Studies on Fuel Production from Fruit Peel Waste – A Review

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Abstract : *Environmental pollution like global-warming and air pollution increases due to extensive use of fossil fuel , which lead to increase price in conventional fuel. So the world requires alternative of fossil fuel. Here bioethanol comes into play because it is both costeffective and renewable. Bioethanol as biofuel has high octane number (108), low boiling point, higher heat of vaporization, comparable energy content and bioethanol can also be use in vehicle without modification of existing engine. The purpose of the study is to extract bioethanol from fruit peels. First we wash Musa acuminata peels (banana peels) with distilled water then we shade dry it peels and after drying it is converted into powder, then we perform hydrolysis on the banana peel powder, which lead to final step fermentation, then we finally extract bioethanol from banana peels. This conducted study show us the process required for extraction of bioethanol, some further study are required for more indepth information of the process.*

Keywords : Pre-treatment, Hydrolysis, Fermentation, Bioethanol, Banana peels.

1. Introduction

Fuel demand for domestic as well as industrial uses is progressive. After covid-19 pandemic we have seen that the prices of petrol, disel, LPG, etc rising day by day. Declination of natural resources of energy production such as coal and other fossil fuels and finding new sources is challenging. The united states is the world's largest producer of bioethanol. Many countries have instructed on ethanol blend with gasoline to be used as a fuel[1]. Also ethanol is very good organic solvent. Hence, ethanol needs are rising to reduce fuel import, boosting rural economies and improving air quality[2]. It is environment friendly, but short supply and increased cost is main hindarance in the production of bioethanol. Most of the feedstocks used for ethanol production are also used as food for human. Many attempts are being made to find new sources of feedstock, which are non- edible and easily available. Under such circumstances a novel approach is essential to use renewable substrate such as fruit waste. There are several fruits available. Out of these, the

peels of certain fruits promise to produce fuel. For our experiment, we have studied banana peels. They are lignocellulosic agricultural waste and can produce bioethanol, a renewable form of energy[3]. The major steps in the bioethanol production are pretreatment and hydrolysis of lignocellulosic biomass. Banana peels contribute 35% from the total weight of ripe as well as unripe banana peels[4].

Materials and Methodology :

We can observe the different methods of bioethanol production from banana peels including selection of peels, pretreatment, proximate analysis of peel powder, hydrolysis, fermentation, etc. Various methods for bioethanol production are mentioned here. Now, we can observe stepwise conversion of banana peels to bioethanol from following methods :-

A) Isolation of microorganisms and its maintainanance :

a) For Asparagilus Niger : Different soil samples from upper surface can collect in 2-3 plastic bags and they were air dried at room temperature approximately 27°C for 1-2 days[13]. Stones and plant residues can be remove. 100 mg of each soil sample in labled test tubes. Test tube contain five ml sterile solution. To minimize bacterial growth 30 mg/L streptomycin was added. Shake test tube in vortex motion. 100 microliter of each of suspension was evenly spreaded on PDA plates with a spreader and incubated at 28°C. Mixed colonies were observed within a week. Pure culture of Aspergillus niger was obtained by streak plate method.

b) For Sacchromyces cervisiae : It can be obtained from local market and maintained on PDA slants at 4°C [21].

B) Pretreatment : It is an important tool for cellulose conversion processes, and is essential to change the structure of cellulosic biomass to make cellulose more available to the enzymes that convert the carbohydrate polymers into fermentable sugars.

It is subdivided in four types as following :-

a) Acidic Pretreatment : It is oldest method for production of bioethanol. HNO_3, H_2SO_4, HCl and H_3PO_4 are used during pretreatment of lignocellulosic biomass. Sodium Hydroxide (NaOH) is the major alkali used in this process. Pretreatment of biomass includes hydrolysis of hemicellulose[18].

b) Biological Pretreatment : White rot fungi is biological agent which separates cellulose from lignocellulosic biomass. Mild conditions of temperature and pressure required. It is Time consuming

process takes 24 hours to complete. Bacteria or fungi secrets enzymes which catalyses degradation of lignin and cellulose. It's not efficient process[25].

c) Organosolv Pretreatment : Extraction of lignocellulosis biomass (LCB) from feedstock with the help of organic solvents and their aqueous solutions is basic aim of organosolv pretreatment method. Ethanol, methanol, acetone, ethylene glycol are used with many more organic solvents and their mixtures in this process. These pretretment needs 60% concentration of organic solvent without catalyst followed by temperature ranges between 160°C to 220°C. Long reaction time of 30 - 120 minutes required[3].

d) Steam pretreatment : It is physiochemical pretreatment technology in which the biomass is heated to 160°C to 260°C in a close vessel under high pressure ranging from 10 bar to 50 bar for minimum time period. Then pressure is quickly released causing explosive effect on cells of banana peels[25].

C) Hydrolysis : Hydrolysis perform to convert lignocellulosis biomass (LCB) i.e. cellulose, hemicellulose into fermentable sugars such as glucose, sucrose, fructose[26]. Membrane reactor is suitable for hydrolysis process because it is used to remove hydrogen from hydrogen producing reaction leads to shift in thermal equillibrium. Yeild of sugar increse when raw materials are finely powdered and posses small particles size. Increase in pH value reduces yeild of sugar.

D) Fermentation : Sacchromyces Cervisieas is also known as Baker's Yeast is widely utilized in the fermentation process [23]. Hexoses sugars e.g. sucrose, maltose, cellbiose, glucose are fermented. 30-70 degreee temperature required. pH ranges from 4 to 7. Obtaining 4% ethanol w/w we required 50°C temperature. 80 g/L sugars are hydrolysed in separate hydrolysis and fermentation process.

E) Filtration : Membrane filtration is used to separate cells from fermentation broth. It can be heated at minimum temperature till 30°C, for 14-22 hours. Ethanol is solvent here. It allows solutes and other particles to separate out from membrane.

F) Distillation : This process is designed in such a manner so that ethanol concentration will increase to highest available purity [26]. Column use can classify the boiling points of water and ethanol to boil further and separate ethanol. The distillation process raise the concentration of alcohol from low purity to high purity. 1L of fermented raw material added in flask and heated at 80°C for maximum 3 hours. Evaporation temperature can be measure by digital heating mantle.

G) Gas Chromatography : The highly sensitive technique based on principle that the sample solution enters a gas stream which transport sample in a separation tube known as column. During the procedure, the oven temperature was maintained at 80°C. The injector and detector temperature is 120°C and 160°C

respectively. The flow rate for carrier gas i.e nitrogen was set at 30 ml per minute. 0.2 microliter volume of injection sample and 0.2 microliter volume of standard ethanol used. From volume of ethanol area of standard sample can be determine[13]. Also by using following formula concentration of unknown sample of ethanol can be determined.

The formula used for calculation of percentage of Ethanol is given below

Concentration of ethanol = Volume of ethanol * Area of unknown sample / Area of standard Ethanol

% of ethanol = 100- [Volume of standard ethanol * Concentration of Ethanol/Volume of standard ethanol*100]

Literature Review :

Freddie Inambao et al investigated the feasibility of bioethanol production from matooke species peels through a fermentation process using Saccharomyces cerevisiae (yeast) [5]. The analysis was carried out for two different varieties of matooke species which are Mbwazirume and Nakyinyika. These species were procured from different parts in Uganda. The peels were separated, washed with distilled water and shade dried for 83 hours. Then it was chipped and ground to a coarse powder and kept at room temperature till further analysis. The hydrolysis process was employed using dilute sulphuric acid in a shaking water bath for about 20-60 min at 50-90 °C followed by filtration at room temperature. Post this, fermentation process was carried out maintaining a pH of 5.0 using 1M NaOH. The solutions were manually shaken and then placed in a shaker water bath at 29-39 °C at 165 rpm for about 10 to 30 hours. After shaking, the mixtures were kept for 18 hours to settle the particles from the liquid. 17 runs per sample were conducted to obtain ethanol. Bioethanol was extracted by distillation and dehydration. For dehydration, anhydrous sodium sulphate was used. The following results were obtained for proximate analysis of two hybrid varieties. Per 100 g, Mbwazirume and Nakyinyika varieties contained 8.51 and 8.71 g moisture, 8.23 and 7.74 g ash content, 5.53 and 4.84 g protein, 3.99 and 3.13 g fat and 72.23 and 72.85 g carbohydrates. It clearly shows a rich amount of carbohydrate content and hence is considered a potential source for bioethanol production.

Amit Ranjan et. al studied the production of bioethanol by Orange and Banana peel using Saccharomyces cerevisiae. For their research, they obtained the Orange and Banana peels from the local market of Allahabad [6]. The peels were wiped with 70% ethanol, washed with distilled water and cut to small pieces. The peels were dried separately and ground to powder. These substrates were treated with 2% NaOH and autoclaved for 1 hour at 121 °C. Further, they were oven dried for 12 hours at 60 °C. The peels were then subjected to enzymatic hydrolysis, incubated on rotary shaker for 24 hours at 50 °C and 75 rpm. To denature the enzyme, the mixtures were boiled for 2 minutes and centrifuged at 5000 rpm for 15 minutes after which

they were fermented. Solid state fermentation (SSF) was employed. The sterilized flasks were inoculated with 3mL for 24 hours and incubated under anaerobic condition. For the fermentation process, pure culture of S. Cerevisiae maintained on Potato Dextrose Agar at 4 °C was used. For the inoculation, 3mL of harvested spores using sterilized water with 0.1% Tween-80 were used. Fermentation process was optimised at different parameters to get the maximum bioethanol production. The parameters were temperature from 10 to 40 °C, pH from 4.0 to 7.0 and incubation period from 48, 72, 96 and 120 hours at 30 °C. The study concluded that the best temperature for Orange peel was 40 °C with 4.07% (v/v) production and that for Banana peel was 30 °C with 2.86% (v/v) production. For the pH, the maximum yield was obtained at 4.0 with 3.90% (v/v) production for Orange peel and 5.0 with 2.90% (v/v) production for Banana peel. 48 hours of incubation time was best suited for Orange peel with 4.04% (v/v) production and for Banana peel, it was 72 hours with 3.78% (v/v) production.

Walia N.K. et. al studied the production of bioethanol from Mango peels [7]. The peels were primarily sun dried and then oven dried for 3 hours at 50 °C. Thereafter the peels were ground to powder, mixed with water (1:3) and left overnight. With the help of cheese cloth, the liquid containing sugars were extracted. The pH and temperature were maintained at 5.0 and 37 °C respectively. The extract was diluted so as to obtain the desired concentration of sugars which was 7-9% (w/v). DNS method was employed to determine the concentration of reducing sugar. To produce bioethanol, 3 mL of DNS reagent was added to 3 mL of glucose sample and the mixture was heated for 5-15 minutes at 90 °C to produce the red-brown colour. In order to stabilize this colour, 1 mL of 40% Rochelle salt solution was added. It was then cooled to room temperature in a cold water bath and absorbance was measured using a spectrophotometer at 575 mm. The extract phase from extraction was subjected to simple distillation and the obtained distillates and residual phase were analysed by gas chromatography to check the Bioethanol concentration. The results showed that the solution produced from mango peel extract after fermentation contained 95% bioethanol.

Yaser Dahman et al. cited a few advantages and drawbacks of bioethanol as an alternative source of fuel [8]. Bioethanol produced from lignocellulosic biomass serves as an alternative promising carbon neutral biofuel. Some advantages of bioethanol as a biofuel include high octane number (108), low boiling point, higher heat of vaporization and comparable energy content. Bioethanol is renewable biofuel that is also oxygenated (35% oxygen) and therefore holds a promising potential to reduce automobile emissions. With many advantages, there are a few drawbacks as well. Bioethanol has low volumetric energy which implies that a vehicle will require more bioethanol per kilometer (50% more) than gasoline. Also bioethanol can degrade certain elastomers. Moreover, bioethanol is difficult to vaporize at low temperature, which means that igniting a car will be difficult at low temperature. It will require large infrastructure change to provide bioethanol. Since bioethanol easily absorbs water , it has a high tendency to corrode metals. Considering

all the aspects, bioethanol today as an alternative source of fuel, being eco-friendly and efficient is in high demand.

Gaurav Shah carried out a research to study the effect of temperature and pH on bioethanol production from waste banana peels [9]. For their study, they collected the waste banana peels from local market in Surat city. Peels were washed with distilled water and chopped into small pieces. For the fermentation process, Saccharomyces cerevisiae was used and for the preparation of inoculum Glucose Yeast Extract (GYE), broth was used. pH was varied with the help of 0.1 N HCl and 0.1 N NaOH. A sample of 5gm chopped banana waste in 100mL distilled water was prepared and it's sugar concentration was measured before autoclaving. Broth was autoclaved at 121 °C for 15minutes maintaining a pressure of 10 psi. Fermentation media for bioethanol was prepared by inoculating 10% inoculum. After every 24h, 5mL sample was withdrawn to check the sugar concentration and ethanol concentration. They studied the effect of temperature on bioethanol production ranging from 25 to 55 °C. Their research depicted that room temperature (around 32 °C) is most suitable for bioethanol production. They got 88 mg/mL production at room temperature. Also, they studied the effect of pH from 5.5 to 7.5 pH on bioethanol production and found that the best results are yield at 6.5 pH. They reported 70 mg/mL bioethanol concentration at 6.5 pH.

A Matharasi et al. studied the determination of bioethanol production from banana waste using indigenous yeast Saccharomyces cerevisiae. For their study, they procured banana wastes such as pseudo stem, peel and spoiled banana from fruit market in Tamil Nadu [10]. These were washed with distilled water and blended to powder using electric blender. To enrich the yeast population, aliquotes of Glucose Peptone-Yeast extract (GPY) were added to liquid media. For the comparative study, 10 yeast strains with morphologically distinct colonies were selected, purified by streaking and stored in Yeast extract-Malt extract Agar (YM) slants containing 5%, 7%, 9%, 11% and 13% alcohol (v/v). Potential yeast isolate was identified by 18s rRNA gene sequencing in Seoul Korea. The cellulosic hydrolysate was inoculated with 1 to 5% of inoculum of Saccharomyces cerevisiae at pH 5. The filtrate was obtained after 7days of incubation at 35 °C. They found that all the 10 isolates showed decent growth. They also found that as the concentration of ethanol increases, the growth rate of the yeast isolates get reduced. No isolate showed growth in 13% ethanol supplemented broth. Also glucose concentration was varied from 5 to 25% and the results were studied. Better growth was seen at 5 to 10% sugar concentration. The highest activity was recorded by the yeast isolate SB10 which was 56.8 µmol/min. Nitrogen sources were also added to enhance the bioethanol production. Among them, ammonium sulphate gave the best yield.

Ashish G. Waghmare et al. performed a research on utilization of unripe banana peel waste as feedstock for ethanol production [11]. Unriped banana peels of hybrid variety of Musa acuminata and Musa balbisiana

were collected from local market, chopped, dried at 60 °C for 24 hours in tray drier and then ground to powder. The powder was sieved through 0.45 mm mesh and kept in airtight container until used for further experiments. AOAC method was employed to determine the protein and ash content. Protein content was determined using Micro Kjeldahl procedure with a nitrogen to protein conversion factor of 6.25. For the determination of fat content, Soxhlet method was used. Concentrated sulphuric acid was used for acid hydrolysis. Three strains of S. cerevisiae (NCIM 3095, NCIM 3570 and NCIM 3059) maintained at MYGP medium were used for fermentation. It was found that the NCIM 3095 strain of S. cerevisiae had maximum conversion efficiency of bioethanol production with 42.8% w/w at 36 hours in acid hydrolysate. Various parameters were optimized by OFAT approach. It was observed that reducing sugar release increased with temperature. 1.5% (v/v) of sulphuric acid was optimized at 120 °C in autoclave at 100 kPa pressure for maximum sugar release. They found the optimum fermentation time to be 36 hours. Also the pH was varied from 4 to 7 and maximum yield was obtained at pH 5.

Udara Arachchige studied the suitable conditions for acid hydrolysis on fruit peels. Hydrolyzation of starch and cellulosic biomass is catalysed by acid [12]. Either concentrated acid (10-30%) or diluted acid (1%) are used for hydrolysis. Concentrated acid operates under low temperature, pressure and long reaction time and vice versa for dilute acid. Dilute acid processes in two steps. The first step converts cellulosic materials to sugars and the next step converts sugars to other chemicals. The first step is performed at mild temperature to maximize 5-carbon sugars from hemicellulose and the second step is conducted at high temperature to maximize 6-carbon sugars from cellulose. Patsalou et al. studied acid hydrolysis of citrus peel waste to produce bioethanol. He concluded that the best yield was obtained by using 0.5% sulphuric acid and 5-6% 9 w/v dry solid citrus peel waste at 116 °C temperature for 10-13 min. The yield of bioethanol obtained was 5.8 g/L. Hydrolysis carried out with concentrated acid completely degrades the cellulose producing glucose and sugars and partly degrades the hemicellulose in the feedstock. The prime advantage of hydrolysis with concentrated acid is the higher sugar recovery efficiency which is over 90%. Danmaliki et al. performed concentrated acid hydrolysis on Banana peels. He carried out the process using 10% sulphuric acid at 120 °C for 6 hours. The maximum yield observed was 1.4% glucose concentration from total sugars and 60 ppm bioethanol.

Ajay Kumar Singh et al. carried out a research on bioethanol production from Banana peels by simultaneous saccharification and fermentation process using Aspergillus niger and Saccharomyces cerevisiae [13]. For their research, they procured the ripe waste banana peels from a local market in Allahabad. The peels were cleaned, chopped to 3-5 cm and disinfected with 70% ethanol. The peels were then sun-dried for a week and finally ground to powder. For the fermentation process, Aspergillus niger was obtained by streak plate method whereas Saccharomyces cerevisiae was obtained from the local market and both maintained at 4

°C on PDA slants. Fermentation was done with 5g powder in 96 mL distilled water which was sterilized and autoclaved at 121 °C for half an hour. 4% (v/v) inoculum of Aspergillus niger and 3% (w/v) inoculum of Saccharomyces cerevisiae was added and the fermentation was done for 7days. The process was studied at different parameters like temperature from 20 to 50 °C at pH 6 and different pH from 4 to 7 at 30 °C. The optimum temperature and pH was investigated and used for fermentation at different yeast concentration ranging from 3 to 12%. Using gas chromatography, bioethanol content was measured every 24 hours. The results depicted that the best yield was achieved at 30 °C temperature with 6.434%. For pH, it was observed that maximum bioethanol production was obtained at pH 6 with 6.287%. They also found that as the concentration of Saccharomyces cerevisiae increases, the time required for fermentation decrease.

Pallavi Sharma et al studied the effect of microwave pretreatment on bioethanol production. For their experiment, they procured the Banana peels from a local market in Allahabad [14]. The peels were cleaned, chopped, disinfected with 70% ethanol, sun-dried for a week and then ground to powder. Samples were prepared with 3 gm Banana peel powder and distilled water with 1:10 w:v ratio. These samples were pretreated by heating for 5min in microwave at different powers like 80, 160 and 320 W. Post which, enzymatic hydrolysis was performed. The samples were autoclaved at 121 °C for 30min and Aspergillus niger inoculum (4% (v/v)) and Saccharomyces cerevisiae inoculum (3% (w/v)) were added to the fermentation process. It was done for a week and a track of ethanol content was kept every 24 hours. Reducing sugar content was determined and it showed maximum content for 160W sample with 400 mg/g and that of unpretreated sample was 180 mg/g. A high performance liquid chromatography (HPLC) system was applied to measure the glucose content. It was found that the maximum sugar content was for 160W sample which was 380 mg/g and that for unpretreated sample was 160 mg/g. On their experimental basis, they concluded that pretreating Banana peels under microwave power at 160W for 5min could significantly elevate the reducing sugar and glucose content in simultaneous saccharification. The microwave pretreatment gave maximum output where the yield obtained for reducing sugar and glucose were increased by 220 and 150 mg/g than the unpretreated Banana peel powder.

Yamunadevi Puraikalan carried out the proximate analysis of Banana (Musa sapientum) peels of the Indian and the USA variety [15]. For the US variety, the Bananas were dried in Dehydro electric food dehydrator. The peels were removed and sliced into small pieces of around 2 inches and we're dehydrated in a dehydrator at 165 °F for 4 hours. Then it was ground to powder and kept in airtight container at room temperature until needed for use. The Banana peel powder for the Indian variety was prepared using Radio Frequency (RF) sterilizer technology. The banana peels were passed five times through the RF sterilizer and the powder was prepared. The analysis of the principles of banana peel powder was triplicated and the

following results were depicted. The Indian variety contained 47.85% carbohydrate, 9.52% moisture, 11.63% protein, 14.41% fiber, 12.95% ash and 3.64% fat whereas the US variety showed 38.06% carbohydrate, 22.06% moisture, 9.42% protein, 11.51% fiber, 12.35% ash and 6.65% fat. It is due this rich carbohydrate content, that banana peels are considered a valuable source of bioethanol production.

M. Tutt et al. studied the influence of different pretreatment methods on bioethanol production from wheat straw [16]. Samples of wheat straw were collected, milled to powder of 1 to 3 mm size and dried to a moisture content of less than 10%. Ash, hemicellulose, cellulose and lignin contents were analyzed. Pretreatment with dilute acid and with alkali was employed. For the dilute acid pretreatment, 0.5 to 1.5% sulphuric acid solution was added to hydrolyse hemicellulose at 130-200 °C during 5-60 minutes. Also, results using nitric acid and hydrochloric acid were analyzed. Alkali pretreatment removes lignin and part of the hemicellulose. Alkalis such as NaOH, KOH and Ca(OH)2 were used at 120-180 °C. They reported that alkali pretreatment yields better result than dilute acid pretreatment. After pretreatment, enzymatic hydrolysis was carried out to convert the cellulose fibres and hemicellulose to fermentable sugars. For the fermentation process, Saccharomyces cerevisiae was added to the samples. The results showed that nitric acid gave the highest cellulose to glucose conversion rate of 316.7 g/kg. The lowest glucose yield of 221.3 g/kg was achieved when pretreated with 1% HCl. But at the same concentration, nitric acid gave 30.1% higher glucose yield. For the wheat straw samples pretreated with sulphuric acid, half the samples were rinsed with water before adding enzymes and rest were not. They concluded that the rinsed samples yielded 267.3 g/kg glucose and unrinsed samples yielded 276.7 g/kg glucose. In case with KOH, rinsed samples gave a glucose yield of 221.7 g/kg while unrinsed samples gave 267.5 g/kg. The rinsed samples pretreated with KOH gave the best bioethanol yield of 104.3 g/kg. 14.5% higher bioethanol yield was seen for the rinsed samples than the unrinsed wheat straw samples pretreated with sulphuric acid. Therefore, they concluded that KOH pretreatment process combined with rinsing the samples before hydrolysis is the most effective method for bioethanol production.

Sócrates Palacios et al. compared the physicochemical pretreatments of banana peels for bioethanol production [17]. Tabasco variety of Banana was obtained from a local market in Mexico. Peels were removed from the fruit at maturation stage number 7. The peels were dried in hot air oven for 48 hours at 72 °C. They were pretreated by processing into three different forms: unmilled, liquefied and pulverized. These three forms were compared and studied. They were treated with sulphuric acid at different concentrations of 0, 0.5 and 1% (v/v), and autoclaved for 15 min at 121 °C. Kluyveromyces marxianus was used for fermentation. Glucose and fructose contents were analyzed through HPLC-IR technique. The results depicted that banana peel contained 86% moisture before drying. As compared with the other two, pulverized form showed highest cellulose content when treated with 0.5% sulphuric acid at 121 °C.

Unmilled banana peel showed highest concentration of hemicellulose when treated with 0.5% sulphuric acid at 28 °C. The liquefied banana peels accounted the highest total sugar levels in comparison with the other two. This was achieved with 0.5% sulphuric acid at 121 °C and 1% sulphuric acid at 28 °C. However, the reducing sugar levels were higher in the liquefied and unmilled banana peels than in pulverized banana peels. Analyzing all the results, they concluded that a pretreatment process with diluted acid and high temperature can dissolve almost all hemicellulose, but not the lignin component.

Mirza Zaheer Baig et al. carried out a comparative study of bioethanol production from acid and enzymatically hydrolysed cotton stalk using co culture of Saccharomyces cerevisiae and Pachysolen Tannophilus [18]. For their study, the cotton stalks were collected, shredded, sundried, debarked, bailed and ground to powder of 1 mm size. They found that it contained approximately 42.40% glucan and 23.20% xylan. Two separate sets were prepared for comparative study of acid hydrolysis and enzymatic hydrolysis. The sugar solutions produced were distinctly fermented to produce bioethanol. Sample obtained during fermentation was centrifuged at 10,000 rpm for 10 min at 4 °C. They found the sugar concentration to be 11 g/L for acid hydrolysis and 24.5 g/L for enzyme hydrolysis. No bioethanol production was detected till 12 hours of fermentation in both the sets. Thereafter bioethanol was steadily produced up to 48 hours. Maximum production was seen when 93.84% from acid hydrolysate and 97.81% from enzyme hydrolysate was utilized by co-cultures. The peak production was noted as 4.96 g/L for acid and 9.56% for enzyme hydrolysate. For acid hydrolysate, the efficiency of fermentation was 87.52% and for enzyme hydrolysate it was recorded 76.85%. The results clearly establish that cotton stalks are a good source for bioethanol production.

Cavendish bananas (*Musa* spp. AAA) were purchased from a local market nearby of University Putra Malaysia, Selangor, Malaysia [19]. The Ripened bananas were selected according to criteria described by Pekke et al. Ripeness of banana was determined from Dole colour Chart (Dole Castle & Cook, Inc) at color stage 4 (when a peel colour more yellow than green). Then peels of fruit were removed and slices of thickness 4,7 and 10 mm made with salad cutter and dipped into lemon juice in lemon:water concentration of 1:4. Banana slices were dried with a conventional oven (Memmert UNB 500, Germany). Various drying temperatures 60,70,80,90 °C were chosen. The sample size of 300 g kept constant throughout the process. Moisture loss of banana slices was measured after interval of an hour. The drying process continued till consistent readings. Dried samples were cooled under laboratory conditions and kept in dessicators for furthur process. Amount of vitamin C (ascorbic acid), vitamin A (beta - carotene), potassium (K) and iron (Fe) was measured according to AOAC method (official method No. 967.21, 960.45 and 968.08). Color was measured with a calorimeter. Statistical software "Statistix 8.1" was used for statistical analysis. Drying rate and thickness are inversely proportional. Thinner were the slice faster was the drying rate. Water

removal rate was very high in first hour for four drying temperature. After three hours rate of water removal decreses. Water of tightly bound type couldn't remove by oven temperatures 60,70,80, °C. When thickness of slice was 4 and 7 mm at 90°C, tightly bound water was removed. Pekke et al. found that at excess thickness i.e. 5 and 8 mm under infrared radiation its hard to remove water at higher temperatures (90°). Nutrient concentration in dried banana slices was more than fresh samples because of increased dry matter. Highest quantity found at 60°C for vitamin A and C was 1.33 and 8.89 mg/100g respectively. At 90°C maximum potassium and iron content were determined. As oven temperature incresed vitamin A and C contents were decreased. Temperature and time parameters are key factors in retension. Prathapan et.al have noted that at higher temperature slices became darker and yellowness was also increased. High temperature was main basis for colour damage during the drying process. The results are in good agreement with Arslan and Özcan (2008) report in savory. The color (*L*, *a** and *b**) was measured with a colorimeter (Konica Minolta CM-3500d Spectrophotometer, USA). In case of oven 60 °C and 90 °C treatments the drying time were 16 and 10 hours, respectively. While, vitamin C concentrations were 8.89 and 2.08 mg/100g which indicated that temperature has massive impact on vitamin C concentration in banana slices as compare to lengthen drying time. These findings are in good agreement with Lee and Labuza (1975); Villota and Karel (1980); Mishkin et al. (1984).. Dried banana chips are good source of vitamin A, C, potassium and iron.Vitamin A and C are highly influenced by temperature and length of drying time. According to the color quality oven 60 °C was found to be the optimum temperature for 4 mm slice thickness as it is much closer to the natural one. Pekke et al. (2013) also reported similar observation about color quality under infrared radiation drying.

Timothy J. Tse et. al reviewed the factors affecting bioethanol yield and studied major fermentation techniques used in commercial production of bioethanol [20]. The choice of yeast plays a major role. Saccharomyces cerevisiae is the most used yeast accounting to its tolerance and robustness. They studied the three major fermentation processes. The first fermentation process is the submerged/liquid state fermentation in which the fermentable substrate is liquified and microbes are grown in that liquid substrate. This process quickly affords a high yield of bioactive metabolites and therefore is utilised in enzyme production. Certain disadvantages to this technique are that it requires inputs of energy and water, large volume bioreactors and distillation columns. Also large volumes of low-value coproducts like thin stillage and wet distiller's grains are generated. Fortunately, these waste byproducts can be managed efficiently. First generation bioethanol production involves this technique. The second fermentation process is Solid-state fermentation (SSF). In this process, the organisms grow on non-soluble material or solid substrates in the absence of free water. Temperature, type of microorganism, substrate used, aeration, water activity and bioreactor used are some of the factors that affect SSF process. Second generation bioethanol production often involves this technique. The third process is Very high gravity (VHG) fermentation. It is a propitious bioethanol production strategy using high sugar musts or feedstock. In general, sugar concentrations can be divided into normal gravity (<180 g/L of total sugars), high gravity (180 to 240 g/L of total sugars) and very high gravity (≥250 g/L of total sugars). In VHG

fermentations, more than 30% of solids are consumed and hence more than 15% (v/v) of bioethanol production could be achieved compared to the average of 10-12% (v/v) that is observed in most distilleries. The advantages of VHG fermentation is that is reduces waste production, energy consumption and water consumption and therefore is propitious and improves environmental sustainability. In addition, the various modes of fermentation which are batch, fed-batch and continuous also affect the fermentation process.

Siti Hajar Mohd Azhar et al. reviewed the importance of yeasts in sustainable bioethanol production [22]. Yeasts hold the ability to catabolize six-carbon molecules such as glucose into two carbon components such as ethanol without proceeding to the final oxidation product which is CO2. They discovered that the total count of yeast species on earth are expected around 150,000. Yeasts may get inhabited due to severe climatic conditions and low temperature. They are also highly susceptible to stressful conditions like osmotic stress, bacterial contamination, ethanol concentration, etc. Rise in temperature (35-45 °C) and ethanol concentration over 20% create challenges during sugar fermentation. Inability of S. cerevisiae to grow in high alcohol concentration media leads to the inhibition of ethanol production. From thousands of years, yeasts such as Saccharomyces cerevisiae are used for alcohol production. It is cost effective as it gives a high ethanol yield and can withstand high ethanol concentration. In recent times, S. cerevisiae is the most used yeast for bioethanol production. It can tolerate a wide range of pH. However, it can only ferment hexoses but not pentoses. Only a few yeasts from genera Pichia, Candida, Pachysolen and Schizosaccharomyces can ferment pentoses to ethanol. The efficiency of bioethanol production can be increased by using yeasts that are tolerant to inhibitors, ethanol and temperature.

Maria Parapouli et al. described about the yeast Saccharomyces cerevisiae and gave its industrial applications. The term bioethanol is used to define the amount of ethanol produced to be used as a fuel [23]. Bioethanol can be used alone or combined with gasoline. This offers several advantages over petroleum fuel such as higher octane number (108), increased heat of vaporization, higher flame speeds and broader flammability limits. Besides it is less toxic, readily biodegradable and produces lesser air-borne pollutants. Saccharomyces cerevisiae is considered the protagonist in the industrial production of bioethanol. It catabolizes sugars using glycolysis and forms pyruvic acid which by pyruvate decarboxylase is converted to acetaldehyde and carbon dioxide and ultimately reduced to form ethanol by alcohol dehydrogenase and simultaneously releases NAD+. After fermentation using yeasts, the ethanol obtained is isolated by distillation and dehydration. There are three ways to carry out these processes. These include separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and simultaneous saccharification and co-fermentation (SSCF). Certain factors like temperature rise, pH alteration, osmotic stress and ethanol concentration cause S. cerevisiae multiple stress phenomena, which in turn affects bioethanol production. S. cerevisiae holds important applications in various industries. These

include beverage and food industry, bread industry, chocolate industry and bioethanol industry. Therefore, it cannot be denied that Saccharomyces cerevisiae is one of the most important yeast.

Many products remain in the background and yet impact our life strongly. Bioethanol is one of them. In India, ethanol is primarily produced as a byproduct of sugar manufacture [24]. Therefore the major ethanol manufacturers in India are the large sugar companies. Hence, it is obvious that the majority of the ethanol producers lie in sugarcane producing states. Right now in India, mainly bioethanol is produced using three types of raw materials which are sugar and molasses based, second generation material and grain-based. Following are some of the important ethanol producing companies in India. Bajaj Hindusthan Sugar Ltd (BHSL), a Mumbai-based company is the largest sugar and ethanol producer in India. The company produced 90900 KL of ethanol during FY 21, over 50% more than the previous year. Balrampur Chinni Mills Ltd, a Kolkata-based company is also among the largest producers. The company is planning to increase the ethanol production from molasses with no residual sugar and sugar cane syrup to increase the contribution of ethanol in blending with petrol. Dalmia Bharat Sugar and Industries Ltd with a total crushing capacity of 35000 TCS reserves itself in the top league of the sugar industry. Shree Renuka Sugars Ltd having the ethanol distillation capacity of 930 KLPD marks itself in one of the top ethanol manufacturers in India. Triveni Engineering and Sugar Industries is also a major ethanol manufacturer and also a significant supplier of ethanol to the oil companies in India for blending with petrol.

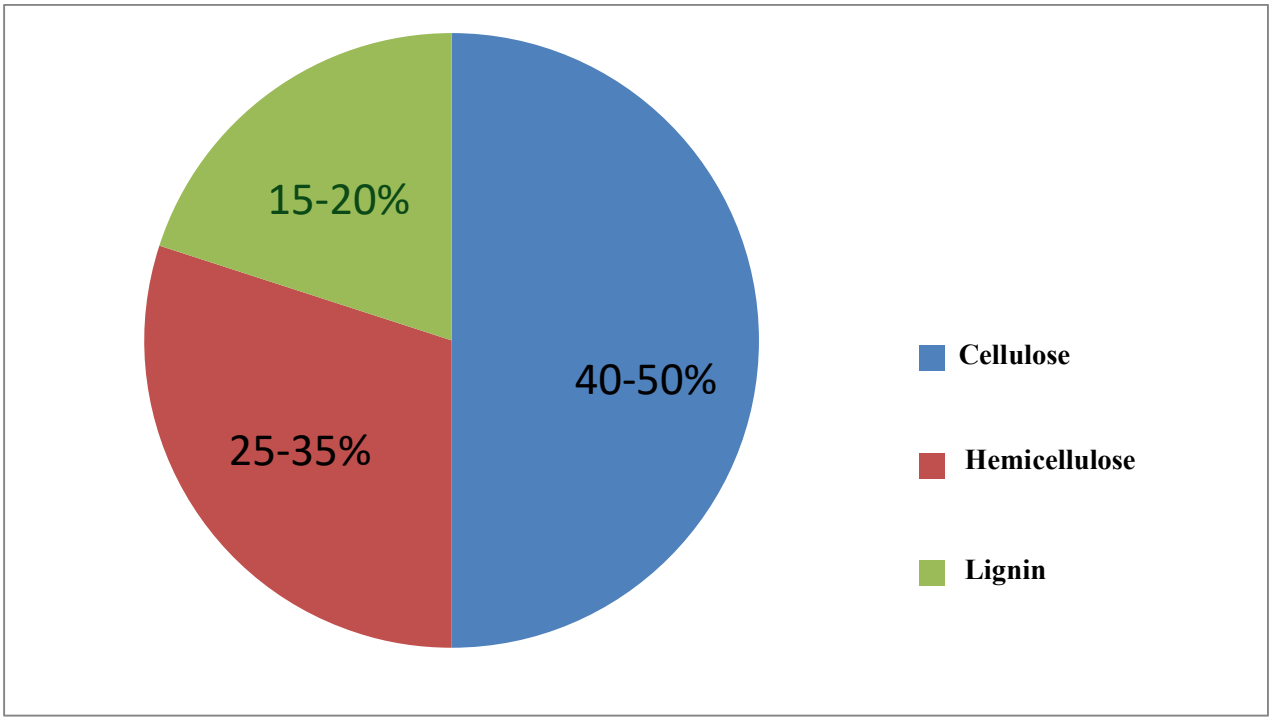
Table 1: Percentage of ethanol production from banana peels at 24 hours interval for seven days by different Aspergillus niger strains at pH 6 and at 30ºC.

Production of ethanol (in %)							
Strain/Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**
A	**0.638**	**0.891**	**2.377**	**3.010**	**4.800**	**5.733**	**6.005**
B	**0.645**	**1.001**	**2.888**	**3.457**	**4.997**	**5.923**	**6.288**
C	**0.506**	**0.933**	**2.239**	**2.948**	**4.289**	**5.295**	**5.466**

Table 2 : Bioethanol production (%v/v) at best optimum conditions.

Parameters	**Banana peel**	**Orange peel**
pH	**2.90**	**3.90**
Temperature (°C)	**2.86**	**4.07**
Incubation period (hours)	**3.78**	**4.04**

Pie chart for lignocellulosic biomass content



Graphs based on Table No. 1

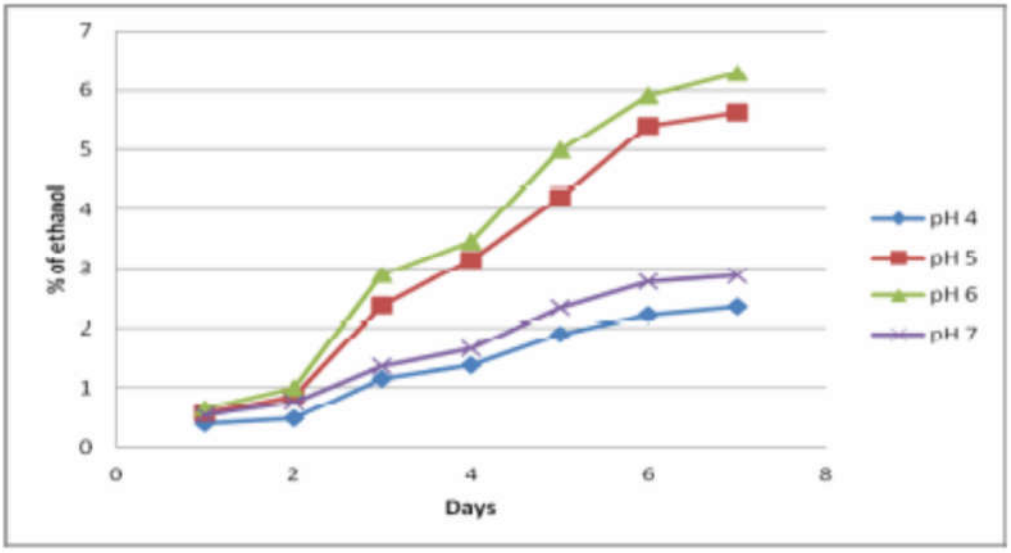


Figure.1 Variation in ethanol yield from banana peels with the change in pH (4 to 7) for seven days by *Aspergillus niger* strain B at 30°C

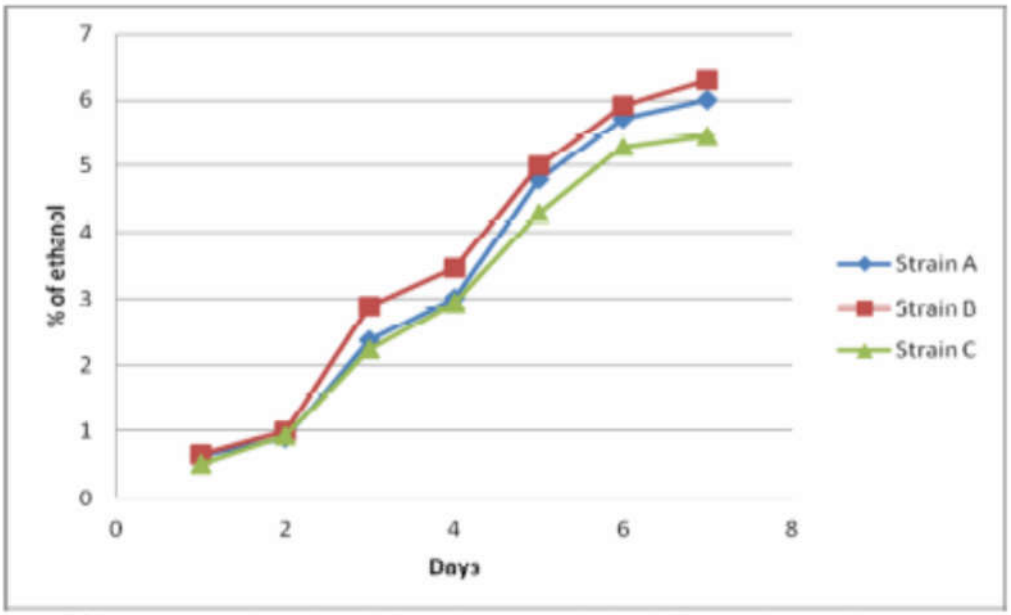


Figure.2 Variation in ethanol yield from banana peels by different *Aspergillus niger* strains at pH 6 and at 30°C

Conclusion

Banana peels are feasible and low cost raw material for bioethanol production. In our study we found that unripe banana peels gives more ethanol yeild than ripe banana peels. Concentration of ethanol is 35.6 g/L and 6.540% for unripe and ripe banana peels respectively. Optimum conditions for fermentative production of ethanol by acid hydrolysis are fermentation time 36 hours, temperature 30°C and pH 5. Among various yeast strains Sacchromyces Cervisiae NCIM 3095 is most convenient yeast strain having high fermentation efficiency and glucose to ethanol conversion rate. Comparative study of hydrolysis visualizes acid hydrolysis is finest method and holds best among different methods of hydrolysis. 90% glucose yeild obtained when lignocellulosic biomass hydrolysed by concentrated acid hydrolysis. It carries minimum time of 2-3 hours. Enzymatic hydrolysis and alkaline hydrolysis are not beneficial as during reaction toxic compounds form in little quantity and gives low sugar yeild after fermentation which convey that they are not beneficial processes. Amid varieties of agricultural waste like cotton stalk, wheat straw, sugarcane baggasse, other fruit peels, etc we can observe that banana peels are proven as best raw material for bioethanol production. This is due to their accesibility, tolerance to environmental condition as well as susceptibilty after treatment with different micro-organisms. Optimistically we can perceive that Banana peels, a second generation source of biofuel production is most convenient and useful raw material in bioethanol production.

Acknowledgements

Authors are very thankful to all members of Datta Meghe College of Engineering

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