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Production of Ethyl-alcohol using Saccharomyces Cerevisiae from Potato

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Abstract- The steady rise in global crude oil prices, the increase in threats to the environment due to air pollution, global warming, and the threat of crude oil prices have had a shattering effect on developing countries, especially in oil-imported countries such as India. From a long-term point of view, it is mandatory to manufacture more fuel-efficient fuels. Ethyl-alcohol is esteemed source of energy. Maize is a major source of ethyl-alcohol because of its high starch content in seeds and grains. Ethyl-alcoholis a renewable alcohol-based fuel that can be produced from starch, sugar, and cellulosic biomass. The present study of our review focuses on production of ethyl-alcoholfrom potato thus obtaining the energy-efficient effect of ethyl-alcoholsynthesis. A variety of substances can be used for ethyl-alcohol production. Ethyl-alcohol based biofuels can be produced using agricultural waste with the help of enzymes. Ethyl-alcohol fuel derived from starchy and agricultural lignocelluloses biomass is gradually being upgraded to reduce petroleum consumption due to depletion of petroleum resources as well as reducing the impact of fuel in nature. In this review we are focusing on production of ethyl alcohol from ground potato using yeast (saccharomyces cerevisiae) and water as chemicals and reagent.

Keywords- **Biofuel, Ethyl-alcohol, fermentation, ground-potato, hydrolysis, yeast**

I. INTRODUCTION

Anxiety regarding the use and reduction of fossil fuels, results in search for alternative fuels. Presently, Ethyl-alcohol has attracted worldwide attention due to its potential use as a transport fuel. Ethyl-alcohol is commonly produced in the fermentation group, especially its saccharomyces cerevisiae and its interspecies hybrids, which provide low productivity. High ethyl-alcohol production from cheap and renewable sources and low energy inputs are important factors in alcohol fermentation research. Ethyl-alcohol is an alcohol -based fuel that can be produced from starch, sugar, as well as cellulosic biomass. Eventually, with the latest advances in cellulosic technology, ethyl-alcohol can also be produced from agricultural waste products for instance moba bagasse, rice hull, potato waste, and brewery waste.

Due to the gradual depletion of fossil fuels, bioethyl-alcohol has received much research from its use as a unique energy source worldwide. Bioethyl-alcohol is produced by the fermentation of renewable fuel sources or fuel additives. In addition, bioethyl-alcoholis considered to be a biomass-based product (Grassi et al., 1999), instead of toxic methyl tert-butyl (MTBE) and tert - amyl methyl ether (TAMES). Bioethyl-alcoholis already being used as a fuel ingredient in many parts of the world. the production of bioethyl-alcohol as biofuel is a recent need, because in many parts of the world such as the United States and Brazil, much research is being done on this project. Global production of bioethyl-alcoholhas reached 51,000 million and the United States and Brazil have reached the top position with India in fourth place. Bioethyl-alcohol is produced from a variety of agricultural waste and sugars containing crops, vegetables and fruits such as sugarcane (Brazil), corn grains (United States) and starchy agricultural waste (India). According to the United States Department of Energy for all energy units used in bioethyl-alcohol production, 1.3 unit of energy is restored.

Substrates for sugar and cellulosic substances are expensive or involve many steps in the production of bioethanol. Therefore, plants that contain a lot of starch are often used for bioethyl-alcohol production such as crops such as corn, barley, wheat, rice, tuber crops, potatoes and sweet potatoes. Bioethyl-alcohol production also depends on the types of yeast such as saccharomyces cerevisiae, the appropriate substrate and the methods used for bioethyl-alcohol production, greatly increasing the efficiency of bioethyl-alcohol production (ethyl-alcohol production, potato chips are cheap and rich because they are rich less than other grains. After proper processing, a good level of ethyl-alcohol can be produced from potatoes used for both fuel and consumption purposes. Rotten potatoes are used for the production of bioethanol. As a product, rotten potatoes are found in 5 to 20% of crops grown in potato farming (Kimmo and Lisa 1999) In Pakistan, millions of discarded potatoes and rotten potatoes are produced annually. Bioethyl-alcohol production using different pH, temperature and a mixture of starch hydrolysis enzymes Ethyl-alcohol today is a very important product of biotechnology in terms of volume and market [. Its market grew from less than 1 liter to 109 liters in 1975 to more than 39 liters to 109 liters in 2006 when petrol ethyl-alcohol was the main source. A similar trend is expected to follow within the next few years [10]. The production of Ethyl-alcohol grains such as corn and wheat are a process established in the industry. However, we have not received any report on the production of ethyl-alcohol residues in bread. The current work is aimed at examining two key phases, the secretion and the secretion of saccharification, in converting bread residues into standard fermented.

II. LITERATURE SURVEY

The steady rise in global crude oil prices, the increase in threats to the environment due to air pollution, global warming, and the threat of crude oil prices have had a devastating effect on developing countries, especially in oil-imported countries such as India. From a long-term energy security perspective, it is necessary to develop more fuel-efficient fuels compared to petroleum-based fuels. [1] Ethyl-alcoholis another source of energy for growing needs. Maize is a major source of ethyl-alcohol because of its high starch content in seeds and grains. Ethyl-alcoholis a renewable alcohol-based fuel that can be produced from starch, sugar, and cellulosic biomass. The current study of our research focuses on enzymatic hydrolysis of corn, thus finding the energy-efficient effect of ethyl-alcohol synthesis. [2]

The synthesis of starch hydrolysis from fresh potato extracts by HC1 and H2SO4 with different concentrations of plant material and acid solution has been investigated. At last, the final concentration of sugar reduction in hydrolyzates depends on the type, acidity of the plant, the acid content of the plant as well as the acid solution but not on the type of potato [3] High dextrose corresponding to 94%, a maximum constant reaction rate of 0.089 min-1 and and a 5-hydroxymethylfurfural (S-HMF) yield of 0.04 g / l. attained by using 1 M HCI per plant material ratio and acid solution of 12 (w / v) Ethyl-alcohol yield of 31 g / l. obtained from fermented hydrolysate made under suitable hydrolysis conditions for commercial baker bakery at 28-C for approximately 18 hours. [4]

Due to the gradual decline in fuel resources and the impact of this waste on the environment, there is a need to use potato waste to extract resources from the waste and clean the environment. In this study, potato waste was investigated as a source of bioethyl-alcohol [5] 100 g of potato powder mixed with 1 L of distilled water in two separate pots to make potato mud. Bioethyl-alcohol production was investigated using the effect of pH, temperature and a mixture of digestive enzymes after dehydration and fermentation. The results show that essential bioethyl-alcohol ($p < 0.05$) was produced at 35 C and at pH 5.5. This study also revealed that enzyme blends significantly improved ($p < 0.05$) bioethyl-alcohol production compared to untreated mixtures High bioethyl-alcohol production is due to the presence of sugar in potatoes. [6]

A comprehensive, ecological explanation is yet to be found and is presently being investigated. If State Government regulations are implemented in accordance with the Kyoto Protocol, an obligatory mix of ethyl-alcohol and traditional petrol at up to 10% will result in the need for great amounts of ethyl-alcohol [8] The amount of starch, cellulose, hemicellulose, and fermented sugar in PPW is adequate to allow it to be used as an ethyl-alcohol stock. To assess fermentation and synthesis of ethyl-alcohol, a number of PPW clusters were immersed in water containing various enzymes and/or acids and fertilized by Saccharomyces cerevisiae var bayanus. After fermentation, 18.5 g LI was extracted using enzymatic hydrolysis and a combination of three enzymes, which reduced sugar and yielded 7.6 g L1 ethyl-alcohol. The findings reveal that PPW, a potato industry product, has a lot of potential for ethyl alcohol synthesis. [9]

Using starch as an amylase enzyme, the bacteria active in the synthesis of amylase (ESI selective) was separated from active mud. Bacillus mojavensis was identified as the virus based on morphological, physiological, and 16S rDNA analysis. At 30 ° C, medium-toxic potato solid induced biomass abundance and strong amylase activity by inoculating 20 percent B. mojavensis with a 20 percent inoculum size. [10]

Abundance of B biomass. mojavensis and its production of a-amylase were reached at the beginning of the standing phase; the production of a-amylase is accompanied by an increase in certain levels of cultural growth and a decrease in starch content in solid potato waste. After 3 days in a liquid condition at pH 6 and 30 ° C, B. mojavensis was able to degrade the starch content in solid potato trash. After 3 days of fermentation with Saccharomyces cerevisiae, different fillings of solid potato chips pre-crushed with B. mojavensis resulted in a high

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conversion of starch to potato powder to sugar, resulting in a high ethyl-alcohol concentration. [11]

The best potato waste concentration for sugar reduction and ethyl alcohol generation was 50%. After 3 days of incubation, the highest production of ethyl-alcohol in potato waste occurred at 30° C, pH 3.5, and inoculum size 10%. Under the right conditions, the use of B. mojavensis and bakery yeast in the manufacture of ethyl-alcohol in a potato waste bin will be used not only for the creation of cheap clean energy, but also for the removal of some undesired waste. [12]

III. MATERIALS AND METHODS

Plastic bag to collect and transport samples to the laboratory, knife to cut potato samples, boiling water bath to cook the sample pieces, oven to dry the sample, crusher to crush the dried sample, sieves to sieve the crushed sample to a particle size of 2mm, balance to weigh the samples and yeast, vessels to hold the samples for distillation, graduated cylinders for volume measurement, distillation set ups to distil, conical flask for preparing soy sauce**.**

Chemicals and Reagents

Yeast or Saccharomyces cerevisiae.

Water For washing the samples, materials and for preparing the solution.

General method

The standard test procedure was followed as follows. The basic steps for the production of ethyl-alcohol from potatoes.

Sample Collection

A sample of potatoes and baking yeast was collected from the local market town of Tepa.

Sample preparation

The samples obtained had to be prepared and packaged for
pretreatment and distillation. The sample preparation process contains: Reducing hand-made size (knife cutting), drying, grinding and sifting after sample collection. A sheet of red and white potatoes per kilogram was used for the preparation of the sample. The drying of the samples was done in a 250degree centigrade oven for easy crushing. Separately, each of the samples were milled. The particle size of the mixed sample was 2 millimeters. The sample size of particles larger than 2mm was milled and until the total particle size was 2mm. The sample was kept at a low temperature until the next stage of testing.

Fermentation The purpose of the study was to measure ethyl-alcohol production by Saccharomyces cerevisiae using potato. Pure solution goes for fermentation. under the anaerobic conditions the process of fermentation was carried out.

List of components for distillation experiment

Distillation vessel, condenser, beaker, thermostat that supports flat metal bar, heating mantle

Simple Distillation Process

The final step in the synthesis of ethyl alcohol from ground potatoes was distillation. It is a cleansing step. Distillation is a method used to separate two mixed liquids based on different boiling point. In this experiment, the separator was used to simplify the distillation at a temperature of 80 degrees centigrade.

IV. ASSESSMENT TASK

Sample collection and treatment

Rotten potato and potato dumps were collected at the District Bennu vegetable market and on their local dumping grounds. The debris was cut into pieces, washed, dried, and mechanically.

Formation of slurries and liquification

Separate beakers to form potato slurry. 30 ml of amylase enzymes are added to one potato solution and the other contains only potato mud. Both solutions were heated to 95 to 110 ° C for 30 to 40 minutes in an autoclave to dissolve the starch and the pH was adjusted to 5.8.

Saccharification

After cooling, the slurries are mixed with 30 ml of glucoamylase enzyme for conversion of starch into fermented sugar and commercial yeast (S. cerevisiae) is added to both solutions as described.

Fermentation and distillation

1000 g of potato powder is added to 2940 ml of distilled water twice. After mixing well, both solutions were sealed (anaerobic fermentation) as shown in Figure 1 and placed at 23 ° C, and 28 to 35 ° C to measure the effect of heat in the production of ethyl alcohol. During this time, the sugar units are converted into bioethyl-alcohol by yeast and a mixture of enzymes. After fermentation, the unfermented residue was filtered while the filtrate (ethyl alcohol) was purified with a rotating evaporator at 78.5 ° C. Obtained ethyl alcohol was used as a biofuel after

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adding 3 to 5% fuel to obtain anhydrous ethyl-alcohol (Kroum0v. Et al., 2006) while solid unripe residues (stillage) were used as essential animal feed stocks.

Fig 1. Potato waste

Fig 2. Potato peel waste

Fig 3. Fermented Bio-ethanol

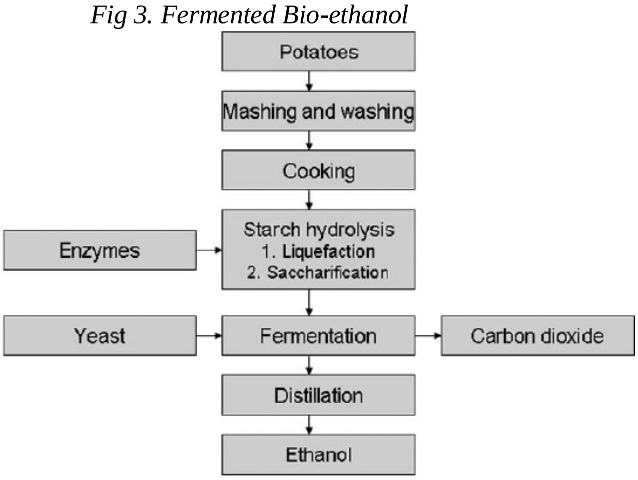

Fig4: Flow chart representing ethyl- alcohol produced from potato waste

Enzymes:

- Liquozyme-added for the liquification of potato mash at liquefaction stage
- Viscozyme-added for reduction of viscosity of potato mash Alpha amylase-added for the hydrolysis of starch at liquification
- Glucoamylase-added for the same at saccharification

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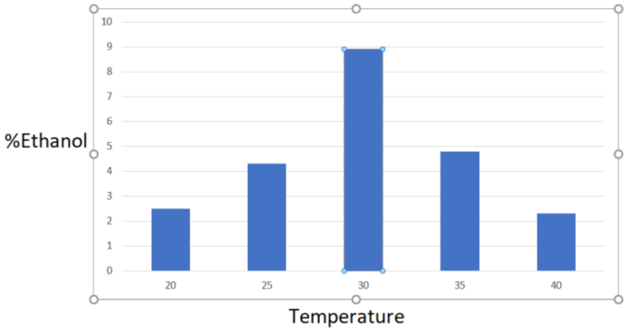
- Yeast (saccharomyces cerevisiae)-added for the fermentation.
- Lime and Soda- to control and maintain PH.
- Heating is done to reduce bacteria level.



Reducing sugar and Temperature / Ethanol	%Bioethanol Production at different Temperature and Reducing sugars				
	Temperature (°C)				
	20	25	30	35	40
%Ethanol	2.5 ± 0.02	4.3 ± 0.14	8.9 ± 0.05	4.8 ± 0.09	2.3 ± 0.01
control(untreated)	0.0	0.0	0.0	0.0	0.0

Reference: Ethyl-alcohol Production from solid Potato Wastes.
Fig5. Egy.J. Plant Pro.Res.2(4)21-55(2014)

V. RESULT AND DISCUSSION

Fehling Factor

Fehling factor = Burette reading/100
= 5.3/100
= 0.053

Starch Percentage after Milling
Fehling factor = 0.053
Dilution factor = 500/3=166.66
Burette reading = 12.4
Starch%= ((0.053x100x166.66)/12.4) ×0.9
= 64.1%

Liquification

The initial stage in the conversion of starch into simple dextrin molecules is liquefaction. There are three sub-processes to it.

• Pre-Liquefaction: This entails partial liquefaction of starch in the presence of enzyme at a temperature well below that of gelatinization.

• Jet Heating: This method involves heating the starch slurry with live steam to immediately raise its temperature. This causes starch molecules to gelatinize and open up, allowing enzymes to operate on them. Jet cooking also sterilizes the slurry.

• Post-liquefaction: To complete the liquefaction process, the jet-cooked slurry is kept at high temperature in the presence of enzymes.

Saccharification

The production of sugars is known as saccharification. The degradation of dextrin is carried out enzymatically. A second enzyme acts on dextrin to further break it down and liberate sugar. The saccharified slurry from the saccharification section is pumped into the fermenter and diluted with water to the desired sugar content. It is then inoculated with the appropriate amount of yeast. Urea and DAP are used to add assimilable nitrogen to the medium. The Fermenter's temperature is kept constant thanks to a plate heat exchanger.

Liquefaction Process

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Reagents: -
Starch powder - 250 gm
Water - 750 ml
Liquozyme - 87.5 ml
• Take 250 gm Starch powder
• Add 750 ml water
• Maintained pH 6.0-6.5 by adding caustic & lime • Heat slurry at 105-110 0 C for 2-3 hrs.
• Add 87.5 ml liquozyme enzyme

Sugar Percentage after Saccharification
• First filter sample
• Taken 25 ml sample in burette
• Taken 5 ml Fehling A& B in conical flask.
• Taken 20-30 ml water.
• Put on heating plate till boil
• Added dropwise sample from burette in conical flask till color changes to reddish brown

Calculations
Sugar % = (Fehling factor/burette reading) * 100
= (0.053/1.2) × 100
= 4.8%

Fermentation

- After the hydrolysis is done, filter the solution & maintain the pH
- Lid the flask mouth using cotton plug so as to make it air tight and finally allow the flask in autoclave for sterilization for about 15 min at press of 15 lb.
- After cooling the media, the pure culture of dry yeast is added to an amount of 0.25 gm
- Add 0.25 gm Mgso4,0.38 gm urea and 87.5 ml saco zyme enzyme
- Keep flask in incubator at 32 0c for about 48 hrs.

Alcohol percentage after fermentation

- Take 250 ml fermented wash + 250 ml water.
- Distilled sample and maintain temp 78 0c.
- After that collected 250 ml distillate in measuring cylinder.
- The deeper sykes hydrometer and thermometer in measuring cylinder.
- Write thermometer and hydrometer reading.
- According to this two reading see spirit table reading.

Calculation
Alcohol % = (100 + spirit table reading) * 0.5714

0bservations
Thermometer reading = 100 F
Hydrometer reading =880 F
Then spirit table reading over proof = 68.5
Alcohol % = 100 / 175 = 0.5714
= (100 +68.5) × 0.5714
=96.28%

Material balance

Over Hydrolyser
Density of water = 993 gm/cm3

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Volume of water = 2940 ml Assume 60% of starch are converted for glucose molecule.
Amount of glucose = (628.18 X 60)/100 = 376.90 gm.

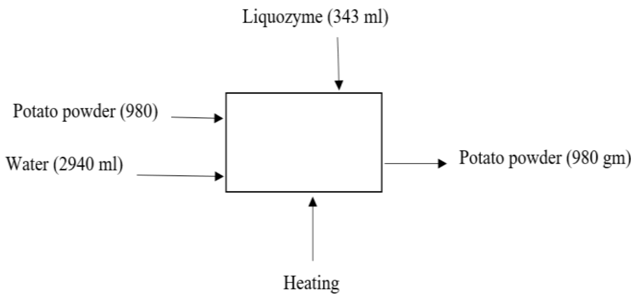

Fig6. Over Hydrolyser

Energy Balance

Energy Balance data
ΔHF $C2H5OH$ = -277.5 Kcal/mol
ΔHF HCl = -167.169Kcal/mol
ΔHF CO2 = -393.53 Kcal/mol
ΔHF C6H12O6 = -1104.57 Kcal/mol
ΔHF H2O = -285.8 Kcal/mol
ΔHF C6H10O5 = -2221 Kcal/mol

Energy Balance - over Hydrolyser

(C6H10O5) n + 2H2O → C6H12O6 + C6H12O6
ΔHR = 2ΔHF (C6H12O6) – (2 X (-285.5) +(-2221))
= (-2X 1104.57) – (2X (-285.5) + (-2221))
= (-2209.14 – (-571.6 - 2221))
= -2209.14 + 571.6 +2221
= 583.46 KJ/mol
Q = ΔHR = 583.46 KJ/mol
Q = m. cp. ΔT
583.46 = m X 4.2 X 77
M = 1.804 KJ/mol

Over fermenter

For fermentation reaction we are feeding 11609.70 gm 0f sterilize sludge
According to fermentation reaction 2 % of glucose are unconverted
Amount of glucose unconverted = (total glucose feed × 2)/100
= (376.90 ×2) /100
= 7.6 gm
The reaction occurred in fermenter.
C6 H12 06 2C2 H5 OH +2C02
Mol of C6 H12 06 =2 moles of C2 H5 OH
Moles of glucose reacted =376.90 / 180
=2.09 moles of glucose
2.09 moles of glucose =2×2.09 moles of ethanol
=4.18 moles of ethanol
Amount of ethanol formed = 4.18 × mol. Wt.
= 4.18 ×46
=192.6 gm
Total amount of ethanol produced in 11609.70 gm of maize
Amount of CO2 formed
Moles of glucose = 2 moles of C02
= 2×2.09
=4.18 moles of CO2
Amount of CO2 formed = 4.18 ×44
=183.92 m

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Energy Balance - over Fermentation

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

$\Delta HF = \Delta HF$ PRODUCT - ΔHF REACTANT

$\Delta HR = 2\,(\Delta HF\ C_2H_5OH + \Delta HF\ CO_2) - \Delta HF\ C_6H_{12}O_6$

$= 2\,(-227.5 - 393.57) - (-1104.57)$

$= -1342 + 1104.57$

$= 237.43$ KJ/mol

Q = m. cp. 273.43

= m X 4.2 X 32m

= 1.766 KJ/mol

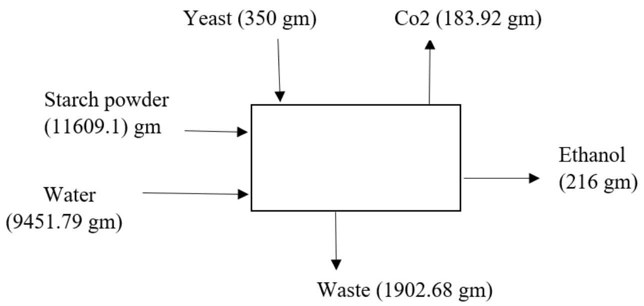

Fig7. Over fermenter

Over crusher

- 64.1% starch = (64.1 × 980) /100

  =628.18 gm.
- 10% water = (10 ×980)/100

  =98 gm.
- 26 % waste = (26 ×980) /100

  =254.8 gm

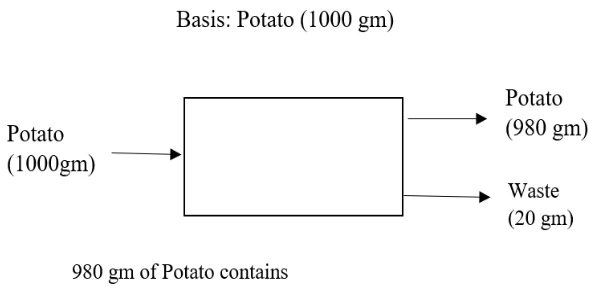

Fig8. Over Crusher

RESULT

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Sr. no	Material Balance	Value
1.	Over Crusher	64.1% Starch = 628.18 gm 10.1% water = 98gm 26% waste = 254.8gm
2.	Over Hydrolyser	Density of water = 993 gm /cm3 Volume of water = 2940 ml Amount of glucose = 376.90 gm
3.	Over Fermenter	Amount of glucose unconverted = 7.6 gm Amount of ethyl-alcohol formed = 192.6gm Amount of CO2 formed = 183.92 gm

Sr.no.	Energy Balance	Value
1.	Over Hydrolyser	Q = ΔHR = 583.46 KJ/mol M = 1.804 KJ/mol
2.	Over Fermenter	Q = ΔHR = 237.43 KJ/mol M = 1.766 KJ/mol

Sr.no.	Percentage yield of alcohol	Value
1.	Overall	96.28%

VI. CONCLUSION

Ethyl-alcoholhas gotten a lot of press because of its potential as a transportation fuel. In the current study on alcoholic fermentation, high ethyl-alcohol productivity from less expensive and renewable sources, as well as low energy input, are essential factors. Because of the amount of starch found in potatoes, 96.28 percent of ethyl-alcoholis created. The material balance over the fermenter reveals a good amount of ethyl-alcohol production, and the energy balance reveals that the process is energy efficient. Researchers are scrambling to identify new and low-cost ethyl-alcohol production sources as a result of the energy crisis. Potatoes were utilized to make ethyl-alcohol in this investigation. Potatoes have a large level of reducing sugars, according to research. It is our first attempt in Pakistan to create bioethyl-alcohol from starchy agricultural wastes, which will offer bioethyl-alcohol for biofuel while also reducing environmental pollution. A comparison study of bioethyl-alcohol generation from potato wastes was conducted. It was discovered that potato wastes contain more starch and produce more bioethyl-alcohol via enzymatic hydrolysis.

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