temperature of 70 ^{o}C. At these optimum operating conditions, the maximum delignification efficiency (77.82±2.1%), starch recovery (80.3±1.2%) and cellulose recovery (89.7±1.5%) were obtained.

The raw and alkali pretreated (optimized parameters) PPW were also characterized using instrumental characterization. The morphology (SEM) study verifies that the crystalline natures of raw PPW with some regular shapes, has changed to amorphous structure which is porous and clean due to the removal of impurity like extractives, waxes and lignin. The functional group (FTIR) analysis results also demonstrated that lignin could be effectively eliminated by treating potato peel using alkali solution. Also the XRD result confirms that crystal structure of PPW transformed into a more amorphous form as it was broken down into its oligomer form.

In the hydrolysis process effects of acid concentration, hydrolysis time and temperature were studied to determine the maximum possible concentration of sugar (glucose) from pretreated potato peel. After the completion of the hydrolysis the maximum glucose concentration of 46.5 mg/ml was obtained at 15% H_2SO_4, 65 ^{o}C and 60 min. whereas, the glucose concentration from untreated PPW was around 29.9 mg/ml. the glucose concentration increase by 55.5% when treating raw PPW using alkali pretreatment prior to hydrolysis. The removal of lignin from the

PPW and an increase in surface area of treated PPW contributed to the improvement of the hydrolysis process.

Finally, In order to produce bioethanol, the pretreated PPW hydrolysate was fermented under anaerobic conditions with the help of microorganisms, specifically the yeast organism S. cerevisiae. Consequently, at optimum operating parameters of pH 5, temperature 30 °C, agitation 150 rpm, and time 72 hr, bioethanol concentration of 21.04 mg/ml and yield of 88.5% were attained at the end of fermentation.

5.2. RECOMMENDATIONS

In this study it can be seen that producing bioethanol from potato peel waste is a promising avenue for sustainable energy production and waste valorization. By optimizing process parameters and integrating efficient technologies, this approach can contribute significantly to reducing waste accumulation, mitigating environmental impact, and meeting renewable energy demands.

Based on the current investigation the following recommendations are forwarded:

- Because potato peels contain both cellulose and starch, they require a different pretreatment mechanism than other lignocellulosic biomasses. Researchers must compare and determine the best pretreatment method for producing bioethanol from potato peels.
- Research must be done to find other microorganisms that can convert reducing sugar into bioethanol in order to increase the yield of bioethanol from potato peels.
- To maximize the yield of bioethanol from the leftover potato peel waste, the hydrolysis variables must be optimized in addition to the pretreatment procedure.
- For the aim of commercialization, an economic feasibility analysis of the entire conversion process is required.

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APPENDIXES

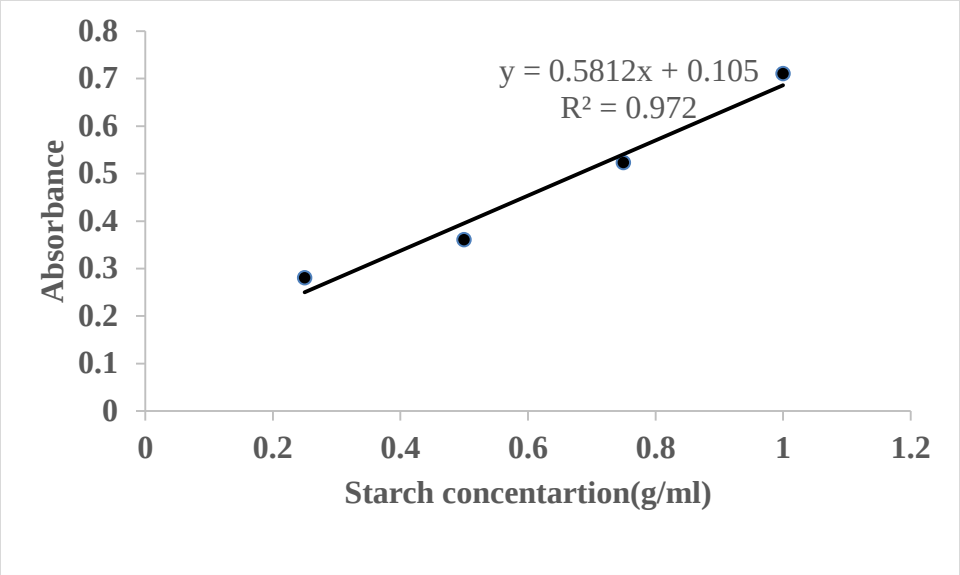
APPENDIXE A: Properties of Ethanol

Physical Properties	Description
Molecular formula	CH_3CH_2OH
Molar mass	40.06844(232)g/mol
Appearance	Colorless clear liquid
Density	$0.789g/m^3$
Melting point	-114.3
Boiling point	78.4^oc
Solubility in water	Fully miscible
Ack2idity(pka)	15.9
Viscosity	1.2mpa.s(cp)at 20^oC
Dipole moment	5.64fc.fm(1.69D)(gas)
EU classification	Flammable(F)
Flash point	286.15K(13^oC)

APPENDIXE B: Criteria for preparation of glucose solution

Tube	**1**	**2**	**3**	**4**	**5**	**6**
Standard solution (ml)	0	20	40	60	80	100
Distilled water (ml)	1	80	60	40	20	0
Sample from standards (m)	1	1	1	1	1	1
Phenol(5%)ml	1	1	1	1	1	1
H_2S0_4 (96%)ml	5	5	5	5	5	5

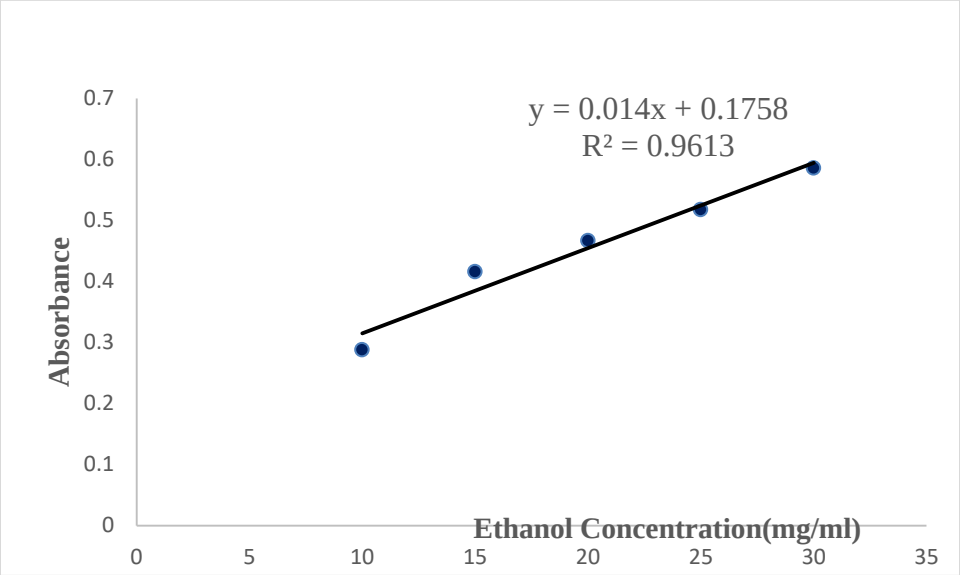
APPENDIXE C : Standard starch curve



APPENDIXE D: Criteria for preparation of ethanol solution

Tube	1	2	3	4	5	6
Standard solution from stock(micro liter)	5	10	15	20	25	30
Distilled water (micro liter)	95	90	85	80	75	70
DNS regent(ml)	10	10	10	10	10	10

APPENDIXE E: Ethanol standard curve



APPENDIXE F: UV analysis instrument

APPENDIXE G: FTIR analysis instrument

APPENDIXE H: SEM analysis instrument

APPENDIXE I: XRD analysis instrument