CONVERSION OF WASTE BANANA TO BIOFUEL

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Abstract:

Bioethanol - biodiesel can be the best alternative fuel for regular conventional fuel. Waste materials like scraped banana biomass can be used to produce bioethanol which are generally discarded due to the imperfection during grading processes or because of over rain or flood like situations. In the present investigation, unfruitful banana waste was pretreated physically as well as chemically using amalgam of enzymes like cellulase and pectinase. Along with this *Saccharomyces cerevisiae* was used for the fermentation of the enzymatically hydrolyzed biomass, providing anaerobic condition. Effect of various operating conditions like different pH, temperature, fermentative days and combination of enzymes on the production of bioethanol was observed. Out of all experimental sets performed, maximum yield of 7.9% was obtained at pH 5.5 and 35^0C temperature along with amalgam of enzymes (pectinase and cellulose) than any of the enzymes alone. The outcome of whole investigation states rotten as well unfruitful banana waste due to heavy rain, flood or other drastic conditions are suitable for bioethanol production and so as renewable energy. In addition, use of waste banana as substrate do not compete with food supply of consumers. The produced bioethanol can be used for the production of sanitizers and can also be used in institutional laboratories for sprit lamps and surface cleaners. Produced bioethanol can be used in normal petrol engines without any drastic modifications. The whole procedure acts as environmental recycling process as the byproduct of whole procedure can be used as manure to the crops directly.

Key words: Bioethanol, banana waste, biomass, fermentation, yeast, enzyme, pH, temperature.

Introduction:

India is 1st in the largest banana producing countries in the world with 29 million tons per year on average between 2010-2017 [1]. But about 20-30% of overall production gets wasted every year [2]. And on other hand developing countries like India need of fuel is increasing day by day with the increase of development and population rate. As the stock of non-renewable source of energy is getting vanished soon and prices are getting higher to the Everest there should be an option for full filling the need of increasing population.

Bioethanol production can be the greater way to get out of this problem. Biomass of banana which is thrown out as waste can be used for the production of bioethanol using yeast called *Saccharomyces cerevisiae* providing anaerobic condition, which gives greater result with the application of combination of enzymes (cellulose and pectinase). Banana peel is rich source of starch called cellulose which can be degraded with the

application of cellulose enzyme. And hence the banana peel can be the greater source of carbon source for bioconversion.

In developing countries like India garbage management is also a very big problem. This project may be at smaller level but can help as environmental waste management. With the combustion of the fossil fuel release of carbon mono oxides and other harmful elements and gasses occurs and which affects ozone layer. With the use of this biofuel this problem also can be eliminated. This waste banana fruit can be used as a renewable source of energy as environmental recycling process.

Materials:

Raw Material

Fruit Waste (FW) collected from fruit and vegetable markets, households, hotels, and juice centers contains 40% organic carbon, 6.9% proteins and 8.5% fat, 60% sugar, 21.8% proteins, and 15.7% lipids [3]. Hydrolysis of carbohydrate in FW may result in the breakage of glycoside bonds, releasing polysaccharides as oligosaccharides and mono-saccharides, which are more amenable to fermentation. Total sugar, protein, and lipid contents in FW are in the range of 35.5e69%, 3.9e21.9%, and 10e40%, respectively [4].

On the contrary of the same information the unfruitful banana wastes was collected form wholesale vegetable and fruit market, Cotton Market, Malaviya square, Amravati and local banana producing farmers from the nearby villages.

Enzymes and Chemicals

Cellulase: Plant body is abundantly made up of starch called cellulose which can get hydrolyzed to simpler sugar like glucose and fructose with the application of enzyme cellulase. The specific reaction involved is the hydrolysis of the 1,4-beta-D-glycosidic linkages in cellulose, hemicellulose, lichenin, and cereal beta-D-glucans [5]. That can further get fermented using yeast called *Saccharomyces cerevisiae* providing anaerobic condition for the production of alcohol. Enzyme with 100U/ml concentration was produced by serial dilution.

Pectinase: Pectin is a type of structural fiber present in the fruiting body of the plant and mostly present in peel portion of the fruit [6]. Pectin polysaccharide is one among the main components of the first cellular wall up the center lamella of plant tissues. The degradation of pectin polysaccharide contributes to fruit softening [7, 8]. Pectin polysaccharide is broken down by pectinase into small monomers of galacturonic acid that are negatively charged. That results in the immediate increase in turbidity but decrease in viscosity as well [9]. Enzyme with 100U/ml concentration was produced by serial dilution.

KMS powder: Colorless food materials such as fruit juices, squashes, apples and raw mango chutney are generally preserved with potassium bisulphite. Sulphur dioxide liberated during the reaction of potassium bislphite with acid of the juice which effectively kills the harmful micro-organisms present in food preventing its spoiling [10]. Potassium Metabisulphite is used for avoiding other contaminations of microbes.

DNSA- 3, 5- Dinitrosalicylic acid; for reducing sugar estimation.

$K_2Cr_2O_7$- Potassium dichromate; for alcohol estimation.

0.1N NaOH& 0.1 N HCl; for pH maintenance.

Yeast: Yeast called *Saccharomyces cerevisiae* is used for the fermentation of waste collected by providing anaerobic condition. Optimum pH and temperature for the yeast growth is 4.0-6.0 and between 32^0C-35^0C respectively [11].

Miscellaneous: Plastic wares & glass wares, Weighing machine, Refractometer, pH meter, Spectrophotometer, Orbital shaking incubator etc

Method: Rotten banana waste was collected and measured 500gm. It was properly chopped physically along with pills. Further it was homogenized by blending it with the help of mixer grinder. Physical pretreatment include milling or grinding to breakdown biomass size and crystal structure to increase the surface area for the exposure to enzymes going to be used. Grinding reduces the biomass size and crystallinity of cellulose [12]. Total soluble solid (TSS) was measured and noted 10-12% and distributed into 5 different air tight jars with 100gm of biomass each. 10ml of 100U/ml concentration working solution of cellulase and pectinase was added to each homogenized slurry mixture. pH of homogenized m`ixture was measured and maintained at 6.5 with the help of 0.1N NaOH and 0.1N HCl as its the optimum pH for cellulase [13] and stable relative pH for pectinase [14] along with 50^0C temperature as its optimum for both cellulase and pectinase [15] into rotary shaking incubator for 72hrs. So because of pectinase and cellulase there will be immediate increase in turbidity but decrease in viscosity [9] and cellulose will be amenable, which is present below the recalcitrant lignin and pectin layers respectively. TSS was measured and noted 8-9% and again incubated for 48hrs and TSS was found to be 5-6%. At this stage presence of reducing sugar was estimated by DNSA method [16, 17]. Above whole procedure was commonly carried out in each experimental set and total 3 experimental sets were performed namely EX-I, EX-II and EX-III where pH 5.0, 5.5 and 6.0 and temperature 30^0C, 35^0C and 40^0C was maintained respectively. Meanwhile 50ml of activated (mix one to two tablespoon sugar into lukewarm water along with dried yeast and allow foaming) yeast (*Saccharomyces cerevisiae*) was added to each container and fermented in rotary shaking incubator for 7, 9 and 11 days in respective experiments. After specific reaction time the mixture was filtered with muslin cloth and collected to the single jar. Filtrate was centrifuged at 10,000rpm to get crystal clear fermented product. Centrifuged filtrate was distilled into soxhlet unit providing 75-80^0C temperature, as boiling point of ethanol is 78.37^0C. Alcohol estimation was done by potassium dichromate ($K_2Cr_2O_7$) titration method [18-20].

Analytical Assay:

Total Soluble Solids (TSS) Total soluble solids were measured at each stage of fermentation by refractometer ranging from 0 to 30% brix unit. The result was reported as % brix.

pH Determination The changes of pH was determined and maintained by using pH meter at each stage of fermentation.

Glucose Estimation: The presence of reducing sugar in test sample is commonly estimated by dinitrosalicylic acid colorimetric assay method (DNSA method) [16, 17]. In the, present study the DNSA protocol was applied to estimate the presence of reducing sugar in test sample after the application of enzymes to the homogenized biomass in comparison with standard reducing sugar estimation protocol. Three experimental sets were arranged, each with different concentration of test samples like 1gm, 2gm and 3gm per milliliter respectively. Where, stocks of biomass' test samples were taken from biomass treated with amalgam of cellulase and pectinase with 100U/ml of concentration of each. From these stocks different concentrations of sample like 20µl, 40µl, 60µl, 80µl and 100µl were taken to which 80µl, 60µl, 40µl, 20µl and 00µl of distilled water was added respectively which was followed by the addition of 3ml of DNS (1gm dinitrosalicylic acid, 200mg crystalline phenol and 50mg sodium sulphate in 100ml of 1% NaOH solution) reagent addition to each tube and kept in water bath for 10 min till the colour develops. 1ml of Rochelle salt (40% sodium potassium tartrate) was added to the warm solution and absorbance was measured at 520nm on spectrophotometer after cooling.

Bioethanol Concentration: As ethanol boils at 78.37^{0}C, taking the reference; filtrate was continuously boiled between 75-80^{0}C in distillation unit. Since, boiling point of water is 100^{0}C, the collected liquid in the extractor was estimated to be ethanol and further confirmed with potassium dichromate titration test [18-20].

Result:

During the whole investigation, after reducing sugar estimation in all the experimental sets, the yield of bioethanol at different pH, temperature, fermentative days and amalgam of enzymes respectively.

In experiment one (EX–I), the homogenized biomass was hydrolyzed with mixture of enzymes, which was fermented for 7 days at pH 5.0 and 30^{0}C temperature, yield obtained was 6.9 % bioethanol (Table 1).

Table 1. Bioethanol concentration obtained with pH 5.0 and temperature 30^{0}C

Sr. No.	Parameters Used	Incubation Conditions
1.	pH	5.0
2.	Temperature	30^{0}C
3.	Fermentative Days	7 days
4.	Bioethanol Conc. Obtained	6.9%

With the increased fermentative days with varying pH and temperature in experiment two (EX–II) with 5.5 and 35^{0}C respectively, yeild of 7.9% of bioethanol concentration was obtained, which was highest of all the three experiments carried out (Table 2).

Table 2. Bioethanol concentration obtained with pH 5.5 and temperature 35^{0}C

Sr. No.	Parameters Used	Incubation Conditions
1.	pH	5.0
2.	Temperature	30^0C
3.	Fermentative Days	9 days
4.	Bioethanol Conc. Obtained	7.9%

In experiment three (EX–III) in fermentation period of 11 days, along with increased pH and temperature of 6.0 and 40^0C respectively, depletion in yield was observed with 7.4% bioethanol concentration.

Table 3: Bioethanol concentration obtained with pH 6.0 and temperature 40^0C

Sr. No.	Parameters Used	Incubation Conditions
1.	pH	6.0
2.	Temperature	40^0C
3.	Fermentative Days	11 days
4.	Bioethanol Conc. Obtained	7.4%

After following all the results the highest yield of biofuel was obtained at pH 5.5 and 35^0C temperature. Following the results another experimental set (EX-IV) was designed with pH 5.5 and 35^0C temperature that was found to be optimum, single enzymes was used instead of amalgam and further similar protocol was followed as in EX-I, EX-II & EX-III to study the respective results (Table 4).

Table 4: Bioethanol concentration obtained with the use of single enzyme.

Sr. No.	Parameters Used	Incubation Conditions
1.	pH	505
2.	Temperature	35^0C
3.	Fermentative Days	9 days
4.	Bioethanol conc. obtained in application of **cellulase**	7.1%
5.	Bioethanol conc. obtained in application of **pectinase**	6.4%
6.	Bioethanol conc. obtained in application of **amalgam (EX-II)**	7.9%

Conclusion:

Unfruitful, drenched, waste banana directly can be used for bioethanol production. This can be the side business to farmers providing raw material as bioethanol to bigger sanitizers or alcohol based cleaning kits producing companies. Biofuel produced can be used as less carbon emitting fuel extender into vehicles. Bioethanol produced can also be provided to the institutions as well as small and large scale laboratories for

their spirit lamps or surface sterilization kits. The whole procedures can be used as environmental recycling process and byproduct can also be used as manure to the crop field itself completing cycle.

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