"STUDY ON PRODUCTION OF BIOETHANOL FROM ONION PEEL"

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CERTIFICATE

This is to certify that **Mr. Arindam Paul** , Final year Masters of Food Technology and Biochemical Engineering examination student of Department of Food Technology and Biochemical Engineering, Jadavpur University, Class RollNo**. 002110902001** , Registration No. **140023 of 2017-18** , Examination Roll No. **M2FTB22002** has completed the Project work titled, **"STUDY ON PRODUCTION OF BIOETHANOL FROM ONION PEEL"** under the guidance of **Dr. Debabrata Bera** during his Master's Curriculum. This work has not been reported earlier anywhere and can be approved for submission in partial fulfilment of the course work.

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Abstract

The depletion of natural fuels and environmental concerns have led to a rising demand for renewable and sustainable energy sources. Bioethanol, derived from biomass such as bio waste, has significant attention as a potential alternative fuel. This project focuses on the production of bioethanol from onion peel waste, a readily available agricultural byproduct with limited utilization .

The study begins with the collection and characterization of onion peel waste to determine its chemical composition and suitability for bioethanol production. Various pretreatment methods, including acid hydrolysis, are explored to enhance the accessibility of sugar fractions within the onion peels. Subsequently, fermentation using selected yeast strains is used to convert the available sugars into ethyl alcohol.

Optimization of process parameters such as pH, temperature, inoculum size, and fermentation time is carried out to maximize ethanol yield. The ethanol produced is then subjected to purification processes, including distillation, to obtain high-purity bioethanol suitable for use as a fuel additive. Finally optimizing all the five parameters it is found that Fermentation time of 48 hr , temperature 30 ° C, inoculum concentration 10%, pH 4.5 , and substrate concentration 15% yield maximum amount of ethanol (4.839% v/v).

The economic feasibility and environmental impact of ethanol production from onion peel waste are also evaluated. The project aims to contribute to sustainable waste management practices while providing a potential source of renewable energy. This research represents a significant step towards utilizing agricultural waste streams to produce bioethanol, thus reducing greenhouse components emissions and dependence on fossil fuels.

Chapter – 1

Introduction

1. **Introduction**:

1.1. Problems of Agricultural Waste:

Agricultural waste in India poses a multitude of challenges that impact the environment, economy, and public health. These challenges stem from the vast agricultural sector, diverse crop patterns, and resource management practices. The improper handling and disposal of agricultural waste exacerbate these problems, necessitating urgent attention and sustainable solutions [1].

One of the foremost issues is air pollution. The burning of crop residues, such as rice straw, after harvest leads to the release of harmful pollutants like particulate matter, carbon dioxide, and methane. This contributes to the already severe air quality problems in many Indian cities, impacting respiratory health and overall well-being [2].

Moreover, improper disposal of agricultural waste impacts soil health. When residues are not properly managed, they can hinder soil structure and nutrient balance, reducing agricultural productivity in the long run. This threatens food security and sustainable agricultural practices.

Water pollution is another significant concern. Runoff from fields with excessive use of agrochemicals and inadequate waste management can contaminate water bodies, affecting aquatic ecosystems and human access to clean water. The runoff may carry pesticides, fertilizers, and pathogens, leading to biodiversity loss and health risks.

Inefficiencies in waste management also contribute to greenhouse gas emissions. Decomposition of organic waste in landfills and uncontrolled settings generates methane, a significant greenhouse gas that is responsible for global warming and climate change. This can exacerbate the challenges already faced due to changing weather patterns and extreme events.

Furthermore, the financial impact of agricultural waste cannot be overlooked. The lack of proper waste management leads to missed opportunities for resource recovery and recycling. Organic waste, if managed effectively, can be turned into compost or biogas, providing valuable inputs for agriculture and energy generation. Failure to harness these resources perpetuates an unsustainable cycle of waste generation.

Public health is at risk due to inadequate waste management practices. Improper disposal can lead to the breeding of disease-carrying vectors like mosquitoes, contributing to the spread of vector-borne diseases. Communities near waste disposal sites may experience higher health risks due to exposure to pollutants and pathogens [3].

Addressing these challenges requires a multi-pronged approach. Encouraging sustainable agricultural practices like zero-tillage farming and crop residue management can help reduce waste generation. Implementing effective waste management systems, such as composting and biogas production, can mitigate environmental and health risks while promoting resource recovery. Education and awareness campaigns for farmers, communities, and policymakers are crucial to drive these changes.

In conclusion, agricultural waste in India presents a complex set of challenges that encompass environmental, economic, and health concerns. Addressing these issues requires collaborative efforts from governments, agricultural communities, researchers, and environmental organizations. By implementing sustainable waste management practices and fostering a culture of responsible resource utilization, India can effectively mitigate the problems associated with agricultural waste and move towards a more resilient and sustainable agricultural future.

1.2.Quantity of different agricultural waste :

India is ranked at the top for wide solid waste generation (nearly 350 - 990 million tons/year) India being an agro-based country, about 620 million tons of agricultural waste is generated every year.

Indian crops (corresponding agro-wastes)	Annual availability (Kt/year)
Rice waste	161893.00
Rice straw	141120.00
Rice husk	20773.00
Wheat (straw)	122991.00
Sugarcane wastes	114761.00
Sugarcane bagasse	73775.00
Sugarcane tops and leaves	40986.00
Maize waste	33720.00
Maize straw	28396.00
Maize cobs	5324.00
Banana waste	67776.00
Banana fruit peels	393.00
Banana pseudo-stem	67383.00
Mustard waste	16877.00
Mustard press cake	2681.00
Mustard seedpod	1355.00
Mustard stalks	12841.00
Sesame (stalks)	1207.70
Soybean husk	671.00
Coconut waste	9060.00
Coconut fronds	7769.00
Coconut shell	726.00
Coconut coir pith	565.00
Areca nut (fronds, husk)	1000.80
Groundnut (shells)	1385.00
Bajra(stalks, cobs, husk)	15831.80
Jowar (cobs, stalks, husk)	24207.80
Ragi (straw)	2630.20
Cotton waste	38281.00
Cotton stalks	35397.00
Cotton hull	2884.00
Pulses* (stalks, husk)	13462.90

*Arhar, gram, masoor, moong, urad

Fig 1.1 Quantity of Different Agricultural Waste

These wastes are generated from the uncontrolled application of intensive farming process and excessive use of chemical fertilizers etc. The nature of waste generated is dependent on the way of agricultural activities applied.

The unscientific method of farming

Methodologies has adversely affected the global environmental condition. The classification and the categorization of agro-wastes, based on the agricultural activity are discussed in Figure.

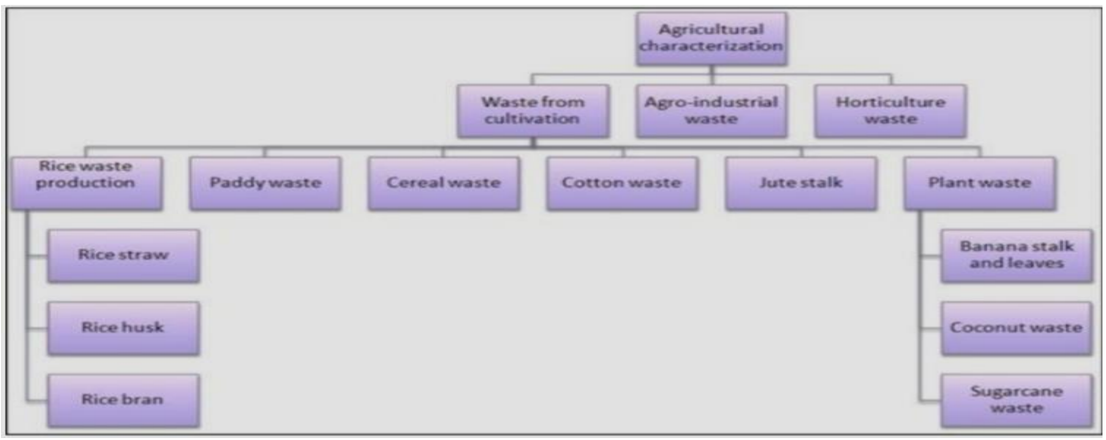

Fig 1.2 Different Agricultural Waste

1.3.Scope of valorisation of agricultural waste:

The valorization of agricultural waste involves turning waste materials into valuable products or resources. In the context of agricultural waste, valorization can have several potential scopes:[4]

i. Bioenergy Production: Agricultural waste can be converted into biofuels like biogas, bioethanol, and biodiesel through processes such as anaerobic digestion and fermentation.
ii. Bio-Based Materials: Waste can be used to produce bioplastics, fibers, and composite materials, reducing the dependence on fossil-based resources.
iii. Fertilizer and Soil Amendments: Some agricultural waste can be transformed into organic fertilizers or soil conditioners, enriching soil quality and promoting sustainable agriculture.
iv. Animal Feed: Certain waste materials can be treated and used as supplementary animal feed, reducing the pressure on natural resources used for livestock feed.
v. Chemical Extraction: Valuable chemicals, enzymes, and compounds can be extracted from agricultural waste for use in various industries, including pharmaceuticals and cosmetics.

vi. Mushroom Cultivation: Some agricultural waste, like crop residues, can serve as substrates for mushroom cultivation, providing an additional source of income.
vii. Bioremediation: Agricultural waste can be employed for bioremediation purposes, helping to clean polluted soils or water bodies.
viii. Composting: Composting agricultural waste can lead to nutrient-rich compost that can enhance soil fertility and structure.
ix. Carbon Sequestration: Incorporating agricultural waste into the soil can help sequester carbon, contributing to climate change mitigation.
x. Research and Innovation: The research and development of new technologies and processes for agricultural waste valorization can create opportunities for entrepreneurship and innovation.

The scope of agricultural waste valorization is vast and continuously evolving as new technologies and approaches emerge. It offers the potential to address environmental concerns, reduce waste, and create economic value from materials that would otherwise be discarded.

1.4.Composition of Onion Peel:

Onion peels, the outer layers of onions that are typically removed before cooking, contain various compounds. These compounds can include [5] :

i. Fiber: Onion peels are rich in dietary fiber, which can contribute to digestive health and help regulate blood sugar levels.
ii. Flavonoids: Flavonoids are antioxidants found in onion peels that has important health benefits, including anti-cancer and anti-inflamatory properties.
iii. Quercetin: Quercetin is a type of flavonoid present in onion peels that has been linked to various health benefits, including cardiovascular health and immune system support.
iv. Organosulfur Compounds: These compounds are responsible for the pungent odor of onions and are associated with potential health benefits, such as anti-microbial and anti-inflammatory effects.
v. Vitamins and Minerals: Onion peels contain small amounts of minerals and vitamins, including vitamin C and B vitamins.
vi. Polyphenols: Polyphenols are another group of antioxidants found in onion peels that may contribute to their potential health-promoting properties.

It's important to note that while onion peels do contain these beneficial compounds, they are typically not consumed directly due to their tough and papery texture. However, there are efforts to explore ways to utilize onion peels in food production, such as incorporating them into teas, extracts, or powders to harness their potential health benefits.

1.5.Availability of Onion Peel Waste :

Onion peel waste is widely available in India due to the country's significant onion production. Onions are a staple in Indian cuisine and are grown in large quantities across various regions. As a result, onion peel waste is generated abundantly from households, restaurants, food processing units, and markets where onions are sold. This waste is often discarded, leading to potential environmental and economic issues [6].

Efforts to utilize onion peel waste have been explored in various contexts. It contains bioactive compounds that could have potential applications in the food, cosmetic industries and pharmaceutical. Researchers have investigated extracting valuable components such as antioxidants, dietary fiber, natural dyes from onion peels. These compounds can be used in various products, including supplements, functional foods, and natural colorants.

Moreover, onion peels can also be utilized in sustainable practices such as composting and as a substrate for biogas production. Composting onion peels along with other organic materials can help create nutrient-rich soil amendments. Similarly, using onion peel waste in biogas production can generate renewable energy while reducing the environmental impact of organic waste.

Given the availability of onion peel waste and the potential benefits of its utilization, there is an opportunity to explore innovative solutions for its management. This could not only contribute to minimizing waste and environmental pollution but also offer economic value through the extraction of valuable compounds and energy production.

1.6.Scope of Onion Peel Waste Utilisation:

Onion peel waste, often considered a byproduct of food processing and consumption, holds significant potential for various utilizations, ranging from agricultural applications to industrial and medicinal uses. This overlooked resource can be harnessed in a multitude of ways, contributing to sustainability, economic value, and waste reduction.

i. Agricultural Applications:

Onion peels are rich in organic matter and essential nutrients like potassium, calcium, and sulfur. They can be composted or incorporated into soil as a natural fertilizer, enhancing soil structure and promoting plant growth. The peels can also act as a natural pest repellent due to their strong odor, reducing the need for chemical pesticides.

ii. Animal Feed:

Dried and processed onion peels can be included in animal feed formulations as a source of dietary fiber and nutrients. However, care must be taken to prevent excessive consumption, as onion peels contain compounds that could be harmful to certain animals.

iii. Food Industry:

Extracts from onion peels contain bioactive compounds like flavonoids and antioxidants, which have potential applications in the food industry. These compounds can be used as natural food additives or preservatives, enhancing both the nutritional value and shelf life of various products.

iv. Cosmetics and Pharmaceuticals:

The bioactive compounds in onion peels also have potential applications in the pharmaceutical industries and cosmetic industries. They can be utilized in skincare products for their antioxidant and anti-inflammatory properties, as well as in the formulation of herbal medicines.

v. Natural Dyeing:

Onion peels have been traditionally used as a natural dye, producing shades of yellow and orange. This eco-friendly dyeing process could find application in the textile industry, reducing the environmental impact of synthetic dyes.

vi. Biogas Production:

Through anaerobic digestion, onion peels can be converted into biogas, a renewable energy source composed mainly of methane. This has the potential to contribute to local energy generation and waste management.

vii. Paper and Packaging:

The cellulose content present in onion peels can be extracted and utilized for producing biodegradable paper and packaging materials, reducing the dependency on non-renewable resources.

viii. Environmental Remediation:

Some studies suggest that onion peels might have the ability to adsorb heavy metals and pollutants from water and soil, potentially contributing to environmental remediation efforts.

ix. Craft and Art:

Artisans and craft enthusiasts can explore the aesthetic value of onion peels by incorporating them into handmade paper, natural dye art, and other creative endeavors.

x. Educational Use:

Onion peels can be used as an educational tool in biology classrooms to study cell structure under a microscope due to their translucent nature.

In conclusion, onion peel waste has extensive scopes for utilization across various sectors, promoting sustainability and reducing waste. However, successful implementation requires research, innovation, and awareness to harness the full potential of this resource, contributing to a more circular and eco-friendly economy.

1.7.Different applications of Onion Peel Waste :

Onion peels, often overlooked and discarded, possess a variety of applications that range from culinary uses to medicinal and even artistic endeavors. These thin, papery layers that enshroud the pungent vegetable have garnered attention for their potential benefits, showcasing their versatility beyond the kitchen.

In the culinary world, onion peels can be repurposed as a natural food coloring agent. The outer layers, rich in pigments like anthocyanin, can be dried and ground to create a spectrum of hues, from pale yellow to deep red. These natural colorants are free from synthetic additives, making them a healthier alternative for enhancing the visual appeal of dishes, baked goods, and beverages.

Moreover, onion peels can contribute to sustainable gardening practices. When composted, they enrich the soil with organic matter, aiding in moisture retention and promoting nutrient availability. As the peels decompose, they release valuable nutrients back into the earth, creating a cycle of nourishment for plants [7].

Beyond the garden, onion peels have found their way into natural remedies and wellness practices. They contain compounds with potential health benefits, such as quercetin, a flavonoid known for its anti-inflammatory and anti-oxidant properties. Some herbalists and holistic practitioners suggest that onion peel infusions may aid in immune system support, respiratory health, and digestion. However, it's important to note that scientific research on these claims is still ongoing.

Craft enthusiasts have also discovered creative uses for onion peels. They can be used to create unique patterns and designs on fabrics through a technique called "onion skin dyeing." By boiling the peels and immersing cloth in the resulting colored liquid, one can achieve earthy, organic patterns that are both visually appealing and environmentally friendly.

In addition to their artistic potential, onion peels possess practical applications in household tasks. The natural pigments in the peels can be harnessed as a natural dye for Easter eggs, providing a chemical-free option for decorating during the holiday season. Furthermore, the astringent properties of onion peels can be utilized as a mild abrasive for cleaning purposes, helping to scrub away stains and residue from surfaces.

In summary, onion peels, often disregarded as waste, have a multitude of applications that extend far beyond the kitchen. From providing natural food coloring to offering potential health benefits, contributing to sustainable gardening practices, inspiring creative crafting endeavors, and aiding in household tasks, these unassuming layers of the onion offer a world of possibilities. As awareness of sustainability and holistic well-being grows, onion peels stand as a testament to the ingenuity of finding value in the overlooked and underappreciated.

1.8. Current Status of Utilisation of Onion Peel Waste in National and International Scenario:

The utilization of onion peel in both national and international scenarios has been gaining attention due to its potential applications in various fields. In India, which is one of the largest onion producers globally, there has been a growing interest in finding sustainable ways to utilize onion peel waste generated during onion processing. Researchers and entrepreneurs have been exploring innovative methods to harness the bioactive compounds present in onion peels for various purposes.

Onion peels are known to contain bioactive compounds such as phenolic acids, flavonoids, and antioxidants, which have potential health benefits. In the national context, there have been efforts to develop value-added products from onion peels, such as natural food colorants, dietary supplements, and nutraceuticals. These products not only contribute to reducing waste but also tap into the health-conscious consumer market.

Internationally, the utilization of onion peel has garnered interest for its potential in diverse sectors. Researchers and industries around the world have been investigating the incorporation of onion peel extracts into cosmetics, pharmaceuticals, and even animal feed. The antioxidant properties of onion peels make them a valuable ingredient in skincare products, while their potential antimicrobial and anti-inflammatory effects could lead to applications in pharmaceutical formulations. Additionally, the use of onion peel extracts in animal feed could have positive implications for livestock health and productivity.

1.9. Bottlenecks in Effective Utilization of Onion Peel Waste :

The effective utilization of onion peel waste faces several bottlenecks that hinder its widespread application and commercialization. here are five key bottlenecks:

i. Lack of Infrastructure and Processing Facilities: One significant challenge is the limited infrastructure and processing facilities dedicated to onion peel waste. The collection, storage, and proper processing of onion peels require specialized equipment and technologies. Without adequate facilities, the potential value of onion peel waste remains largely untapped.

ii. Extraction and Preservation Techniques: Efficient extraction and preservation of bioactive compounds from onion peels are essential for their utilization in various applications. Developing cost-effective and environmentally friendly extraction methods that maintain the potency of these compounds is a technical challenge that researchers and industries need to address.

iii. Quality and Consistency: Maintaining consistent quality and composition of bioactive compounds from onion peels is crucial for their use in food, cosmetic, and pharmaceutical products. Variability in onion peel waste due to factors like onion variety, growing conditions, and post-harvest handling can affect the reliability and efficacy of end-products.

iv. Regulatory Hurdles: The utilization of onion peel waste in various industries, such as food, cosmetics, and pharmaceuticals, often requires compliance with regulatory standards and safety assessments. Navigating through the regulatory framework can be time-consuming and costly, particularly when introducing novel ingredients or products.

v. Limited Awareness and Market Demand: Despite the potential benefits of onion peel waste, limited awareness among consumers and industries about its value and potential applications can hinder market demand. Creating awareness and generating market interest for products derived from onion peels is necessary to drive commercial adoption and investment.

Researchers, entrepreneurs, and policymakers continue to work on addressing these bottlenecks to unlock the full potential of onion peel waste utilization.

1.10. Proposal to overcome those limitations:

Here are ten points on overcoming the obstacles in the effective utilization of onion peel waste:

i. Investment in Research and Development: Increased funding and research efforts directed towards finding innovative extraction, processing, and preservation methods can lead to more efficient utilization of bioactive compounds from onion peels.

ii. Technological Advancements: Develop and adopt advanced technologies such as supercritical fluid extraction, enzymatic treatments, and nanotechnology to enhance the extraction efficiency and preservation of bioactive compounds.

iii. Infrastructure Development: Establish dedicated processing facilities and infrastructure for handling onion peel waste, including collection centers, processing units, and storage facilities to ensure proper handling and utilization.

iv. Standardization and Quality Control: Develop standardized protocols for the collection, processing, and extraction of onion peel waste to ensure consistent quality and composition of extracted bioactive compounds.

v. Collaboration and Partnerships: Foster collaborations between research institutions, industries, and government bodies to share knowledge, expertise, and resources for the development of onion peel waste utilization technologies.

vi. Awareness Campaigns: Launch public awareness campaigns to educate consumers, industries, and policymakers about the benefits and potential applications of onion peel waste-derived products.

vii. Regulatory Streamlining: Work closely with regulatory authorities to establish clear guidelines and standards for the use of onion peel-derived ingredients in various industries, facilitating the regulatory approval process.

viii. Value-Added Product Development: Invest in research and product development to create a diverse range of value-added products, such as natural food colorants, dietary supplements, skincare products, and pharmaceutical formulations.

ix. Market Research and Demand Generation: Conduct market research to identify potential demand for onion peel waste-derived products and develop targeted marketing strategies to promote their adoption.
x. Capacity Building and Training: Provide training programs and workshops for farmers, processors, and entrepreneurs to enhance their skills and knowledge in onion peel waste utilization, fostering a skilled workforce in this emerging field.

These strategies, along with continued innovation and collaboration, can contribute to overcoming the obstacles and promoting the effective utilization of onion peel waste in various industries and applications.

Chapter-2

Project Objective

2. Project Objective :

The production of ethyl alcohol from onion peel waste is an innovative and sustainable approach that addresses both environmental and economic concerns. This project aims to achieve several key objectives, leveraging the potential of onion peel waste as a valuable resource for bioethanol production. The objectives can be summarized as follows:

i. Waste Valorization: One of the primary objectives of this project is to valorize onion peel waste, a byproduct generated in large quantities from food processing industries and households. By converting this waste into bioethanol, a renewable and cleaner energy source, the project aims to contribute to waste reduction and minimize its environmental impact.
ii. Bioethanol Production Optimization: The project aims to optimize the bioethanol production process from onion peel waste. This involves conducting comprehensive research to identify the most suitable techniques and conditions for efficiently converting the waste material into bioethanol. Factors such as pretreatment methods, enzymatic hydrolysis, and fermentation parameters will be investigated to achieve maximum ethanol yield.
iii. Resource Efficiency: Resource efficiency is a critical aspect of sustainable production. The project aims to explore methods to enhance the efficiency of resource utilization in the bioethanol production process. This includes minimizing energy consumption, water usage, and other resources while maximizing the ethanol output.
iv. Technological Innovation: The project seeks to bring technological innovation to the forefront by adopting cutting-edge techniques and equipment for the bioethanol production process. By incorporating advanced technologies, the project aims to improve overall process efficiency and increase the feasibility of large-scale implementation.
v. Environmental Impact Reduction: One of the overarching goals of the project is to reduce the overall environmental impact of bioethanol production. By utilizing onion peel waste as a feedstock, the project aims to reduction in greenhouse gas emissions, as well as the demand for conventional fossil fuels, thereby contributing to mitigating climate change.
vi. Economic Viability: The project aims to evaluate the economic viability of producing bioethanol from onion peel waste. This involves conducting a comprehensive cost-benefit analysis, considering factors such as raw material acquisition, processing costs, and potential revenue from bioethanol sales. The goal is to demonstrate that this approach can be economically sustainable in the long run.
vii. Waste Management Solution: Addressing the issue of waste management is a crucial objective of this project. By providing a viable solution for repurposing onion peel

waste, the project aims to contribute to a circular economy where waste is transformed into a valuable resource.

viii. Promoting Renewable Energy: The project aligns with the global shift towards renewable energy sources. By producing bioethanol from onion peel waste, the project aims to promote the use of a cleaner alternative to traditional fossil fuels, thereby reducing the reliance on non-renewable resources.

ix. Feasibility Assessment: The feasibility of scaling up the bioethanol production process is a significant objective. The project aims to provide insights into the scalability of the process and identify any potential challenges that may arise when transitioning from small-scale to large-scale production.

In conclusion, the project's objectives revolve around waste valorization, bioethanol production optimization, resource efficiency, technological innovation, environmental impact reduction, economic viability, waste management solutions, promotion of renewable energy, educational outreach, and feasibility assessment. By achieving these objectives, the project aims to contribute to a more sustainable and greener future while addressing the challenges of waste management and energy sustainability.

Chapter-3

Literature Review

Literature Review :

Sl. No.	Title of Paper	Reference	Description
1	Optimizing bio-ethanol production from cabbage and onion peels waste using yeast (Saccharomyces cerevisiae) as fermenting agent [21]	International Journal of Life Science Research Archive Temam G. G., Desta L. E. and Aschalew K. G. 2021 https://doi.org/10.53771/ijlsra.2021.1.1.0012	Authors showed that various factors like pre-treatment with acid, inoculum size, fermentation time and substrate concentration alters the amount of bio-ethanol production.
2	Bio-ethanol Production from Green Onion by Yeast in Repeated Batch [22]	Indian Journal of Microbiology Reza Robati 2013 10.1007/s12088-013-0374-3	This study was basd on the effective yield of ethanol from Green onion ‘‘*Saccharomyces cerevisiae*’’ in repeated batch. The results indicated that the total sugar present in onion juice was 68.4 g/l. The highest rate of productivity, ethyl alcohol yield and final bio-ethanol percentage was 7 g/l/h , 35 g/l and 90 %, respectively.
3	Potential in Bioethanol Production from Various Agro Wastes Fermenting by Microorganisms Using Carrot Peel, Onion Peel, Potato Peel and Sugar Beet Peel as Substrates [23]	Archives of Ecotoxicology Dr.isaie Mushimiyimana, Fidel Niyitanga, Celestin Sirimu 2021 https://doi.org/10.36547/ft.132	The product obtained was purified using distillation at 79 °C. The ethanol is then measured by specific dichromate method and Gas Chromatography. It yielded highest ethanol in the substrate of carrot peel was 16.9 % at 21st day .
4	Production of bioethanol from agro waste by saccharification and fermentation [24]	Jordan Journal of Agricultural Sciences Isaie Mushimiyimana, Padmavathi Tallapragada	Saccharification followed by fermentation process generated maximum ethanol in the substrate sugar beet peel 15.79% at 28 th day .

		2017 https://doi.org/341378765	
5	Bioethanol Production from Agro Wastes by Acid Hydrolysis and Fermentation Process [25]	Journal of Scientific & Industrial Research I Mushimiyimana and P Tallapragada 2017 http://doi.org/123456789/34355	The fermented product was purified by distillation at 78 C and the fraction was collected. The ethanol was obtainedby dichromate method. Highest yield of ethanol was obtained from onion peel 14.52% on 28th day.
6	Onion waste based-biorefinery for sustainable generation of value-added products [26]	Bioresource Technology Narashans Alok Sagar , Yogesh Kumar , Ramveer Singh , C. Nickhil , Deepak Kumar , Praveen Sharma , Hari Om Pandey , Suvarna Bhoj , Ayon Tarafdar 2022 https://doi.org/10.1016/j.biortech.2022.127870	This article tells the recent advances in the biorefinery processes for valorization of onion processing waste and production of different value-added products from various classes of onion waste. The review also suggests the current bottlenecks faced by the biochemical industry for the utilization of onion waste and perspectives to minimize them.
7	Enzymatic Co-Fermentation of Onion Waste for Bioethanol Production Using Saccharomyces cerevisiae and Pichia pastoris [27]	Journal of Multidisciplinary Digital Publishing Institute. Iqra Shahid, Ghulam Hussain, Mehwish Anis, Muhammad Umar Farooq, Jaroslaw Krzywanski, Muhammad Usman 2023	This determines the practicability of bioethanol production from onion by *Saccharomyces cerevisiae* and Pichia pastoris and their novel co-culture through fermentation. The maximum bioethanol concentration was achieved through a monoculture of

		https://doi.org/10.3390/en16052181	Saccharomyces cerevisiae, i.e., 30.56 g/L. The ethanol productivity was determined based on the ethanol concentration and fermentation time ratio.

Table 3.1 Literature Review

Chapter-4

Experimental Work and Methodology

Experimental Work and Methodology

Determination of Moisture Content :

Fig 4.1 Determination of Moisture Content

The determination of moisture content in onion peel is a crucial process in various industries, particularly food processing and agriculture. This method typically involves a straightforward yet precise technique known as drying [8]. To begin, a representative sample of onion peel is collected. Care is taken to ensure the sample is homogeneous, as moisture content can vary within the peel. The sample is then weighed accurately. This initial weight is recorded as the wet weight.

Next, the sample is set in an oven or a moisture analyzer. The oven is placed to a specific temperature, usually around 105°C (221°F). Over some time , typically 24 hours, the object is subjected to this constant heat. During this time, moisture within the onion peel gradually evaporates. After the allotted time, the sample is removed from the oven, and its weight is measured again. This weight is recorded as the dry weight. The amount of moisture is calculated by differencing the dry weight from the wet weight and expressing it as a percentage of the wet weight.

The drying method is widely accepted for its simplicity and reliability in determining moisture content, making it an essential technique for quality control and compliance with industry standards in the production of various onion-based product.

Determination of Ash Content:

Fig 4.2 Determination of Ash Content

The determination of ash content in onion peel by incineration is a crucial analytical process widely employed in food and agricultural industries. This method assesses the mineral and inorganic content in onion peels, providing insights into its nutritional composition and suitability for various applications [9].

The process begins by obtaining a representative sample of dried onion peel, which is essential to ensure accurate results. The sample is then weighed and placed in a crucible. To initiate incineration, the crucible is subjected to high temperatures in a muffle furnace, typically around 550-600°C. During this heating process, organic matter in the onion peel sample combusts and evaporates, leaving behind inorganic residues or ash.

Once the incineration is complete, the crucible is cooled in a desiccator to prevent moisture absorption, and the remaining ash is weighed. The ash content is calculated with respect to the initial sample weight. This information aids in evaluating the peel's nutritional value and suitability for various applications, such as animal feed or composting, making it a valuable analytical technique in the agricultural and food industries.

Determination of Crude Fibre Content:

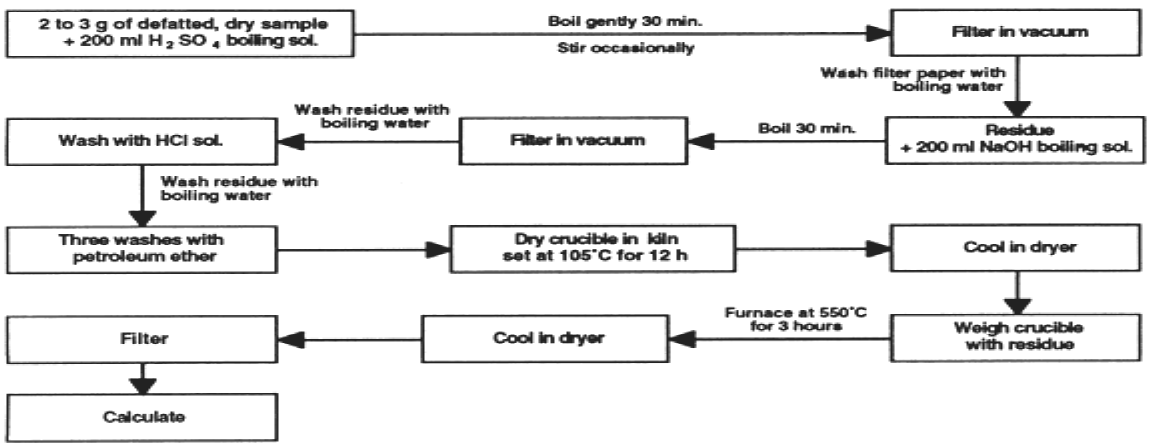

Fig 4.3 Determination of Crude Fibre Content

The evaluation of amount of crude fiber in onion peel involves a multi-step process that combines acid and alkali treatments followed by incineration [10]. This method is essential for understanding the nutritional composition of onion peel, which can have potential applications in animal feed or food processing. Here's a detailed explanation of the procedure:

Sample Preparation: Begin by obtaining a representative sample of onion peel, ensuring it is free from any foreign materials or contaminants. The sample is then dried at a controlled temperature to remove moisture, setting oven at 105°C until weight is unchanged.
Acid Treatment: A portion of the dried sample is weighed and placed in a flask. Sulfuric acid (H2SO4) is added to the flask, and the mixture is heated. The acid hydrolyzes the complex carbohydrates, leaving cellulose, lignin, and other fibrous components intact. The mixture is boiled for a specified period and then filtered to separate the liquid portion.
Alkali Treatment: The residue from the acid treatment is washed and transferred to another flask. Sodium hydroxide (NaOH) solution is added, and the mixture is heated again. This step dissolves the cellulose portion, leaving behind lignin. The substrate is filtered, and the solid residue is obtained.
Incineration: The solid residue obtained after the alkali treatment is incinerated at a high temperature, usually in a muffle furnace set at 550-600°C. This process burns away all organic matter, leaving behind ash.
Calculation: The difference in weight between the original dried sample and the ash obtained after Incineration represents the crude fiber content. Crude fiber content is measured with respect to the dry weight of the onion peel sample.
This method allows for the determination of crude fiber content in onion peel, which consists mainly of cellulose and lignin. It's a crucial step in nutritional analysis and quality control for various applications in the food and agricultural industries.

Determination of Total Flavonoids Content :

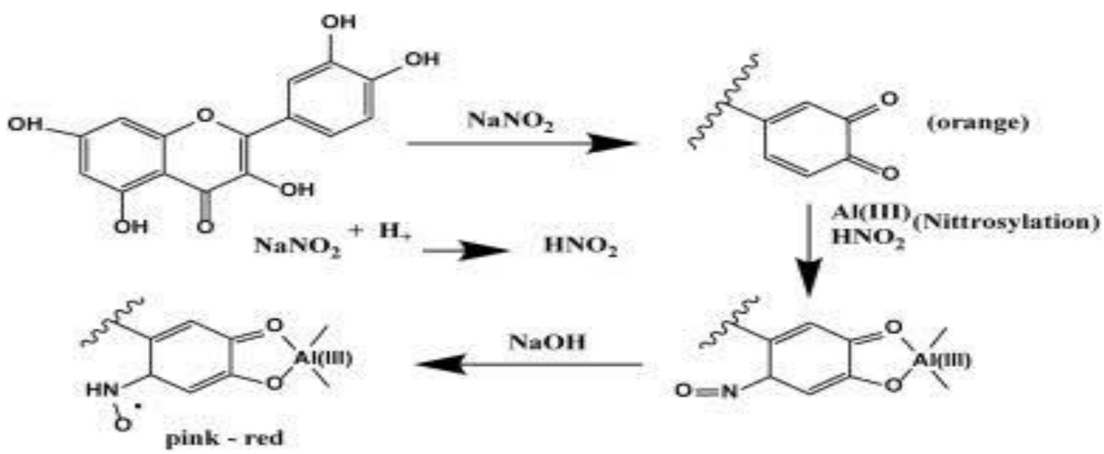

Fig 4.4 Determination of Total Flavonoids Content

The evaluation of the total flavonoid content in onion peel by aluminum chloride is a widely employed method to estimate the existence of flavonoids [11], that are natural compounds studied for their antioxidant properties and potential health benefits. Here's a detailed explanation of the procedure:
Sample Preparation: Begin by obtaining a representative sample of onion peel, ensuring it is clean and free from any contaminants. The sample is then dried and ground into a fine powder using a mortar and pestle or a grinder. The powder is stored in airtight containers to prevent degradation.
Extraction: A portion of the powdered onion peel is weighed accurately and placed in a flask.
Ethanol or methanol is added to the flask to create an extraction solution. Flavonoids are soluble in these solvents. The flask is sealed and left to macerate for a specified period, allowing the flavonoids to dissolve into the solvent.
Filtration: After maceration, the extract is filtered to separate the liquid portion (containing the extracted flavonoids) from the solid residue.
Reaction with Aluminum Chloride: A measured volume of the extract is transferred to a new container. Aluminum chloride (AlCl3) solution is added to the extract. The AlCl3 forms complexes with the flavonoids, resulting in a color change from pale yellow to intense yellow or orange.
Absorbance Measurement: The color change in the reaction mixture is quantified by measuring its absorbance at a specific wavelength (usually around 420-450 nm) by a UV-visible spectrophotometer. The calibration curve is obtained with standard solutions of known flavonoid concentrations to correlate absorbance with flavonoid content.
Calculation: The total flavonoid content in the onion peel is reported based on the absorbance values and the calibration curve. Results are typically stated in mg of flavonoids per g of dry onion peel (mg/g). This method provides a quantitative assessment of the total flavonoid in onion peel, which can be valuable for evaluating its potential health benefits and its use in various food and pharmaceutical applications.

Determination of Antioxidant Content using DPPH reagent:

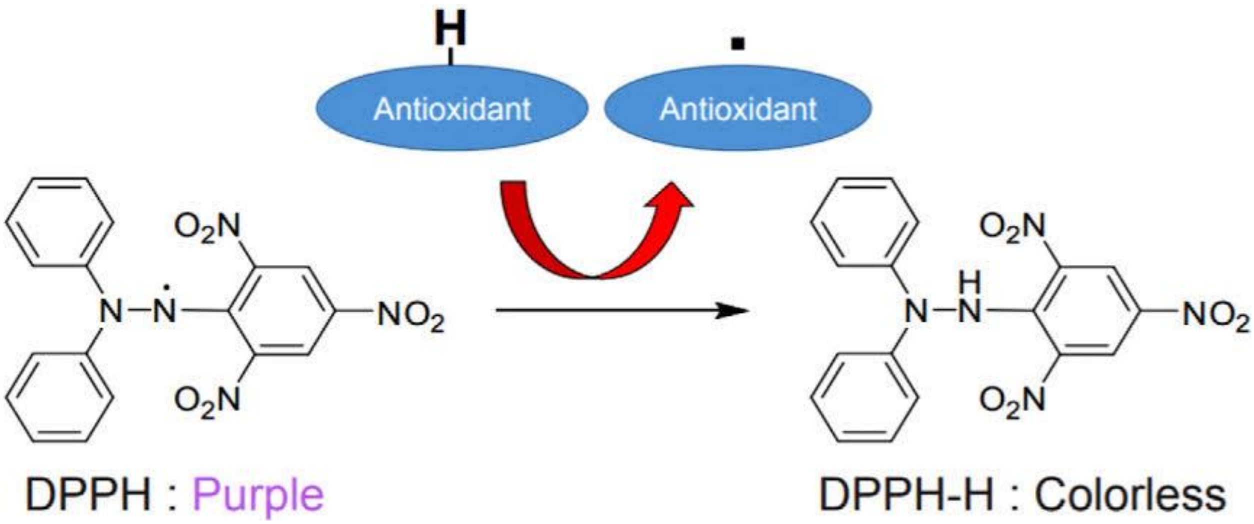

Fig 4.5 Determination of Antioxidant Content using DPPH reagent

The determination of antioxidant content in onion peel is crucial due to the potential health benefits associated with these compounds. One common method for assessing amount of antioxidant is the 2,2-diphenyl-1-picrylhydrazyl assay [12]. This method is widely used to measure the ability of substances to scavenge free radicals, which are highly reactive molecules responsible for oxidative damage in cells. To perform the DPPH assay on onion peel extracts, the following steps are typically followed:

Sample Preparation: Onion peels are collected, dried, and grounded into powder. This powder is then mixed with a suitable solvent, such as ethanol or methanol, to extract the antioxidant compounds.

DPPH Solution: A DPPH solution is obtained by dissolving DPPH in the chosen solvent. The solution appears deep purple due to the presence of unpaired electrons on the DPPH molecules.

Reaction: A small amount of the extract is added to the DPPH solution. The antioxidants in the extract react with the DPPH radicals, causing a color change from purple to yellow. This change is monitored spectrophotometric ally.

Measurement: The decrease in absorbance (usually around 517 nm) is recorded over time. A lower absorbance indicates a higher antioxidant capacity.

Calculation: The antioxidant activity is calculated as the percentage of DPPH radical scavenged by the onion peel extract. This can be determined using a standard curve of known antioxidant concentrations.

Data Interpretation: The results are expressed in terms of antioxidant activity, often reported as Trolox Equivalents or IC50 value.

The DPPH method provides valuable update of of antioxidant potential of various substances. High antioxidant activity indicates the presence of compounds like flavonoids and phenolic acids, which aids in the health-improving properties of onion peels. This information is valuable for both nutritional and pharmaceutical applications, helping to harness the health benefits of these natural antioxidants.

Determination of Reducing Sugar Content using Lane & Eynon Method:

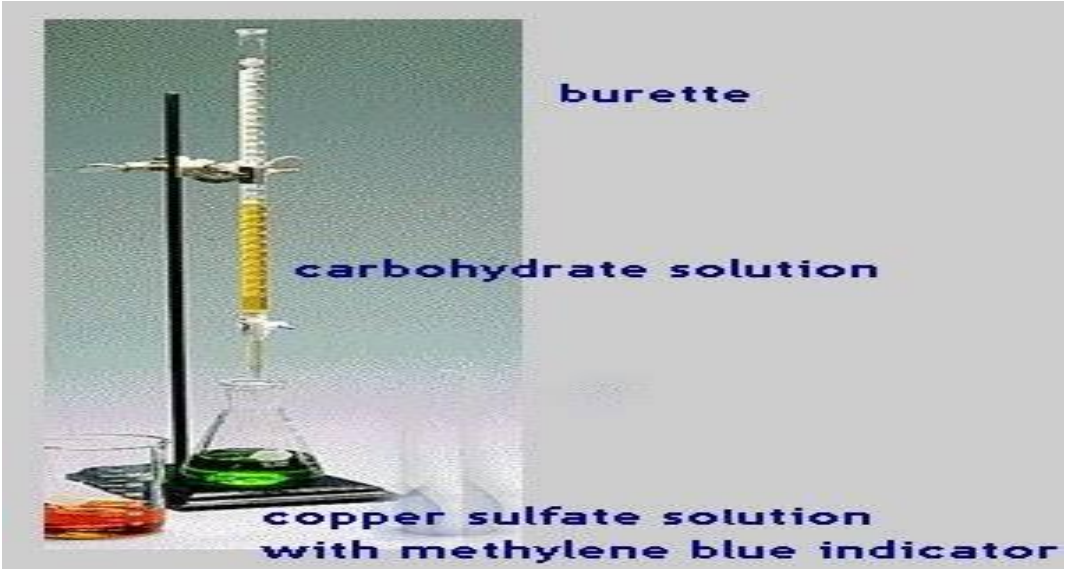

Fig 4.6 Determination of Reducing Sugar Content using Lane & Eynon Method

Determining the sugar content in onion peel using Fehling reagents involves a chemical reaction that quantifies reducing sugars present in the sample []. Here's a simplified explanation of the method:
Sample Preparation: First, onion peels are collected, dried, and ground into a fine powder. A known amount of this powder is then weighed accurately.
Extraction: The powdered onion peel is mixed with distilled water to create an aqueous extract. The extract is then filtered to remove solid particles, leaving behind a clear solution.
Fehling Reagent Preparation: Fehling reagent consists of two solutions, Fehling A (copper sulfate) and Fehling B (alkaline tartrate solution). These solutions are mixed in equal proportions shortly before use.
Reaction: A known volume of the onion peel extract is placed in a test tube or flask. Fehling reagent is added to the extract, and the mixture is heated on a hot plate. Reducing sugars in the onion peel extract (such as glucose and fructose) react with the Fehling reagent, causing a color change from blue to brick-red or orange.
Endpoint: The reaction is considered complete when the solution turns from blue to a stable brick-red or orange color, indicating that all reducing sugars have reacted.
Quantification: The amount of reducing sugars is measured by measuring the volume of Fehling reagent required to reach the endpoint. A calibration curve with known sugar concentrations can be used to calculate the sugar content in the onion peel extract.

This Fehling method is a classic and reliable technique for determining sugar content, commonly used in food science and agriculture to assess the sweetness of various plant materials, including onion peels. It provides valuable information for quality control, nutritional analysis, and research purposes.

Ethanol Fermentation Process by Saccharomyces cerevisiae :

KH_2PO_4	0.1%
$(NH_4)_2SO_4$	0.5%
$MgSO_4 \cdot 7H_2O$	0.05%
Yeast Extract	0.1%
pH	4.5

Table 4.1 Composition of Fermentation Media

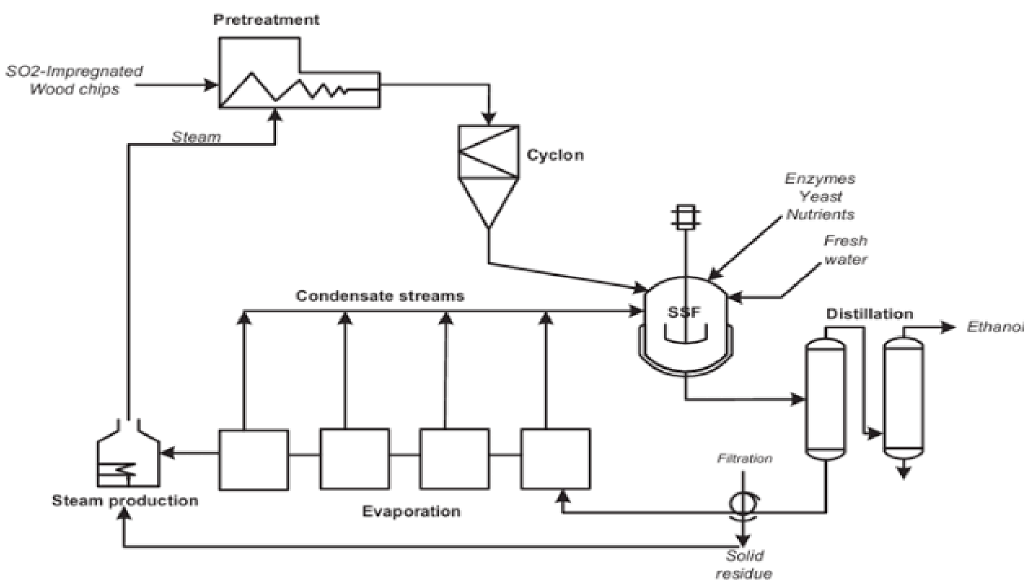

Fig 4.7 Schematic Diagram of Ethanol Fermentation

Ethanol, also known as ethyl alcohol, is a widely used chemical compound with a variety of applications, ranging from fuel production to alcoholic beverages. One of the most common methods for producing ethanol is through fermentation, a biological process that converts the sugars into alcohol and carbon dioxide by microorganisms. *Saccharomyces cerevisiae* is a prominent microorganism used in this process. This article delves into the ethanol fermentation process, subsequent distillation, and the determination of alcohol content using an alcoholmeter, with a focus on the role of *Saccharomyces cerevisiae.*

Ethanol Fermentation Process:

Introduction to *Saccharomyces cerevisiae* : *Saccharomyces cerevisiae*, a single-celled fungus, has been employed for centuries in various applications , like the production of bread, beer, and wine. Its ability to convert sugars into ethanol and carbon dioxide makes it a crucial microorganism in ethanol production.

Substrate Selection: In ethanol fermentation, the choice of substrate is vital. Common sources of fermentable sugars include sugarcane, corn, wheat, and grapes. These substrates are rich in glucose and fructose, which are readily metabolized by *Saccharomyces cerevisiae.*

Sugar Breakdown: The initial step in ethanol fermentation is the breakdown of sugars. *Saccharomyces cerevisiae* utilizes enzymes to convert complex sugars into simpler forms, primarily glucose and fructose. This process is essential to ensure a steady supply of fermentable sugars.

Fermentation: Once the sugars are broken down, fermentation begins. *Saccharomyces cerevisiae* metabolizes glucose and fructose through a series of enzymatic reactions, producing ethanol and carbon dioxide as byproducts. The quation for this reaction is:

$C_6H_{12}O_6$ (glucose) → 2 C_2H_5OH (ethanol) + 2 CO_2 (carbon dioxide)

Temperature and pH Control:

Optimal fermentation conditions are crucial for maximizing ethanol yield. The temperature and pH levels are carefully controlled to maintain the yeast's activity. Typically, a temperature range of 25-30°C and a pH of around 4-6 are maintained throughout the fermentation process.

Ethanol Production: As the fermentation progresses, ethanol accumulates in the liquid medium. The concentration of ethanol in the fermentation broth gradually increases until it reaches a desired level or the yeast's tolerance limit, at which point the fermentation process is halted.

Distillation of Ethanol: After fermentation, the ethanol-containing liquid, known as "mash" or "wash," contains a mixture of ethanol, water, and various impurities. To obtain a higher concentration of ethanol, distillation is employed.

Distillation Process: The distillation process involves heating the mash to separate the components based on their boiling points. Ethanol has a lower boiling point than water, allowing it to vaporize and be collected separately.

Collecting Ethanol As the vaporized ethanol rises through the distillation column, it is condensed back into a liquid form. The resulting liquid, known as "distillate" or "rectified spirit," contains a significantly higher ethanol content than the original mash.

Determination of Alcohol Content:

Accurately measuring the alcohol content of the distillate is crucial for various applications, including regulatory compliance, quality control in the beverage industry, and fuel blending. This is where the alcoholmeter comes into play.

Alcoholmeter Principle:

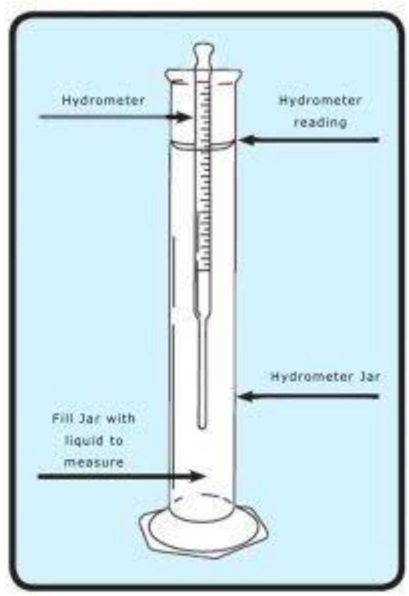

Fig 4.8 Alcoholmeter

An alcoholmeter is a specialized hydrometer designed to measure the alcohol content of a liquid. It operates on the principle that the buoyancy of an object in a liquid is directly proportional to the density of the liquid [13]. The denser the liquid, the higher the object (alcoholmeter) will float.
Calibration: Before using an alcoholmeter, it must be calibrated to ensure accurate readings. This is typically done using a sample of known alcohol content, often referred to as a "proof standard" in the beverage industry.
The Measurement Process: To determine the alcohol content of the distillate, the alcoholmeter is carefully immersed in the liquid. The point at which the alcoholmeter settles in the liquid indicates the alcohol content, usually expressed as a percentage by volume (ABV).
Correcting for Temperature: Temperature can affect the accuracy of alcoholmeter readings, so it is essential to correct for temperature variations. Most alcoholmeters come with temperature correction tables or scales to adjust the reading based on the sample's temperature.
Interpreting Results: Once the alcoholmeter reading is obtained and corrected for temperature, the alcohol content of the distillate is determined. This information is valuable for regulatory compliance and quality control purposes.

Chapter-5

Result and Discussion

Result and Discussion:

Composition of Onion Peel :

Table 5.1 shows the composition of onion peel. It was found that onion peel contained higher amount of carbohydrate (77.4%) and low protein (0.88%), ash (0.517%) and crude fiber (18.72%). Previous study of Ly et al. revealed that onion peel had a protein and ash content higher than that of garlic peel (0.57%) (0.49%) respectively while carbohydrate content (93.26%) was found more in garlic peel [26].

Characteristics (%)	Onion Peel
Ash	0.517%
Carbohydrate	77.4%
Crude Fibre	18.72%
Fat	0.08
Moisture Content	12.195
Protein	0.88

Table 5.1 Proximate Composition of onion peel

Total Flavonoids Content of Onion Peel :

Total flavonoid content of onion peel ethanolic extract are shown in Table 5.2. From Fig 5.1.1 it shows that amount of total flavonoid content in onion peel is 68.89 mg QE/ g dw sample. Previously it was reported total flavonoid of 19.50 mg QE /g dw from outer scales of onion [27]. Onion has been reported as one of the major sources of dietary flavonoids . The brown skin of red onion contain the highest level of phenolics . Flavonoids are known to reduce the risk of inhibit the initiation, heart disease, and progression of tumors . Phenolic compounds and antioxidant are prominent components of aromatic plants that are engaged in cooking to enhance the sensory quality of foods [29].

Extract	Total Flavonoids
Onion Peel Extract in Ethanol	68.89 mg QE/ g dw.

Table 5.2 Total Flavonoids Content of Onion Peel

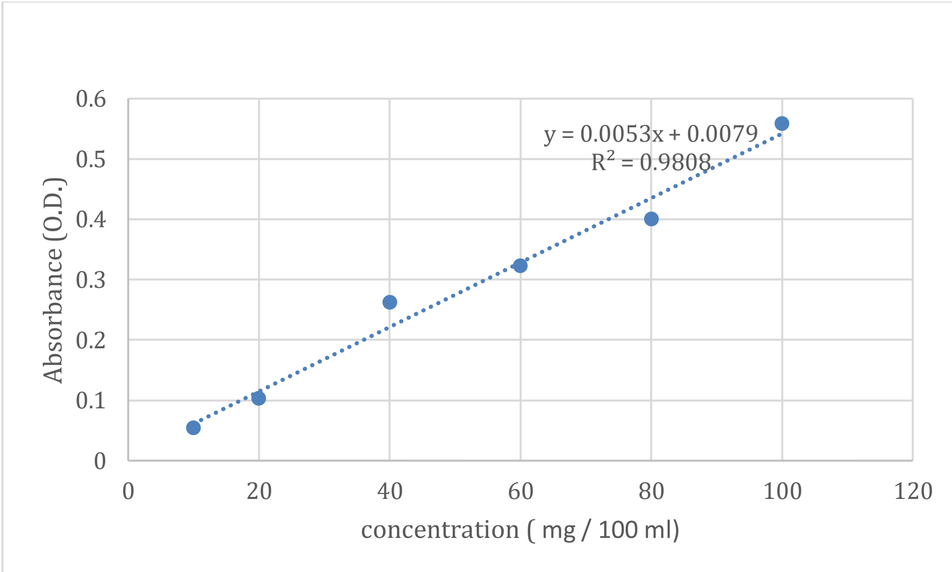

Fig 5.1.1 Plot of Concentration vs Absorbance for Total Flavonoids Content

From the above linear equation,

Therefore, Total Flavonoids content of Onion Peel = 68.89 mg QE / g dw sample.

Antioxidant Content of Onion Peel:

Table 5.3 represents the antioxidant activity of onion peel extract. In addition, brown skin, the top and bottom sections of onion showed better antioxidant capacity than inner fleshy leaves [26]. The flavonoid, quercetin, an antioxidant found in onions has been shown to eliminate free radicals in the body, inhibits low-density lipoprotein oxidation and helps to circumvent the harmful effects of heavy metal ions [28].

Here % Antioxidant Activity = (Ac-As) / Ac

Where Ac = Control Reaction Absorbance

As = Testing Specimen Absorbance

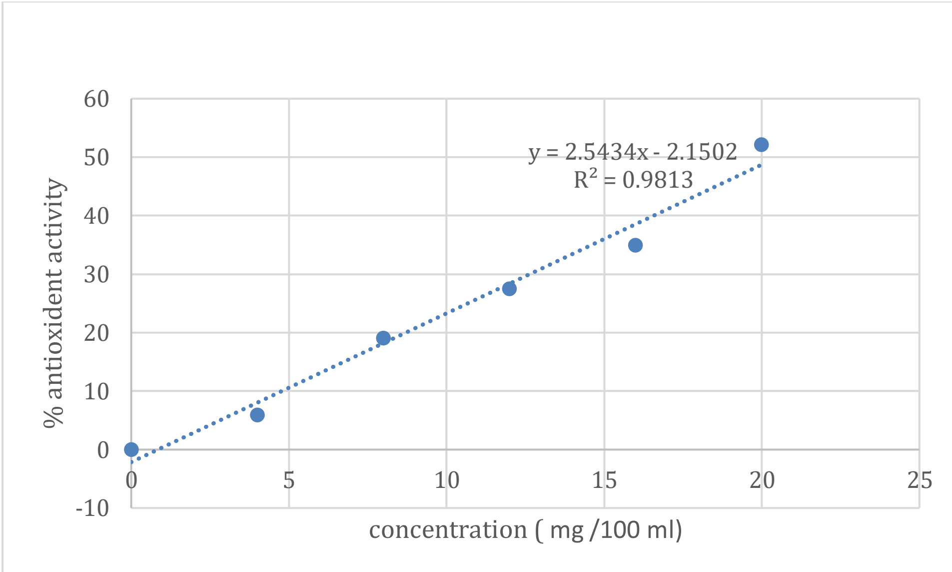

Fig 5.1.2 Plot of antioxidant activity vs concentration of onion peel

Now, using the above linear equation,

Antioxidant Content = IC 50 = 20.504 mg/ g dw sample

Optimization of various parameters of Ethanol Production:

Fermentation Time Optimization: Five different fermentation time is considered keeping rest of the parameters fixed to obtain optimum fermentation time. This is done for both substrate and control (taking sucrose instead of onion peel). The results are shown in Table 5.3 and 5.4.

For Different Fermentation Time (Sample):

Time (hr)	Temperature (°C)	Inoculum Concentration (%)	pH	Substrate Concentration (%)	Cell mass (g/ l)	Residual Sugar (%)	Theoritical Alcohol Content (%)	Actual Alcohol content (%)	Efficiency (%)
24	35	10	4.5	15.24	2.3	5.58	6.25	5.2	83.2
48	35	10	4.5	15.24	2.7	5.45	6.33	5.95	93.9
72	35	10	4.5	15.24	3	5.106	6.56	4.07	62
96	35	10	4.5	15.24	3.1	5	6.62	3.9	58.9
120	35	10	4.5	15.24	3.3	4.8	6.75	3.85	57

Table 5.3 Parameters for Different Fermentation Time (for sample)

For Different Fermentation Time (Control) :

Sl. No.	Time (hr)	Temperature (°C)	Inoculum Concentration (%)	pH	Substrate Concentration (%)	Cell mass (g/ l)	Residual Sugar (%)	Theoritical Alcohol Content (%)	Actual Alcohol content (%)	Efficiency (%)
1	24	35	10	4.5	15.24	2.1	4.213	7.13	4.11	57.6
2	48	35	10	4.5	15.24	2.6	4.07	7.23	4.02	55.6
3	72	35	10	4.5	15.24	2.2	3.94	7.31	3.94	53.9
4	96	35	10	4.5	15.24	2.7	2.31	8.36	3.85	46.1
5	120	35	10	4.5	15.24	3.3	2.243	8.41	3.79	45.1

Table 5.4 Parameters for different fermentation time (for control)

A 48-hour fermentation time is often considered optimal for ethanol production due to several factors [17]. Firstly, it allows yeast or other microorganisms to consume a substantial amount of sugar, maximizing ethanol yield. Beyond this point, diminishing returns occur as the yeast begins to produce unwanted byproducts like acetic acid. Secondly, a longer fermentation period can lead to increased contamination risk, reducing ethanol quality. Previously it was observed [17] that 8 day fermentation period yields maximum ethanol yield from onion and cabbage peel.

Fermentation Temperature Optimization :

Three different fermentation temperature is considered keeping rest of the parameters fixed to obtain optimum fermentation temperature. This is done for both substrate and control (taking sucrose instead of onion peel). The results are shown in Table 5.5 and 5.6.

For Different Temperature (Sample) :

Sl. No.	Time (hr)	Temperature (°C)	Inoculum Concentration (%)	pH	Substrate Concentration (%)	Cell mass (g/ l)	Residual Sugar (%)	Theoritical Alcohol Content (%)	Actual Alcohol content (%)	Efficiency
1	48	25	10	4.5	15.24	2.3	4.9	6.69	4.237	63.33
2	48	30	10	4.5	15.24	2.6	4.8	7.047	4.348	61.7
3	48	35	10	4.5	15.24	2.7	4.8	6.754	4.286	63.46

Table 5.5 Parameters For Different Temperature (Sample)

For Different Temperature (Control) :

Sl. No.	Time (hr)	Temperature (°C)	Inoculum Concentration (%)	pH	Substrate Concentration (%)	Cell mass (g/ l)	Residual Sugar (%)	Theoritical Alcohol Content (%)	Actual Alcohol content (%)	Efficiency)%)
1	48	25	10	4.5	15.24	1.7	4.21	7.13	3.51	49.2
2	48	30	10	4.5	15.24	2.2	4.12	7.19	3.82	53.1
3	48	35	10	4.5	15.24	2.3	4.03	7.25	3.43	47.3

Table 5.6 Parameters For Different Temperature (Control)

At 30°C, yeast metabolism is optimized, promoting efficient conversion of sugars into ethanol and carbon dioxide[18]. Additionally, this temperature minimizes the risk of contamination by unwanted microorganisms, as many harmful bacteria and fungi thrive at lower or higher temperatures. Moreover, 30°C strikes a balance between fast fermentation rates and the production of high-quality ethanol, making it the preferred temperature for ethanol production in industrial settings. Previous work demonstrated that the best performance of ethanol production is at 37°C with fermentation of 86.27% [32] using sucrose as substrate.

pH of Fermentation Optimization :

Five different pH of fermentation is considered keeping rest of the parameters fixed to obtain optimum pH of fermentation . This is done for both substrate and control (taking sucrose instead of onion peel) and the results are shown in Table 5.7 and 5.8.

For Different pH (Sample) :

Sl. No.	Time (hr)	Temperature (°C)	Inoculum Concentration (%)	pH	Substrate Concentration (%)	Cell mass (g/ l)	Residual Sugar (%)	Theoritical Alcohol Content (%)	Actual Alcohol content (%)	Efficiency)%)
1	48	30	10	3.5	15.2	2.5	5.217	6.459	3.845	59.53
2	48	30	10	4	15.2	2.7	5.106	6.531	4.268	65.35
3	48	30	10	4.5	15.2	2.8	4.8	6.728	4.861	72.33
4	48	30	10	5	15.2	2.5	5	6.59	4.109	62.35
5	48	30	10	5.5	15.2	2.2	5	6.59	3.703	56.19

Table 5.7 Parameters For Different pH (Sample)

For Different pH (Control) :

Sl. No.	Time (hr)	Temperature (°C)	Inoculum Concentration (%)	pH	Substrate Concentration (%)	Cell mass (g/ l)	Residual Sugar (%)	Theoritical Alcohol Content (%)	Actual Alcohol content (%)	Efficiency)%)
1	48	30	10	3.5	15.2	2.1	2.376	8.297	3.703	44.63
2	48	30	10	4	15.2	2.1	2.353	8.312	4	48.12
3	48	30	10	4.5	15.2	2.1	2.308	8.341	4.054	48.61
4	48	30	10	5	15.2	2.1	2.353	8.312	4.054	48.77
5	48	30	10	5.5	15.2	1.9	2.33	8.327	3.245	38.97

Table 5.8 Parameters for Different pH (control)

A pH of 4.5 is considered optimum for ethanol fermentation for several reasons. Firstly, itmcreates an environment conducive to the activity of yeast, typically *Saccharomyces cerevisiae*, which is commonly used in ethanol production. At this pH, yeast enzymes responsible for ethanol production, such as alcohol dehydrogenase, work most efficiently [19]. Previous research work indicates that pH 4.5 provides optimum ethanol and biomass yield as compared to pH 5 using glucose syrup as substrate [31].

Inoculum Concentration Optimization :

Three different inoculum concentration is considered keeping rest of the parameters fixed to obtain optimum inoculum concentration . This is done for both substrate and control (taking sucrose instead of onion peel) and the result is shown table 5.9 and 5.10.

For Different Inoculum Concentration (Sample) :

Sl. No.	Time (hr)	Temperature (°C)	Inoculum Concentration (%)	pH	Substrate Concentration (%)	Cell mass (g/ l)	Residual Sugar (%)	Theoritical Alcohol Content (%)	Actual Alcohol content (%)	Efficiency)%)
1	48	30	5	4.5	15.24	2.3	4.898	6.691	3.846	51.48
2	48	30	10	4.5	15.24	2.8	4.8	6.754	4.651	68.86
3	48	30	15	4.5	15.24	3.1	4.615	6.874	4.321	62.86

Tablr 5.9 Parametrs For Different Inoculum Concentration (Sample)

For Different Inoculum Concentration (Control) :

Sl. No.	Time (hr)	Temperature (°C)	Inoculum Concentration (%)	pH	Substrate Concentration (%)	Cell mass (g/ l)	Residual Sugar (%)	Theoritical Alcohol Content (%)	Actual Alcohol content (%)	Efficiency)%)
1	48	30	5	4.5	15.24	1.9	3.243	7.761	3.521	45.37
2	48	30	10	4.5	15.24	2.3	2.449	8.275	4.268	51.58
3	48	30	15	4.5	15.24	2.7	2.308	8.367	4.109	49.1

Tablr 5.10 Parametrs For Different Inoculum Concentration (Control)

A 10% inoculum concentration is considered optimum for ethanol fermentation due to a delicate balance between microbial activity and substrate availability. In this concentration, there are enough yeast cells to efficiently convert sugars into ethanol without overwhelming the system. Too low of an inoculum may result in slow fermentation or susceptibility to contamination, while too high of an inoculum can lead to competition for nutrients and reduced ethanol yields [17]. The 10% concentration allows for a healthy yeast population to thrive, ensuring a robust fermentation process, higher ethanol production, and reduced risk of unwanted byproducts, making it the ideal choice for ethanol production. It was reported that 6% inoculum concentration resulted optimum ethanol yield [32] using banana peel as substrate.

Substrate Concentration Optimization :

Four different substrate concentration is considered keeping rest of the parameters fixed to obtain optimum substrate concentration . This is done for both substrate and control (taking sucrose instead of onion peel) and the results are shown in Table 5.11 and 5.12.

For Different Substrate Concentration (Sample):

Sl. No.	Time (hr)	Temperature (°C)	Inoculum Concentration (%)	pH	Substrate Concentration (%)	Cell mass (g/ l)	Residual Sugar (%)	Theoritical Alcohol Content (%)	Actual Alcohol content (%)	Efficiency)%)
1	48	30	10	4.5	10	2	3.158	4.426	3.623	81.8
2	48	30	10	4.5	12	2.2	3.871	5.259	4.478	85.15
3	48	30	10	4.5	15	2.6	4.615	6.718	4.839	72.03
4	48	30	10	4.5	18	2.7	6.66	7.336	4.687	63.89

Table 5.11 Parameters For Different Substrate Concentration (Sample)

For Different Substrate Concentration (Control):

Sl. No.	Time (hr)	Temperature (°C)	Inoculum Concentration (%)	pH	Substrate Concentration (%)	Cell mass (g/ l)	Residual Sugar (%)	Theoritical Alcohol Content (%)	Actual Alcohol content (%)	Efficiency)%)
1	48	30	10	4.5	10	1.7	2.069	5.131	3.906	76.12
2	48	30	10	4.5	12	1.9	1.967	6.491	4.109	63.3
3	48	30	10	4.5	15	2.3	2.264	8.239	4.285	52
4	48	30	10	4.5	18	2.4	5.106	8.342	4.166	49.94

Table 5.12 Parameters For Different Substrate Concentration (Control)

A 15% substrate concentration is often considered optimum for ethanol fermentation due to several key factors. At this concentration, the balance between substrate (usually sugars) and yeast activity is ideal. Higher concentrations can overwhelm the yeast, leading to reduced yield and potential stress on the microorganisms. Conversely, lower concentrations result in underutilization of yeast's fermentation potential [19]. From earlier work it was reported highest substrate concentration upto 12% resulted in higher ethanol yield using leaves of *Mangifera indica* (mango), *Syzygium cumini* (jamun) as substrate. [35].

The following plots (in Fig 5.3.1 and 5.3.2) are obtained using Michaelis- Menten Equation for both sample and control of varying substrate concentration.

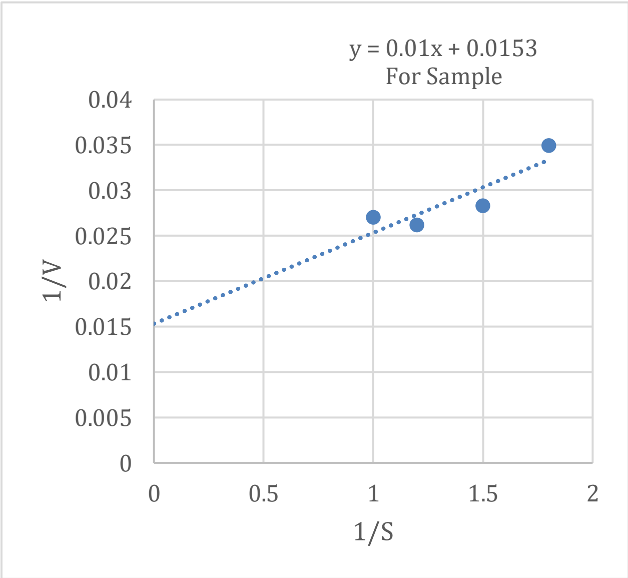

Fig 5.3 1/V vs 1/S plot for onion peel

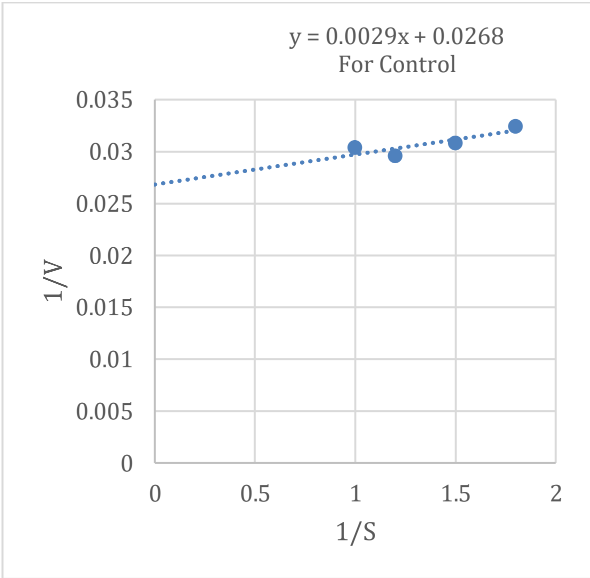

Fig 5.3 2/V vs 1/S plot for control

Optimizing all the five parameters it is found that fermentation time of 48 hr, temperature 30 °C , pH 4.5, inoculum concentration 10% and substrate concentration of 15% yield maximum amount of ethanol (4.839 % v/v).

Discussion:

Fermentation time plays a pivotal role in ethanol production, a critical process in the biofuel industry. The duration of fermentation directly influences the yield and quality of ethanol. Longer fermentation periods typically result in higher ethanol concentrations, as yeast or bacteria have more time to convert sugars into alcohol. However, extended fermentation can also lead to the production of undesirable byproducts like acetic acid, reducing ethanol's purity. Balancing fermentation time is essential to maximize ethanol output while maintaining efficiency. Careful monitoring and control of fermentation duration enable producers to optimize their processes, striking a delicate equilibrium between quantity and quality in ethanol production.

The fermentation temperature plays a pivotal role in ethanol production. It directly influences the efficiency and quality of the process. Generally, yeast used for fermentation thrives within a specific temperature range, typically between 25°C to 35°C. Cooler temperatures slow down fermentation, while higher temperatures can be detrimental, leading to the production of unwanted byproducts and yeast stress.

Optimal temperature control is crucial for maximizing ethanol yield. Elevated temperatures can accelerate the process but might compromise ethanol purity. Conversely, cooler temperatures can produce a cleaner ethanol product but may extend fermentation time. Striking the right balance between temperature, yeast strain, and other factors is essential to achieve highmethanol production rates while maintaining product quality.

The pH of the fermentation media in ethanol production is a critical factor that influences the efficiency of the process. Typically, the optimal pH range for ethanol fermentation by yeast strains like *Saccharomyces cerevisiae* is between 4.0 and 5.0. Maintaining the appropriate pH level is essential because it affects yeast metabolism and ethanol yield. Too acidic or alkaline conditions can inhibit yeast growth and fermentation. pH control is achieved through the addition of acids or bases as needed, ensuring a favorable environment for yeast to convert sugars into ethanol. Accurate pH management is pivotal for maximizing ethanol production in the biofuel industry.

In ethanol production, inoculum concentration plays a critical role in the fermentation process. It refers to the quantity of microorganisms, typically yeast strains like *Saccharomyces cerevisiae*, introduced into the fermentation broth. The inoculum concentration affects fermentation efficiency and ethanol yield. A higher concentration of yeast accelerates fermentation, as more cells are available to convert sugars into ethanol. However, excessively high concentrations can lead to increased competition for nutrients and oxygen, potentially compromising yeast performance. Finding the optimal inoculum concentration is essential to strike a balance between fast fermentation rates and efficient ethanol production, ultimately impacting the economics of bioethanol production.

Substrate concentration plays a pivotal role in ethanol production. As the concentration of fermentable sugars, typically derived from crops like corn or sugarcane, increases, so does the potential ethanol yield. This phenomenon is due to the higher availability of substrates for yeast fermentation. However, there's a limit to this benefit. Beyond a certain point, substrate concentration can inhibit fermentation by creating stressful conditions for the yeast, impacting ethanol productivity and purity. Striking the right balance between substrate concentration and yeast health is essential for maximizing ethanol production. This delicate equilibrium is crucial in optimizing the efficiency of biofuel production processes

Chapter-6

Conclusion and Future Outlook

Conclusion and Future Outlook

In conclusion, the endeavor to produce bioethanol from onion peel waste has yielded significant insights into the realm of sustainable energy production and waste management. This project aimed to address the dual challenges of finding alternative renewable energy sources and managing agricultural waste effectively. Through a systematic exploration of the process, its feasibility, efficiency, and potential impacts, this study has contributed to the growing body of knowledge in green technologies.

Throughout the course of this project, key findings have emerged that underscore the viability of utilizing onion peel waste as a valuable feedstock for bioethanol production. The thorough characterization of onion peel waste provided essential data on its chemical composition, confirming the presence of essential sugars and cellulose-rich materials suitable for bioconversion. The subsequent bioethanol production process using yeast fermentation showcased promising results, with notable ethanol yields obtained from the converted sugars in the onion peel waste.

One of the remarkable achievements of this project lies in the efficient utilization of an otherwise discarded agricultural byproduct. By transforming onion peel waste into a valuable resource like bioethanol, the project addresses both environmental and economic concerns. The successful conversion of waste into bioethanol offers a tangible solution to reducing the burden on landfill while simultaneously contributing to the generation of renewable energy. This aligns with the broader goal of achieving sustainability and reducing our reliance on fossil fuels.

Furthermore, the project sheds light on the potential environmental benefits associated with bioethanol production from onion peel waste. With the current global emphasis on mitigating climate change and reducing greenhouse gas emissions, the use of bioethanol as a cleaner alternative to conventional fossil fuels becomes all the more relevant. By reusing agricultural waste to produce bioethanol, this process could lead to a reduction in net carbon emissions and contribute to a more circular and environmentally conscious approach to energy production.

Looking ahead, this project paves the way for future research and development in the field. While the results obtained are promising, there is room for optimization and fine-tuning of the production process. Exploring advanced fermentation techniques, optimizing reaction conditions, and investigating potential byproducts or co-products from the bioconversion process could enhance the overall efficiency and economic viability of the process.

In conclusion, the production of bioethanol from onion peel waste presents a sustainable solution that aligns with the principles of waste reduction and renewable energy generation. This project serves as a testament to the potential of converting agricultural waste into a valuable resource, with implications spanning waste management, renewable energy, and environmental sustainability. As we continue to explore innovative ways to address the challenges of the modern world, projects like this underscore the importance of thinking creatively and leveraging the untapped potential in our surroundings. Through the convergence of science, technology, and environmental consciousness, we can forge a path toward a more sustainable and harmonious future.

References

Reference:

1. Maji, S., Dwivedi, D. H., Singh, N., Kishor, S., & Gond, M. (2020). Agricultural waste: Its impact on environment and management approaches. Emerging eco-friendly green technologies for wastewater treatment, 329-351
2. Kumar, P., & Joshi, L. (2013). Pollution caused by agricultural waste burning and possible alternate uses of crop stubble: a case study of Punjab. Knowledge systems of societies for adaptation and mitigation of impacts of climate change, 367-385.
3. Khan, N., Yaqub, G., Hafeez, T., & Tariq, M. (2020). Assessment of health risk due to pesticide residues in fruits, vegetables, soil, and water. Journal of Chemistry, 2020, 1-7.
4. Amran, M. A., Palaniveloo, K., Fauzi, R., Satar, N. M., Mohidin, T. B. M., Mohan, G., ... & Sathiya Seelan, J. S. (2021). Value-added metabolites from agricultural waste and application of green extraction techniques. Sustainability, 13(20), 11432.
5. Shabir, I., Pandey, V. K., Dar, A. H., Pandiselvam, R., Manzoor, S., Mir, S. A., ... & Trif, M. (2022). Nutritional profile, phytochemical compounds, biological activities, and utilisation of onion peel for food applications: a review. Sustainability, 14(19), 11958.
6. Eraky, M., Nasr, M., Elsayed, M., Ai, P., & Tawfik, A. (2023). Synergistic interaction of tween 20 and magnesium@ functionalized graphene oxide nano-composite for dual productivity of biohydrogen and biochar from onion peel waste. Renewable Energy, 216, 119082.
7. Celano, R., Docimo, T., Piccinelli, A. L., Gazzerro, P., Tucci, M., Di Sanzo, R., ... & Rastrelli, L. (2021). Onion peel: Turning a food waste into a resource. Antioxidants, 10(2), 304.
8. Nielsen, S. S. (2010). Determination of moisture content. Food analysis laboratory manual, 17-27.
9. Marshall, M. R. (2010). Ash analysis. Food analysis, 4, 105-116.
10. Naumann, K. (1938). Determination of crude fibre. Landwirtschaftliche Versuchsstation, 129, 171-176.
11. Matić, P., Sabljić, M., & Jakobek, L. (2017). Validation of spectrophotometric methods for the determination of total polyphenol and total flavonoid content. Journal of AOAC International, 100(6), 1795-1803.
12. Chen, Z., Bertin, R., & Froldi, G. (2013). EC50 estimation of antioxidant activity in DPPH assay using several statistical programs. Food chemistry, 138(1), 414-420.
13. Hertiavi, M. A., Marwoto, P., Iswari, R. S., & Cahyono, E. (2021). The Use of a Modest Alcohol Meter. Jurnal Penelitian Pendidikan IPA, 7(SpecialIssue), 126-130.
14. Lin, Y., Zhang, W., Li, C., Sakakibara, K., Tanaka, S., & Kong, H. (2012). Factors affecting ethanol fermentation using *Saccharomyces cerevisiae* BY4742. Biomass and bioenergy, 47, 395-401.
15. Şener, A., Canbaş, A., & ÜNAL, M. (2007). The effect of fermentation temperature on the growth kinetics of wine yeast species. Turkish Journal of Agriculture and Forestry, 31(5), 349-354.
16. Wu, Y., Wang, C., Zheng, M., Zuo, J., Wu, J., Wang, K., & Yang, B. (2017). Effect of pH on ethanol-type acidogenic fermentation of fruit and vegetable waste. Waste Management, 60, 158-163.
17. Laluce, C., Tognolli, J. O., De Oliveira, K. F., Souza, C. S., & Morais, M. R. (2009). Optimization of temperature, sugar concentration, and inoculum size to maximize ethanol production without significant decrease in yeast cell viability. Applied Microbiology and Biotechnology, 83, 627-637.
18. Thatipamala, R., Rohani, S., & Hill, G. A. (1992). Effects of high product and substrate inhibitions on the kinetics and biomass and product yields during ethanol batch fermentation. Biotechnology and bioengineering, 40(2), 289-297.

19. Genemo, T. G., Erebo, D. L., & Gabre, A. K. (2021). Optimizing bio-ethanol production from cabbage and onion peels waste using yeast (*Saccharomyces cerevisiae*) as fermenting agent.
20. Robati, R. (2013). Bio-ethanol production from green onion by yeast in repeated batch. Indian journal of microbiology, 53, 329-331.
21. Mushimiyimana, I., Sirimu, C., Niyitanga, F., & Niyonsaba, L. (2021). Potential in Bioethanol Production from Various Agro Wastes Fermenting by Microorganisms Using Carrot Peel, Onion Peel, Potato Peel and Sugar Beet Peel as Substrates.
22. Mushimiyimana, I., & Tallapragada, P. (2017). Production of bioethanol from agro waste by saccharification and fermentation. Jordan J Agric Sci, 13(1).
23. Mushimiyimana, I., & Tallapragada, P. (2017). Bioethanol production from agro wastes by acid hydrolysis and fermentation process.
24. Sagar, N. A., Kumar, Y., Singh, R., Nickhil, C., Kumar, D., Sharma, P., ... & Tarafdar, A. (2022). Onion waste based-biorefinery for sustainable generation of value-added products. Bioresource Technology, 362, 127870.
25. Shahid, I., Hussain, G., Anis, M., Farooq, M. U., Usman, M., Fouad, Y., & Krzywanski, J. (2023). Enzymatic Co-Fermentation of Onion Waste for Bioethanol Production Using *Saccharomyces cerevisiae* and Pichia pastoris. Energies, 16(5), 2181.
26. Ifesan, B.O.T., Fadipe, E.A. & Ifesan, B.T. 2014. Investigation of Antioxidant and Antimicrobial properties of garlic peel extract (Allium sativum) and its use as the natural food additive in cooked beef. Journal of Scientific Research and Reports 3: 712-721.
27. Benítez, V., Mollá, E., Martín-Cabrejas, M.A., López-Andréu, J.F., Downes, K., Terry, L.A. & Esteban, R.M. 2011. Study of bioactive compound content in different onion sections. Plant Foods for Human Nutrition 66: 48- 57.
28. Ly, T.N., Hazama, C., Shimoyamada, M., Ando, H., Kato, K. & Yamauchi, R. 2005. Antioxidative compounds from the outer scales of Onion. Journal of Agriculture and Food Chemistry 53: 8183-8189.
29. Tang, X. & Cronin, D. A. 2007. The effects of brined onion extracts on lipid oxidation and sensory quality in refrigerated cooked turkey breast rolls during storage. Food Chemistry 100: 712–718.
30. Techaparin, A., Thanonkeo, P., & Klanrit, P. (2017). High-temperature ethanol production using thermotolerant yeast newly isolated from Greater Mekong Subregion. Brazilian journal of Microbiology, 48, 461-475.
31. Bajpai, P., & Margaritis, A. (1987). The effect of temperature and pH on ethanol production by free and immobilized cells of Kluyveromyces marxianus grown on Jerusalem artichoke extract. Biotechnology and bioengineering, 30(2), 306-313.
32. Laluce, C., Tognolli, J. O., De Oliveira, K. F., Souza, C. S., & Morais, M. R. (2009). Optimization of temperature, sugar concentration, and inoculum size to maximize ethanol production without significant decrease in yeast cell viability. Applied Microbiology and Biotechnology, 83, 627-637.
33. Chen, S. L. (1981). Optimization of batch alcoholic fermentation of glucose syrup substrate. Biotechnology and Bioengineering, 23(8), 1827-1836.
34. Sharma, N., Kalra, K. L., Oberoi, H. S., & Bansal, S. (2007). Optimization of fermentation parameters for production of ethanol from kinnow waste and banana peels by simultaneous saccharification and fermentation. Indian Journal of Microbiology, 47, 310-316.
35. Mutreja, R., Das, D., Goyal, D., & Goyal, A. (2011). Bioconversion of agricultural waste to ethanol by SSF using recombinant cellulase from Clostridium thermocellum. Enzyme research, 2011.
36. Khan, T,H. (1979). Titrimetric determination of reducing sugars with copper (II) sulphate. Analyst, 104(1236), 261-265.