Original Research

Production Of Renewable Future Fuel (Bioethanol) From Various Sources Through Simultaneous Saccharification And Fermentation.

Bheema Naik Angothu[1] *, Kanneboina Navya Sri[2], Gade Laya[3], Vavilla Manaswini[4], Samreen[5], Cheemala Naveen[6], Dr.CH. V. Suresh[7], Dr.K.N.V. Rao[8]

[1*,2,3,4,5,6,7,8]Department of Pharmaceutical chemistry, Nalanda College Of Pharmacy, Charlapally, Nalgonda, Telangana-508001.

***Corresponding Author:-** Angothu Bheema Naik,
*Associate professor, Department of Pharmaceutical Chemistry, Nalanda college of Pharmacy, Nalgonda, Telangana, 508001, Email: bheema.24carats@gmail.com

Abstract
Increase in the need for petroleum results in a remarkable rise in its prices. The main objective to satisfy this need leads to development of easier techniques using cheaper and eco-friendly raw material for the production of bioethanol and in turn reduce the massive usage of fossil fuel. This study was done for the production of bioethanol from guava and palm jaggery to obtain the maximum yield of ethanol by the process of simultaneous saccharification and fermentation. The benefits of performing the enzymatic hydrolysis together with the fermentation, instead of in a separate step after the hydrolysis, are the reduced end product inhibition of enzymatic hydrolysis and reduced investment cost.

Keywords: Simultaneous Saccharification and Fermentation, Bioethanol, Enzymatic hydrolysis.

INTRODUCTION

Bioethanol is the ethyl alcohol or ethanol obtained from bioresources (biomass) by hydrolysis or sugar fermentation processes. It is a principle fuel used as a petrol substitute for road transport vehicles. Although ethyl alcohol or ethanol can also be produced by the chemical process of reacting ethylene with steam, but cannot be said bioethanol because of the lack of renewable source of raw material and the nature of production route. Sugar fermentation is the process widely used. The main sources of sugar required to produce ethanol come from fuel or energy crops. These crops include corn, maize and wheat crops, waste straw, willow and popular trees, sawdust, reed canary grass, cord grasses, Jerusalem artichoke, myscanthus and sorghum plants. There is also ongoing research and development into the use of municipal solid wastes to produce ethanol fuel.[1]

INDIA BIOETHANOL PRODUCTION

India Ethanol market demand stood at 3250 Million Litres in FY2021 and is forecast to reach 5412.06 Million Litres by FY2030, growing at a healthy CAGR of 8.25% until FY2030. With the start of National Biofuel Policy 2018, which has put forth an Ethanol blending target of 10% by 2022 and 20% by 2030 from the current rate of 2-3%, Ethanol demand is set to grow by leaps and bounds in the period of forecast. Over the past five years, the Indian government has been encouraging Ethanol capacity expansion to cut its dependency on imported crude oil and channelize the excess sugar inventories into Ethanol production. These factors will further propel the growth of the Ethanol market in India.[2]

CLASSIFICATION OF BIOETHANOL FEEDSTOCKS (RAW MATERIALS)

The available feed stocks for bioethanol can be categorized into four major types:

1. First generation bioethanol feedstock

It is mainly edible food crops such as rice, wheat, barley, potato, corn, sugarcane, and vegetable oil, for example, soybean oil, sunflower oil, olive oil, canola oil etc

2. Second generation bioethanol feedstock

Second generation bioethanol feed stocks, including lignocellulosic biomass such as forest residue, woody biomass, herbaceous biomass, etc.; non food crops; municipal solid waste; and animal fat.

3. Third generation bioethanol feedstock

Bioethanol produced from algal biomass (i.e., microalgae, microbes) is considered third-generation bioethanol.

4. Fourth generation bioethanol feedstock

Bioethanol produced from captured carbon dioxide by using advanced technologies such as electrochemical synthesis, oxide electrolysis, and petroleum hydro processing is termed fourth-generation bioethanol.[3,4]

MATERIAL AND METHODOLOGY MATERIALS

a. CHEMICALS:

Sulphuric acid
Glycerin
Enzyme
Micro organism

b. EQUIPMENTS:

Distillation unit
Thermometer
Measuring
cylinder Beaker
volumetric flask
Stirrer, Glass rod
Mortar and pestle
Funnel

BIO ETHANOL PRODUCTION PROCESS:

Bio ethanol production process is based on the saccharification and fermentation process.

SIMULTANEOUS SACCHARIFICATION AND FERMENTATION

The simultaneous saccharification and fermentation (SSF) approach has emerged as a more effective method for bioethanol production compared to the conventional separate hydrolysis and fermentation (SHF). This is due to the reduction in energy, time, and capital costs.

In the SSF process both cellulose and fermentation of glucose are carried out in presence of fermentative microorganisms in a single step and the process optimally operates at 37–38°C. This technique reduces the number of steps in the process and is a promising way for converting cellulose to ethanol. Additionally, the SSF method minimizes enzyme inhibition by high glucose concentrations and reduces the risk of microbial contamination.[5,6,7]

EXPERIMENTAL METHOD PRODUCTION OF BIOETHANOL FROM GUAVA

Collection & grinding of raw material

- Guava was collected and chopped into pieces for grinding.
- The pieces were grinded into paste by using grinding machine.

Acidic pretreatment

- Acidic pretreatment is done at three different concentrations of acid were 1%, 2%, 5%.
- Dissolve 2.55 ml (1%), 5.1 ml (2%) and 12.75 ml (5%) of acid in three different flask each containing 250 ml water.
- About 50g guava paste was suspended in each three different concentration of acid solution.
- Stir continuously with glass rod and keep it a side.

Simultaneous saccharification and fermentation

- Pretreated guava sample was autoclaved at 121°C for 30 min.
- Activation of yeast: 10 gr sugar, 4 gr yeast added in 100 ml water. This media was then poured in each flask containing pretreated guava sample.
- Cellulase enzyme was added in each flask and transferred into bottle.
- After sometime S. cerevisiae was inoculated in each bottle for the process of fermentation. The sample were then allowed to incubate for 72h at room temperature.
- Bottle was air locked with balloon and store in cool and dark place.

Filtration and distillation process

- Samples were then filtered by using filter paper and then distillation was done at 70- 80°C to get ethanol.

Sample under SSF

Distillation Process Process

PRODUCTION OF BIOETHANOL FROM PALM JAGGERY METHOD-1

Preparation of sample

- Palm jaggery was collected and crushed into small particles
- Dissolve 250 g of palm jaggery in 1000ml boiled water by continuous stirring
- Add some pieces of raisins into the bottle
- Air lock with balloon and keep in cool and dark place
- After 4 days the yeast and sugar in raisin starts working.

Filtration and distillation

- Samples were then filtered by using filter paper to remove solid substrate from liquid and then distillation was done at 70-80^{o}C to get the ethanol.

METHOD-2

Preparation of sample

- Palm jaggery was collected and crushed into small particles
- Dissolve 250 g of palm jaggery in 1000ml boiled water by continuous stirring

Activation of yeast

- 100ml water boiled at 36-37^{o}C for 10 mins.
- 10 g sugar, 4 gr yeast added in 100 ml boiled water.

Fermentation

- Activated yeast was added to the sample in the bottle
- Then bottle was air locked with balloon and store in cool and dark place

Filtration and distillation

- Sample were then filtered by using filter paper and then distillation was done at 70-80^{o}C to get ethanol

Air locked bottles containing palm Bioethanol Jaggery with raisins and Palm jaggery with yeast

EVALUATION TESTS FOR BIOETHANOL

Physicochemical Parameter 1. Physical/Visual Examination

The appearance of the bioethanol produced was observed by naked eyes.

2. p^H Determination test

Exactly 100 ml of the sample were accurately measured and poured into 250 ml beaker. The pH meter was on and the testing electrode was introduced into the sample in a beaker.
Digital readout of the pH meter displayed the digits and was recorded as the pH of the sample.[8]

p^H Determination test 3. Specific Gravity

An empty bottle was placed on weighing balance and the reading was recorded, the bottle was removed and filled with 20 ml of the distillate which was later placed on a weighing balance, the reading was taken. The bottle was then removed and filled with 20ml distilled water. The reading was recorded and the specific gravity was calculated using the formula. [9,10]

$$\text{Specific gravity} = \frac{wt\ of\ bottle + sample - wt of\ \ empty\ bottle}{wt\ of\ bottle + water - wt of\ \ empty\ water}$$

4. Potassium dichromate test

5ml of distillate sample was taken and 2 drops of potassium dichromate was added into the distillate, heated in a water bath for 30minutes.[11]

Fuel properties of Bioethanol 1. Flame test

Bioethanol was taken into a sprayer and it was sprayed on a burner.[12]

Flame test

2. Flash point test

Bioethanol is taken in a beaker and is heated (under controlled condition). A thermometer is placed in the beaker. The temperature at which it catches fire is termed its flashpoint.

3. Freezing Point

Add 1ml of bioethanol in a test tube and keep the test tubes in a test tube rack and place it in a freezer for 15-20 mins. So that it forms like a gel in this time.

Remove the test tubes from the freezer and note down the temperature by using thermometer.[13]

RESULT AND DISCUSSION

RESULTS:

500ml litre of bioethanol was synthesized from guava and palm jaggery.

Table 1: physicochemical characterization of Bioethanol

Parameters	Bioethanol
Appearance	Colourless
p^H Determination test	7.2 ± 0.2
Potassium dichromate test	Yellowish orange colour
Specific gravity	0.783 ± 0.03 g/cm^2

Table 2: Fuel properties of Bioethanol

Parameters	Bioethanol
Flame test	Burn clearly
Flash test	10 sec
Freezing test	2^0 c

DISCUSSION:

Bioethanol production from guava and palm jaggery was assessed and the experimental bioethanol appears to be colourless and free from any particles, thus indicates that it's within the minimum ASTM specification for bioethanol fuel hence, can be used as fuel for vehicles. The p^H of the sample was measured in order to ascertain the acidity and alkalinity of the bioethanol produced. The result presented in table 1 indicates the p^H of 7.2 for experimental bioethanol falls within the specification (6.5-9.0) for bioethanol fuel which is clear indication that the bioethanol produced is neither acidic nor alkaline.

Specific gravity is the ratio of the density of the substance compared to the density of a reference substance. The specific gravity of the experimental bioethanol was measured in order to indicate the density of it by comparing it to the density of water. The result 0.783 g/cm^2 presented in table 1 indicated the specific gravity of the experimental bioethanol which is in total agreement with the standard obtained from ASTM E100 (range 0.789-0.801).

The bioethanol sample produced was further confirmed using potassium dichromate test. The colour changed to yellowish orange. The flame test was performed it shows that it burns cleanly with bluish flame colour. The flash point gives an idea about the volatility of the substance. The result 10 sec presented in table 2 indicated that the bioethanol catches the fire with in 10 sec at temperature 16.5 ± 0.04^0c. The result of freezing point is at 2^0 c.

CONCLUSION

The main aim of current study was to investigate the potential of Guava and Palm jaggery for bioethanol production. It was found that Guava and Palm jaggery could be a raw material for bioethanol production. The benefits of performing the enzymatic hydrolysis together with the fermentation, instead of in a separate step after the hydrolysis, are the reduced end product inhibition of enzymatic hydrolysis and reduced investment cost.

It is also observed that the bioethanol properties are close to the petrol and satisfies the ethanol standards. It was reported that combustion characteristics of bioethanol are similar to petrol and output of bioethanol is also equivalent to it.

It was concluded that this study discovered the use of Guava and Palm jaggery as substrate for bioethanol production and serve as an alternative fuel.

ACKNOWLEDGEMENT

The authors are grateful to the management of Nalanda college of pharmacy, Nalgonda for providing the facilities to carry out the present research work.

REFERENCE

1. A. K. Sulieman, M. H. Gaily, M. A. Zeinelabdeen, M. D. Putra, A. E. Abasaeed, 2013. Production of Bioethanol Fuel from Low-Grade-Date extract, International Journal of chemical engineering and applications, vol-4.
2. Dublin, 2023. India Ethanol Market Analysis in research and markets.
3. Pobitra Halder, Kalam Azad, Shaheen Shah, Eity Sarker, 2019. Prospects and technological advancement of cellulosic bioethanol ecofuel production, Journal of Biomass and Bioenergy.
4. Santhana Krishnan, Mohamod Faizal Ahmad, Nurr Azmira Zainuddin, Shahabaldin Rezania, 2020. Bioethanol production from lignocellulosic biomass (water hyacinth): a biofuel alternative, pp-123-143.
5. Rawinder Kaur, Himanshu Singh, 2017. Bio-ethanol Production from Rice Husk Using Simultaneous Saccharification and Fermentation and Optimization of Pretreatment Methods. Journal Der pharma Chemica, pp- 1-7.
6. Afifi, M. M. I, Massoud, O.N, El-Akasher, Y.S, 2015. Bioethanol Production by Simultaneous Saccharification and Fermentation using Pretreated Rice straw. Middle East Journal of Applied Sciences, Vol-5, issue-3, pp-769-776.
7. Kim Olofsson, Gunnar Liden, Magnus Bertilsson, 2018. A short review on SSF- an interesting process option for ethanol production from lignocellulosic feed stocks, Journal of biotech. and biofuel, pp- 1-7.
8. Ogala Harrison Chidozie Ekene, Iboyi Nathaniel, 2022. Production and Characterization of Bioethanol from Acid Catalysed Hydrolysis of Cellulosic Biomass (Maize Cob). Journal of Energy, Environmental & Chemical Engineering. Vol. 7, No.3, pp.66-70.
9. Upendra R. S, Pratima Khandelwal, Priyanka. S, Jagadish M L, Nandhini N J, 2013. Production of bioethanol from hitherto underutilized agro waste (Field beans/Green pea pods waste) incorporated zero waste generation technique, International Journal of IRSET, vol-2, issue-10.

10. Abubakar K. Rabiu, Cosmos M. Elinge, Murtala M. Ambursa, Aminu K. Rabiu, Danladi S. Samaila, 2021. Characterization of Bioethanol Fuel from Rice and Corn Straws: A Comparative study, Equity Journal of Sciences and Technology, pp- 74-78.
11. Mustafa Hauwa M., Salihu Dahiru, Bashir Abdulrahman, Ibrahim Abdullahi, 2019. Bio-ethanol production from cassava (manihot esculenta) waste peels using acid hydrolysis and fermentation process. science world journal, pp-45-50.
12. Torgrim Log, Asgjerd Litlere Moi, 2018. Ethanol and Methanol Burn Risks in the Home Environment, Journal of environ Res Public Health, pp- 11-15.
13. https://sciencenote.info/what-is-freezing-point-of-ethanol.