ETHANOL PRODUCATION FROM SWEET POTATO PEELS AND ITS CHARACTERIZATION

A DISSERTATION

SUBMITTD IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE

OF

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

Submitted by:

Dilkush Meena

2K21/MSCBIO/12

Under the supervision of

Prof. Jai Gopal Sharma

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

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CANDIDATE'S DECLARATION

I, Dilkush Meena, Roll No -2K21/MSCBIO/12 student of Master of Science. (Biotechnology), hereby declare that the project Dissertation titled " ETHANOL PRODUCTION FROM SWEET POTATO PEELS AND CHARACTERIZATION "which is submitted by me to the Department of Biotechnology - Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science , is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

Place: Delhi

Date 30/05/2023

Dilkush Meena

2K21/MSCBIO/12

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Place: Delhi

Date; 23/05/2023

Prof. Jai Gopal Sharma
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ACKNOWLEDGEMENT

The success and final outcome of this project required a lot of guidance and assistance from many people and we are extremely fortunate to have got this all along the completion of this project work.

We wish to express our gratitude towards my project Supervisor, Prof. Jai Gopal Sharma, Department of Biotechnology, Delhi Technological University, who provided us a golden opportunity to work under their able guidance. Their scholastic guidance and sagacious suggestions helped me to complete the project on time

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We are thankful to and fortunate enough to get constant encouragement, support and guidance from all teaching staffs of Department of Biotechnology, which helped me in successfully completing my project work. We are also thankful to PhD scholar Neha Tiwari for their constant support and motivation.

Finally, yet importantly, we would like to express our heartfelt thanks to our beloved family and friends who have endured our long working hours and whose motivation kept us going.

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Dilkush Meena

ABSTRACT:-

Fossil fuels (petroleum and coal) are among the natural energy supplies that are being used up quickly and are only expected to be available for a short period of time. Bioethanol is the most promising of the various alternative is enrgy sources that can replace natural enrgy resources because it is biological and renewable, typically derived from energy crops like maize, sugarcane, cassava, and sweet potato as well as byproducts of agriculture and forestry. Alcohol known as bioethanol is created by fermenting and distilling different feedstock (such as sugar, starch, and cellulose).

Which have undergone enzymatic or acid hydrolysis to become simple sugars. One of the most significant starchy crops, the sweet potato (Ipomoea balatas L.) has a short growth cycle (90–120 days) and may grow in a variety of agro-climatic situations. The invention of a simultaneous saccharification and fermentation process is the most significant process advancement developed for the enzymatic hydrolysis of diverse starch-containing crops and biomass. By removing the separate (saccharification) reactor, this approach uses thermo tolerant are yeast strains to reduce the number of reactors required. More significantly, however preventing the issue of product removal brought on by the accumulation of enzymes such celosias and AMG, which stop starch breakdown. It contrast, it concurrently applies fermenting microorganisms and a scarifying enzyme (AMG). AMG processes starch into sugars, and the fermentative microbes then turn the carbohydrates into ethanol. In this study, the effects of time (incubation) on the enzymatic liquefaction and saccharification of sweet potatoes were examined. Additional emphasis was placed on the use of SSF in the ethanol production process using liquefied sweet potato starchy biomass.

Key Words: Sweet Potato, Bio-Ethanol, Fermentation, Hydrolysis, Fermentable Sugar

CONTENTS

List of Table & Figure Captions

CHAPTER 1. Introduction

Ethanol production from sweet potato peels is an emerging field in the realm of biofuel research. Sweet potatoes are widely cultivated worldwide and are a valuable food crop. However, the peels generated as a by-product during sweet potato processing often go to waste. Utilizing these peels for ethanol production presents an opportunity to convert a waste material into a valuable resource, promoting sustainability and contributing to the production of renewable energy.(1)

Bioethanol, a type of alcohol, is a renewable and environmental is friendly alternative to fossil fuels. It can be used as a transportation fuel or blended with gasoline to reduce greenhouse gas emissions and dependence on non-renewable energy sources. (2) Sweet potato peels, which contain starch and other carbohydrates, can serve as a feedstock for ethanol production. Through a series of processes, the starch is converted into fermentable sugars, which are then metabolized by microorganisms to produce ethanol.

The production of ethanol from sweet potato peels involves various stages, including substrate preparation, enzymatic or acid hydrolysis to break down starch, fermentation using specific yeast strains, and distillation to separate ethanol from the fermented solution.(3) Optimizing the fermentation process parameters, such as substrate concentration, pH, temperature, and fermentation time, is crucial for maximizing ethanol yield.

The significance of ethanol production from sweet potato peels lies in its potential to address multiple challenges. Firstly, it offers a sustainable solution to the waste generated during sweet potato processing, reducing environmental impact and promoting a circular economy approach. Secondly, it contributes to the diversification of energy sources and the reduction of greenhouse gas emissions.(4) Thirdly, ethanol production from sweet potato peels can create economic opportunities for farmers and food processing industries, generating additional revenue streams and supporting local economies.

Chapter 2 Literature Review

The majority of nations rely in fossil fuels to supply the rising need for enrgy for heating, transportation, and industrial is processes. Nevertheless, concerns over environmental degradation, supply security, and the increase in global population are forcing people to consider alternate renewable energy sources. Due to the depletion of fossil fuel reserves, an increase in the amount of is greenhouse gases released into the atmosphere generally, or rising petrol prices, ethanol consumption has been steadily rising in many parts of the world. A promising renewable energy source made from biomass is called biofuel. The majority of nations rely in fossil fuels to supply the rising need for in energy for heating, transportation, and industrial processes. Nevertheless, concerns over environmental degradation, supply security, and the increase in global population are forcing people to consider alternate renewable energy sources. Due to the depletion of fossil fuel reserves, an increase in the amount of greenhouse gas released into the atmosphere is generally, and rising petrol prices, ethanol consumption has been steadily rising in many parts of the world. A promising renewable energy source made from biomass is called biofuel.

Recently, scientific research has concentrated on improving the production of biofuels from biomass by addressing issues like the effective use of alternative biomass as feedstock, which includes integrated cogeneration facilities, commercialization of bioethanol production, and integrated production systems capable of using multiple feedstock under a single platform. Food security is unaffected by using food and agricultural waste as a fuel for the manufacture of bioethanol. By reducing greenhouse gas emissions, it offers a sustainable method of managing food waste. The production of bioethanol from these food waste feedstock is crucial for providing sustainable transportation fuel and halting the use of fossil fuels.

Thus, feeding stocks made from agricultural plants for bioenergy instead of fossil fuels increases the sustainability of the energy industry while improving farmer income creation. (7) Mazaherii et al., for instance, confirmed the need to advance bioethanol synthesis from biomass substrates by producing the fuel from pomegranate peel. However, it is always used required to start by locating renewable raw sources with the right carbohydrate compositions. There are three generations of bioethanol (8) depending on the feedstock that are available. In the first generation, bioethanol is produced using substances often found in human diet and animal feed, such as potatoes, oats, soy beans, sugarcane, corn and carrots. (9) The majority of the

lignocellulose materials or agro-industrial waste used to make second-generation bioethanol include maize cobs, wheat straw, sugar cane bagasse, and agave bagasse. Similar to this, aquatic biomass including cyanobacteria, microalgae, and microalgae is used to make third-generation bioethanol.(10), as in the case of Rio et al. employed whole-slurry fermentation to study and create bioethanol from maize waste as a feedstock. Carrot pulp dried was employed as a feedstock for ethanol synthesis in the study by Khoshkho et al. Microalgae were used in the work by Fetyan et al. to investigate the generation of bioethanol using microalgae that had been subjected to a defeated acid pre-treatment. In a different study, wheat straw was mechanically processed and used to produce ethanol by fermenting the hydro lysate, according to Ward et al. (12)

Despite being made from a variety of feedstock's and utilised in a number of processes and applications, bioethanol is still in great demand since it may be used as fuel. Therefore, it is necessary to promote the development of biomass-based ethanol, a fuel that is almost carbon neutral.13) In order to considerably reduce production costs, a more efficient and high yield ethanol fermentation technology must be developed, as alcohol synthesis from sugar-enriched feedstock is still not economically viable. (14) While the available sugary substrates are more expensive than molasses, they can still be successfully used to produce ethanol with very minor process modifications. Bhatia and others(15() stated that the use of sugars as a substrate for the synthesis of ethanol is problematic since it is not economically feasible compared to other substrates because sugars are primarily employed in the manufacturing of food and require regular pH adjustments throughout the process. Waste paper as a feedstock for bioethanol was examined by Dubey et al. The work effectively synthesised bioethanol while rigorously examining acid-pre-treated discarded paper hydro lysate [21]. Similar to this, Miezah et al. proved the use of lignocellulose as a feedstock for ethanol by producing bioethanol from municipal solid wastes. The study examined municipal wastes as a potential supply of ethanol, both hydrothermally treated and untreated [22]. However, because lignocellulose feedstock is only occasionally available, it is not cost-effective to produce bioethanol using it due to storage and transportation costs [23]. In addition, for efficient bioethanol synthesis, inhibitors such furfural, acetic acid, and hydroxybenzayldehyde must be eliminated from the hydrolysates of lignocellulose materials.

Because they are readily available and financially viable, starchy raw materials are preferred [7]. Starch-containing feedstock's such potatoes, maize flour, wheat, rice, barley and sweet potatoes can be used to make bioethanol by first fermenting the starch into sugar and then

ethanol [14, 18, 25, 26]. A complex carbohydrate called starch needs to be broken down into simple sugars in order to produce bioethanol. For the manufacturing of bioethanol, a starch that includes barley, corn, potatoes, wheat, sweet sorghum, rice, and sweet potatoes is employed. They are thought of being inexpensive substrates for biomass-based bioethanol synthesis [11]. The peels of sweet potatoes, carrots, onions, and sugar beets are examples of useful biomass wastes that boost the yield of ethanol production by microbially fermenting the substrate [12, 21, 23, 27]. For instance, dried carrot pulps were used as feedstock for ethanol synthesis utilising yeast Saccharomyces cerevisiae, as reported in the work by Khoshkho et al. [5]. The study demonstrated the possible use of starchy waste feedstock as a substitute source for ethanol production. A. S. T. O. L. F. also used the saccharification and fermentation procedures to produce bioethanol from maize [28]. The study investigated the factors that primarily influence ethanol production, including temperature and biomass dosage, along with the conditions for fermentation. In their investigation, Duque et al. looked into the bioethanol content of enzyme-catalyzed barley and discovered a 38 g/l ethanol concentration [29]. The study demonstrated that during the production procedures, alkali pre-treatment and enzyme concentration have a significant impact on bioethanol yield. In a different sense, improving knowledge of energy conversion systems from the viewpoints of thermodynamics, economics, and ecology is crucial for sustainable development systems.

An effort should be made to raise the thermodynamic efciencies of the plants in order to improve their environmental performance, which is important for sustainability. The methodology for determining ecocosts has several drawbacks, but the outcomes of the exergoeconoenvironmental study are insightful in the generation of energy. Exergoecono environmental analysis being developed in more sophisticated ways may improve the process' efficacy as a holistic strategy for sustainable development [30]. Most essential, when producing ethanol on a large scale, environmentally friendly alternatives must be used. For ethanol manufacturing technologies to be economically viable, low-cost waste biomass must be used(31)

Waste sweet potato peels are a type of starchy agricultural biomass that is appropriate as a starting point for the synthesis of bioethanol. It was determined through research on the dry base characterisation of sweet potato peels that they are a rich source of bioethanol substrate [32]. Potato trash is typically utilised to make cheap animal feed, fertiliser, or biogas raw materials, which results in the wastage of a lot of nutritious components. Antioxidant, antibacterial, apoptotic chemo-preventive, and anti-inflammatory are some of its qualities. The

goal of current research is to increase the use of potato peel recycling in the food processing, phytopharmaceutical, and biosynthetic sectors [24, 32–34]. Since several S. cerevisiae species were recognised to be effective in converting polysaccharides into ethanol and other compounds, they were used in fermentation processes [9]. Finally, distillation uses temperature differences to extract undesired contaminants from bioethanol, and the finished product needed to be characterised.

Additionally, potato (Solanum tuberosum) is a different starchy raw material for the manufacture of bioethanol that doesn't need a labor-intensive pre-treatment [20]. Only a few investigations on the creation of ethanol from used potato peels have been done, to the best of the authors' knowledge. In other studies, numerous researchers looked at the economic analysis of the manufacture of fuel ethanol and found that the reduced ethanol content in the fermented mixture led to significant energy consumption in recovery steps during distillation [25]. Additionally, it was found that fermentation variables had a significant impact on bioethanol yield [26]. This suggests that fermentation is a process that needs more care and can be enhanced by optimising key factors. The yield of bioethanol was also said to be increased by pre-treating biomass feedstock utilising several pre-treatment technologies, including chemical, physical, physicochemical, biological, and ionic liquid [35]. However, the pre-treatment of biomass feedstock can change the fermentation conditions since it impacts factors including temperature, moisture, substrate size, pH, culture media composition, and cellulose crystallinity. This leads to the forecasting of the subsequent work to optimise operational conditions and examine large-scale biofuel production from biomass by applying various pre-treatment techniques. In order to achieve a high production of bioethanol, the current work optimised fermentation parameters, including pH, temperature, and fermentation duration, utilising response surface approach.

In general, the present work optimised the fermentation conditions to manufacture bioethanol from discarded potato peels. The preparation of raw discarded potato peel, starch hydrolysis, distillation, and the identification of the ideal fermentation conditions were the key techniques used. The generated bioethanol was characterised, and a thorough investigation was done into the impact of temperature, pH, and contact time for fermentation on bioethanol yield. Additionally, the response surface methodology was used to optimise the fermentation parameters, and the interaction effects of the parameters were examined (Table 1).

Chapter 3 Materials And Methods

3.1 Materials;-

Waste potato peels were employed in the study, together with S. cerevisiae, NaOH (99.8%), HCl (36.46%), H_3BO3 (98%) H_2SO_4 (98%), NH_3 (28%), and KOH (98%)

Table 1;- Previous Experimenet And This Project

Descriptions	36	97	38	This Project
Feedstock used	Palm plant (Iraqi dates	Waste potato mash	Colocynthis vulgaris Shrad seeds shell	Waste Sweet potato peels
Production method	simultaneous fermentation and hydrolysis without pretreatmentsimultaneous fermentation and hydrolysis without pretreatment	simultaneous fermentation and hydrolysis with pretreatment	Enzymatic hydrolyss	simultaneous fermentation and hydrolysis with pretreatment
Experimental design for optimization	Box–Wilson design	Box–Behnken design	Box–Behnken design	Box–Behnken design
Ranges of optimized parameters	Temperature (25–35 °C), pH (4–6.5), and fermentation time (48–96 h)	Temperature (40–60 °C), dose of enzyme (0.2–1 ml), and saccharifcation time (24–72 h)	Temperature (40–70 °C), pH (3–11), and time (24–216 h)	Temperature (21– 38 °C), pH (4–5), and incubation time (72–96 h)
Maximum yield	33.00 g/l	31.99 g/l	-	31.5% (v/v)

Table 1 Previous investigations and short overviews of the current study

3.2 Raw material characterization

3.2.1 Moisture content determination:-

The oven-dry method was used to gauge the moisture content of the leftover potato peels. Prior to recording the mass of the sample and aluminium foil (W2), known chopped potato samples were placed on a pre-weighed aluminium foil (W1). The moisture % was then calculated in accordance with the information provided by [28, 39, 40] after samples were held in an oven at a temperature of 100 °C for 8 hours to achieve a consistent weight.

$$\% \text{ Moisture} = \frac{W2 - W1,}{W2 - W3} * 100$$

W1 stands for the preweighed sample of potatoes, W2 for the sample weight plus aluminium foil, and W3 for the dried sample plus aluminium foil.

3.2.2 Total ash content determination:-

After weighing the sample free and clear crucibles, 10 g of dry, ground waste potatoes were added to each one, and each weight was noted. The sample-containing crucibles were first brought to a furnace that had already been preheated. To prevent insufficient ashing, the furnace temperature was then steadily raised from 250 to 450 °C after 20 minutes. The crucibles were taken out of the furnace with a tong after one hour and put in desiccators to cool. The ash-containing crucibles were then weighed after being allowed to cool for an hour. Eq. (2) was then used to determine total ash, and an average value was chosen for further investigation.

$$\% \text{ Total ash} = \frac{W3 - W1,}{W2 - W1} * 100$$

W1 is the crucible's weight, W2 is the mass of the crucible and the sample, and W3 is the crucible's weight and the dried, cooled sample.

3.2.3 Total reducing sugar determination:-

For the purpose of determining total reducing sugar, several standard glucose solutions (2, 1, 0.5, 0.25, 0.125, 0, and 1.4% w/v) were created. Test tubes holding 1 ml of each standard glucose solution, and 9ml Distilled water and forcefully mixed, then heated in a water bath at 100 °C for 5 min to achieve the desired red hue. And cool down the room temp. and After cool down the room temp. add 1 ml Distilled water and As recommended by [30, 39], the samples were then filtered using filter paper, and their absorbance was measured using a

spectrophotometer at a wavelength of 540 nm. Finally, a calibration curve was used to determine the concentrations of the samples.

Figure 1:- sugar determination Test

3.2.4 Protein content determination:-

In the first stage, the following reagents were made: Kjeldahl catalyst: a mixture of copper and potassium sulphates in a 1:9 (% v/v) ratio, as well as 96% sulphuric acid solution, 40% sodium hydroxide solution, 0.2 N HCl solution, and 4% H3BO3. A 1.0-ml sample of leftover potatoes was added to the digestion fask. Then 200 ml of 96% concentrated H2SO4 and 5 g of Kjedahl catalyst were added. A sample was then carefully added to 60 cc of distilled water after cooling for 30 minutes. The flask was immediately attached to the condenser's digesting bulb with a tip that was submerged in standard acid and mixed with five drops of indicator in the receiver. The flask was heated and turned once more to ensure that all of the NH3 was distilled. Finally,

$$\% \text{ Protein} = \frac{((A-B) * N * 14 * 6.25),}{W}$$

N is the HCl's normalcy, W is the sample's weight in grammes, 14 is the atomic weight of nitrogen, and 6.25 is the ratio of fish protein to nitrogen and its byproducts. A and B are the volumes of HCl with 0.2 N applied during the sample and blank titration, respectively.

3.2.5 Fat content determination

Before assessing the amount of fat in a prepared waste potato sample, a petroleum ether reagent was applied. The bottle was first weighed after being placed in the incubator for the night at 105 °C with the cover on. After that, a paper filter and 4 g of the leftover potato sample were weighed. The extraction thimble was filled with a waste sample potato, and it was then placed into Soxhlet. About 250 cc of liquid was added to the bottle before it was placed on a heating mantle. The Soxhlet apparatus was attached, the water was turned on to cool, and then the heating mantle was switched for roughly 14 hours. The solvent was evaporated with the aid of a vacuum condenser. Until the solvent had completely evaporated and dried, the bottle was kept at 80–90 °C. Eventually, the bottle was moved to

$$\% \text{ Fat} = \frac{W1,}{W2} * 100$$

W2 is the weight of the sample of potatoes, and W1 is the weight of the fat**.**

3.2.6 Crude fibre content determination:-

Two grammes of defatted material were subjected to successive treatments with boiling solutions of 0.26 N H2SO4 and 0.23 N KOH. The leftovers were then separated by filtration, cleaned, and put into a crucible before being heated to 105 °C for 18 hours. The sample was ashed at 500 °C in an enclosed incinerator, then weighed after removal from the crucible. Finally, Eq. (5) was used to get the crude fibre %.

$$\% \text{ Fibre Content } = \frac{W2 - W1,}{W3} * 100$$

The weight of the sample is represented by W3, the mass of the crucible with the sample is represented by W1, and the mass of the crucible with the sample is represented by W2.

3.2.7 Carbohydrate content determination

To get the total carbs, the percentage sum of the moisture, fat, protein, fibre, and ash levels were subtracted from one hundred [16].

% Carbohydrates = 100% − (% ash + % moisture + % protein + % fat + % fibre)

3.2.7 Thermo gravimetric Analysis

TGA is an investigative technique for assessing a compound's thermal durability. The sample's loss of weight is calculated in this investigation as a function of temperature over a period of time. This approach also provides data on thermal breakdown, adsorption, desorption, and absorption. The thermo gravimetric analyser, a device employed in TGA, is made up of a precision balance enclosed inside a combustion chamber and a specimen holder. In most cases, percentage of weight is monitored while the temperature is raised at a consistent pace. During the thermal reaction in TGA, which is carried out under an inert environment, particularly N2 gas, the temperature often approaches 1000 °C. TGA also offers a curve between temperature and mass %. If the substance is thermally stable, there won't be any observable mass shift and there will be no slope in the TGA record. This method is also employed to gauge the polymers' heat stability

Figure 2:-Thermo gravimetric Analyser

3.2.8 Fourier transform infrared (FTIR) spectroscopy

This method is used to determine the identity of an unknown substance, the proportion of each component in a combination, and the functional group of an organic compound. The theory guiding infrared spectroscopy states that molecules have a tendency to absorb infrared area frequencies. Not all bonds inside a molecule absorb infrared light. Only those bonds are considered infrared active molecules because they exhibit a change in their dipole moment. The primary goal of infrared spectroscopy is to distinguish between the two chemicals in the fingerprint region (below 1500 cm-1) under similar conditions. Due to the molecules' bending and stretching vibrations, this fingerprint region has a significant number of absorption bands.

Therefore, some compounds can be easily identified because they exhibit comparable absorption above 1500 cm-1 but differing absorption in the fingerprint area. Some of the infrared light is absorbed and some is transmitted when it passes through the sample. The resulting spectrum gives that sample a molecular fingerprint. Molecular rotation occurs as a result of the infrared radiation's absorption (less than 100 cm-1), which causes discrete lines to emerge in the spectrum. Molecular vibration occurs when the sample is exposed to more intense radiation (104 to 102 cm 1). In the infrared area, these quantized absorptions generate rotation and vibration. A molecule of an organic compound will exhibit an array of peaks in the infrared spectrum. The compound's structural details are sufficiently revealed by infrared spectroscopy. Infrared spectroscopy is best performed using the Fourier transform. It is related to the interferometer, a basic optical instrument. As a result, a signal known as an interferogram is produced, which is then transformed into the necessary spectrum, which contains information about each molecule.

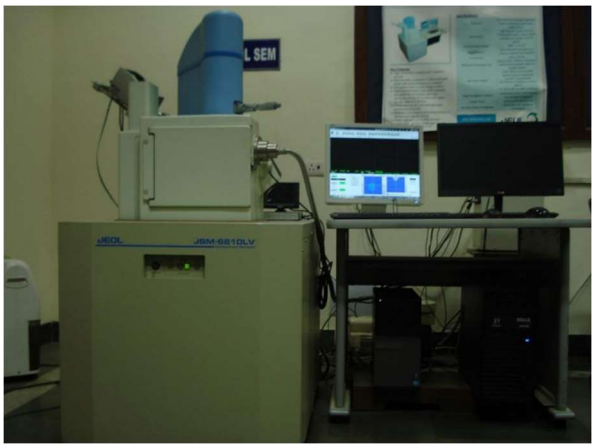

Fig 3:-(FTIR)

3.3 Experimental procedures

3.3.1 Preparation of waste Sweet potato peels sample

One Thousand grams (1000g) of sweet potato peels collected from Rohini Sector 16 , 17 market from a sweet potato local Vender is wastage. These were aseptically collected in a polythene bag and taken to Delhi Technological University is HJB Hostel room and Remove Unnecessary material. Dried for 5 days with sun light. And 38 hour in Room heater. And when these Sweet potato peels are crispy form and then after Grind with these sweet potato peels in Mixer Grinder. Grind when these are not convert powder form. And these powder store in Air tight Container for further analysis. Figure 1 Row Sample of Sweet potato

Figure 4:- Row sample of Sweet Potato peels

Figure 5:- Grind (Powder) sample Of sweet potato peels

3.3.2 Pretreatment;-

In distilled water, reduced waste potatoes (0.5 mm in size) were distributed. According to [17, 32], the liquid mixture of water and the solid powder of potato waste was heated at 121 °C for about 30 minutes.

Figure; - 6 Pre-treated Sweet potato sample

3.3.3 Sulphuric acid (H2SO4) hydrolysis

The potato waste mixture was made into a concentrated solution that ranged from 1 to 15 (% w/w) in distilled water. H2SO4 was used to lower the mixture's pH, which was 5.85, to 4.5. The resulting mixture was then autoclaved for 60 minutes at 121 °C. The samples' temperature was then lowered for roughly 40 minutes to prepare them for further analysis.

$$(C_6H_{10}O_5)_n + nH_2O + Heat \rightarrow nC_6H_{12}O_6$$

Starch *Water* *Glucose*

3.3.4 Preparation of starter culture

200 ml of distilled water were used to dissolve a 3-g yeast extract of S. cerevisiae, 10 g of peptone, and 20 g of dextrose. For 15 minutes, the media were sterilised at 121 °C.

3.3.5 Fermentation

All of the fermentation-related equipment, including the reactor, was sterilised. A temperature of 121 °C was used for the sterilisation for 15 minutes. In the beginning, 200 ml of distilled water was used to dissolve a 3-g yeast extract of S. cerevisiae, 10 g of peptone, and 20 g of dextrose. For each sample including fermentation process tubes, 110 ml of hydrolysates and 100 ml of 10% (v/v) yeast concentration were combined after the fermentation media had been prepared. The prepared sample was then put to the 500-ml flasks with the fermentation media at a 10% ratio (1% cultured media with 10% hydro lysate sample). Finally, it was placed on an incubator that was shaken at various temperatures, pH levels, and incubation times. With a 200 rpm stirring pace. By focusing on the experiment's three key points, the fermentation condition was examined. Usually, one parameter is changed while keeping the others fixed while identifying the best conditions for fermentation operations.

Temperature

Each flask holding a 200-ml sample of hydro lysate was infected with 10% (v/v) yeast isolates and incubated at a different temperature between 21 and 38 °C under stationary conditions to determine the ideal temperature for an enhanced bioethanol yield following the hydrolysis process. The chosen yeast concentration was 10%, and the stirring speed was 150 rpm.

Figure 7 :- Fermentation Different Different samples

PH

Each flask containing a 200-ml sample of hydro lysate was infected with 10% (v/v) yeast isolates and incubated under stationary conditions at a chosen temperature with a pH between 4.5 and 5.5 At a stirring speed of 140 rpm, the necessary fermentation process for a 10% yeast concentration was completed. The ideal pH was found after everything was finished.

Fermentation time

In order to determine the ideal fermentation time for the highest yield of bioethanol following the hydrolysis process, each flask containing a 200-ml sample of hydro lysate was inoculated with 10% (v/v) yeast isolates and incubated over a chosen point of temperature and pH for a different incubation period between 72 and 96 h at stationary conditions.. At a stirring speed of 150 rpm, the necessary fermentation process for a 10% yeast concentration was completed.

3.3.6 Distillation

After fermentation, a distillate yield was obtained through batch distillation using batch distillation equipment. The top-fermented broth was transferred to a bottom flask in an amount of around 200 ml. After that, it was placed on a heating mantle for each run that was fixed to distillation and submerged in tap water for about two hours. Another flask with a higher

temperature was fixed to a distillation column with a temperature set at 78 °C, and the condenser was coupled to a device to cool the ethanol vapour. This approach is based on [18].

3.4 Data analysis

Table 2:- We Chosen Different Parameters

Parameters	Levels		
	Lower (−1)	Medium (0)	Upper (+1)
Temperature (°C)	21	31	38
pH	4.5	5	5.5
Incubation time (h)	72	84	96

At a stirring velocity of 150 rpm and a yeast concentration of 20% (v/v), the importance of crucial factors such as temperature, pH, and fermentation duration on the process was further examined. The last discussion relating to the regression model was discussed utilising a correct association between yield and important fermentation condition in addition to the findings and conclusion of the ANOVA table.

3.5 Bioethanol characterization

Techniques for ethanol analysis were created in order to increase the value of ethanol. Using a Bruker Vertex v70 model spectrophotometer, the IR spectra of the solutions were gathered over the range of 500 to 4000 cm1. 1 cm1 was the nominal resolution. The quality of the ethanol was checked using infrared spectroscopy (IR). By calculating the quality features such as density, pH test, viscosity, functional group, boiling point, fash point, fre point, and specific gravity, the following properties of the produced bioethanol were evaluated.

The alcohol vol by vol percentge was meaured using the following equation:

$$\%Y\ (v/v)=\frac{Vd}{Vg}\ *100$$

where VD is the volume of distillate, Vg is the volume of utilised glucose mixture, and %Y (v/v) is the percentage yield of bioethanol.

3.5.1 Density and specifc gravity test

Weighed was the pycnometer when empty. First, sample (ethanol) was added to the pycnometer, excess was removed, and the density was noted. Second, distilled water was

weighed and recorded after being filled within the pycnometer. Finally, Eq. (11) was used to compute specific gravity. Specific gravity equals (11) bioethanol's density in terms of water.

$$\text{Specific gravity} = \frac{density\ of\ bioethanol}{density\ of\ water}$$

3.5.2 PH test

A pH metre are used to measure the pH of the sample. Before usage, the pH metre was calibrated against a buffer solution with a pH of 7 and maintained in a standard buffer solution. The sample's pH was then measured from the ideal point**.**

3.5.3 Viscosity

First, pipetting was used to allow distilled water to flow through a small viscometer tube while Without air bubbles and note the water's flow rate. A sucker was then used to pull the sample to the designated spot on the capillary Tube after 50 ml of distilled bioethanol had been Filled in the same manner as distilled water. Its time to return (fowl) to the indicated area under the noted time using stop watch.

3.5.4 Boiling point

The exact boiling point of the generated bioethanol was determined using the rotary flask evaporator at temperatures ranging from 75 to 80 °C.

3.6.5 Flash point and fre point

Utilising fash point tester equipment, this test was conducted. A sample of generated ethanol weighing 50 millilitres was put into apparatus with a thermometer. The fash and fre point testing equipment received power. Every five seconds, a small amount of air was allowed to travel through the substance, and a steady stirrer maintained a constant temperature throughout the procedure. After each test cup was cleaned and dried prior to the subsequent test, the temperature value at which the vapour first ignites with the blue flame was recorded as the samples' flash point. A fre point was defined as the point at which vapours form on a test specimen of the material as a result of the effects of an ignition source. The fre point was calculated by applying the ignition source continuously until it ignited the test specimen and sustained burning for approximately 5 s [22, 37].

Chapter 4 Results and discussion

The model's performance with respectable accuracy must be maintained using the parameter values generated through the optimised method. The examination of a mathematical model using a Box-Behnken experimental design of RSM has shown excellent relationships between the variables and the identification of important parameter values.

4.1 The proximate analysis of waste potato

Through a series of studies, the chemical composition of potato waste (substrate) was evaluated in order to assess its suitability for bioethanol synthesis using S. cerevisiae. Table 3 displays the findings of the study of the moisture, ash, fat, fibre, protein, and starch composition of potato trash. The finding

Table 3:- Result table of Ash, Moisture, Starch, Protein, Fat

This Work		References		
		33	29	31
Characteristics	Amount (%)			
Moisture	34.0	37.10	77.10	72.10
Ash	5.62	2.10	-	1.10
Starch	37.7	34.16	18.10	12.10
Protein	9.16	19.87	2.10	2.10
Fat	7.13	5.30	-	10.00

According to the study, there is a significant amount of moisture (34.7%), ash (5.82%), fat (7.33%), protein (9.46%), fibre (3.02%), and starch (39.7%) in potato trash. As shown in Table 3, similar results for wasted potatoes were observed [18, 35, 43]. When using biomass as a feedstock for energy production, moisture content is a crucial factor since it impacts the conversion efficiency and heating value. It is essential for adjusting the caloric values as well as the storage conditions, combustion temperature, and amount of exhaust gas. Additionally, immediate access to data from moisture analyses may be required for quality control, therefore drying is necessary for conversion. When compared to the typical moisture-containing produced biofuel, the acquired value of moisture content (34.71%) is beneficial. Consequently, it is advised that fuel have a low moisture content. The fuel's low moisture content contributes

to its higher heating value and aids in performance at lower temperatures [36]. This suggests that the optimum fuel should have less moisture. In a mufe boiler, conventional dry ashing was burned at high temperatures. The ash content result denotes an inorganic by-product of either full oxidation or ignition of raw materials in an edible substance. The residue, with the exception of specified components, may be used for additional detailed mineral analysis. The amount of ash (5.82%) is comparable to that of the majority of fresh foods [33]. Denaturants, such as acid, alkali, urea, heat, organic solvents, and detergents, have the potential to change the unique structure of proteins. The acquired value is also favourable compared to other compositions described by Suryaningsih and Irma's [33], who investigated the protein content of sorghum for the synthesis of bioethanol. It is necessary to assess the amount of cellulosic fibre to ascertain its cellulose composition and determine whether yeasts can effectively use it or not. The effectiveness of S. cerevisiae, a yeast that produces bioethanol, can prevent the chemicals made from lignocellulose biomass [37]. The wild varieties of this yeast, however, are unable to handle a biomass with a lot of fibre content. To get the monomer sugars present in the biomass, it is necessary to combine thermochemical and enzymatic pre-treatments with the lignocellulose material. Inhibitors formed during the additional pretreatment procedure, such as acetic acid and other organic acids, fur aldehydes, and phenolic, affect a strain's ability to ferment [38]. Reduced wild strain performance yields low sugar content during the fermentation retention time. In addition, the main substrate, which contains starch, is hydrolysed differently depending on the parameters used for optimising the bioethanol process (temperature, pH, and fermentation duration). The yeast S. cerevisiae must devour the starch analysis, and if the content is too high, it requires a high temperature (413–453 K) [47].

The trash potato's composition examination revealed that it contains a significant amount of starch that can be turned into bioethanol. Since denaturants like acids, alkalis, and heat can be utilised during manufacture, it can tolerate their effects. Additionally, the outcomes of a proximate analysis for protein and fat content point to favourable conditions for the generation of bioethanol.

4.2 Optimization of fermentation condition

Table 4 Different sample of Fermentation sample and Result

Run	Temperature (°C	pH	Fermentation time (h)	Experimental yield (% v/v)
1	21	4.5	96	13.25
2	38	5.5	84	20.25
3	38	5	72	26.25
4	38	5	96	22.75
5	31	4.5	96	29.75
6	21	5.5	84	13.5

4.3Total reducing sugar determination:-

Table 5:- Sugar Determination Data

1	Sample	Wavelength
2	2	0.181
3	1	0.074
4	0.5	-0.023
5	0.25	-0.062
6	0.125	-0.084
	0	0
	Unknown samples (mg/ml)	Average Absorbance
	0.295716639	0.1
	0.707578254	0.15

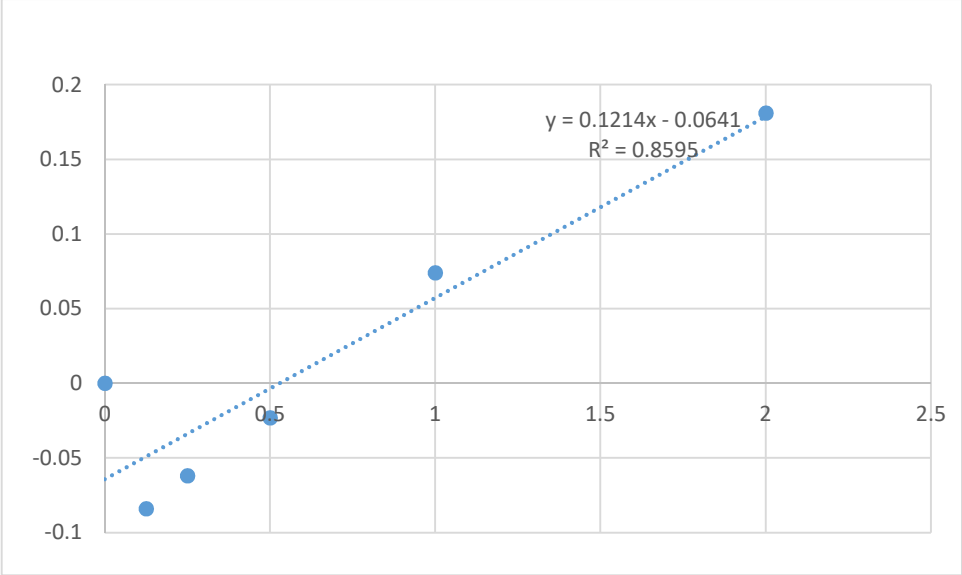

Figure 8 Sugar Determination

4.4 Fourier-transform infrared (FTIR)

Used to describe the functional is group of the ethanol generated, as shown in Fig. 11. The O-H stretching band's highest absorbance was noted at 400, between 2000 or 2500, and at 3900 cm1. The peak of ethanol is , which can be attributed to C-O stretches, ranges in size from 400 to 2500 cm1. The stretching region of 1300-2000 cm1 enlarges the C-H bending region, and contributions from the ethanol and methylene groups may be seen at 3500-4000 cm1. The obtained results were consistent with those in [32].

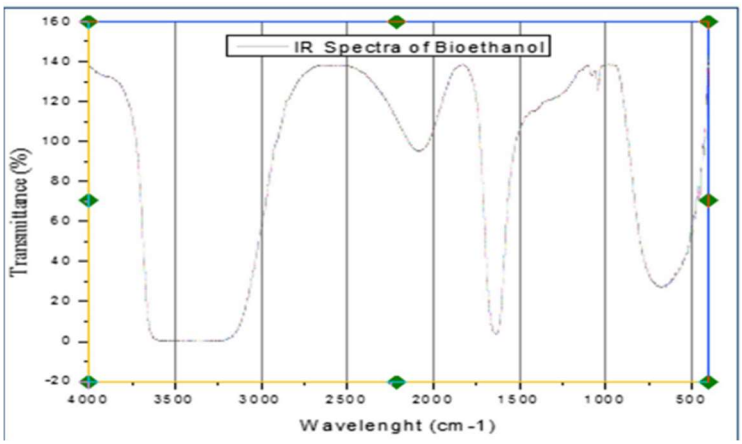

Figure 9:-FTIR of bioethanol

Chapter 5 Conclusions and future directions

Due to its greater availability and high starch content, used potato peel was chosen as a crucial feedstock in the current investigation and effectively converted into bioethanol. The proximate analysis result showed that the chosen raw material was suitable since it had a starch content of 39.65%, a moisture content of 34.71%, an ash content of 5.823%, a protein content of 9.46%, and a fat content of 7.33% that was virtually identical to the theoretical composition of discarded potatoes. For examining several fermentation operating variables (temperature, pH, and incubation time) and their interactions on bioethanol yield, a quadratic model of the Box-Behnken design was shown to be sufficient. The study found that fermentation time, pH, and temperature all have an impact on bioethanol production. A maximum ethanol yield of 34.5% (v/v) was reached at the best conditions, which were 32.199 °C, 4.066, and 72.082 h for temperature, pH, and fermentation time, respectively. This proves that improving fermentation conditions increases ethanol production. However, the study revealed that fermentation duration and temperature had a significant impact on yield. The produced ethanol's density, specific gravity, pH, flash point, viscosity, and boiling point, which were all 0.9987 g/ml, 0.9, 5.1, 36 °C, and 79 °C, respectively, were all quite near to those of typical bioethanol. This high viscosity number indicates that the solution contains more ethanol. Alcohol components were discovered in a generated distillate by FT-IR analysis. This study demonstrates that leftover potato peels make an excellent feedstock for the manufacture of bioethanol and that optimising the fermentation conditions enhances ethanol yield significantly.

The work on technical feasibilities was taken into consideration for the current investigation. The fermentation process is, however, impacted by various factors, including substrate concentration, inoculum size, and mixing pace. Therefore, greater investigation into these process variables is needed to optimise the fermentation environment for the highest bioethanol yield. . Similar to this, scaling up must be carried out to achieve additional recovery at large-scale production, and the processes must be thoroughly explored for economic viability. It is crucial to collaborate on research initiatives with ethanol producers that use ethanol as a fuel for internal combustion engines, such as sugar mills and other higher biofuel producers..

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