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Production of Biofuel from Fruit Wastes as an eco-friendly carbon source

Chaudhari Varsha M[1], More Puja K [1]
[1] Department of Microbiology, P.S.G.V.P.Mandal's SI Patil Arts, GB Patel Science, STSKV Commerce College, Shahada, Dist-Nandurbar 425409, India.
Email: varsharaj2913@gmail.com.

ABSTRACT
Demands for biofuels are increasing day to day with increase in population. Bioethanol is an alternative type of biofuel which is capable of providing energy. The production of bioethanol should be increased using eco-friendly and cheap raw materials as carbon source. Bioethanol can be produced by fermentation utilizing carbohydrate containing substrates. The main aim of the present study was to determine the relatively cheap carbon sources for the production of bioethanol. Different spoiled waste fruits such as banana, apple and pomegranate were used asan alternative substrates for bioethanol production by using strain Saccharomyces cerevisiae. All fruits contains large amount of sugar which can be fermented into bioethanol. Comparative studies were also carried out on bioethanol production from different fruit wastes. Maximum production of bioethanol was found to be in waste pomegranate fruit sample. Optimum conditions like pH and temperature for bioethanol production were also determined. The present study revealed that the large amount of fruits are discarded due to spoilage, as fruits are rich in sugars it should be converted into useful products like bioethanol which can serves as an alternative fuel source.
Keywords: *Bioethanol, distillation, fermentation, fruit wastes, Saccharomyces cerevisiae.*

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INTRODUCTION

Ethanol fuel (C_2H_5OH)is the most common biofuel worldwide. Ethyl alcohol is also found in alcoholic beverages [1].It is also an important industrial ingredient and has widespread use as a base chemical for other organic compounds and used in medical wipes, most commonly as an antibacterial hand sanitizer gels and as an antiseptic [2]. It is most often used as a motor fuel, mainly as a biofuel additive for gasoline. Biofuel is a renewable source of energy and hence can be used as an alternative to conventional fossil fuels. It burns up to 75% cleaner than fossil fuels[3].Due to increasing population and industrialization, the demand of energy is increasing day by day [4]. Production of various fuels by using low cost processes is important from the economic viewpoint and to meet the demand of increasing population. Bioethanol can be manufactured from various low cost agricultural raw materials like potato waste, molasses, banana waste, waste food grain etc. [5].

The production of bioethanol using conventional fermentation feedstock, lead to a serious competition with human-animal food consumption. To avoid this competition, it is important to explore various alternative feedstock especially those from inedible waste materials. Potentially, fruit wastes such as damaged fruits, peels represent alternative cheap carbon source for alcohol production. Utilization of fruit waste for bioethanol production is one of the best options [6].The fruit wastes are cheapest, the least expensive and easily available raw materials for the production of bioethanol [7]. Fruits contain sugar and acts as a potential energy sources, from which bioethanol can be obtained. For example pineapple waste has been used as raw material and converted to bioethanol [8]. Bioethanol production from banana [9] and pineapple peels [10] were also investigated. Production of bioethanol from waste banana peels was also studied[11]. The waste fruit contain valuable components such as sucrose, glucose, fructose and other nutrients [12] which can be utilized for bioethanol production.

The present experimental study was conducted on bioethanol production using waste fruits - spoiled fruits such as banana, apple and pomegranate through fermentation by *Saccharomyces cerevisiae* yeast.

These fruits were non-consumable and easily available raw material for bioethanol production. *Saccharomyces cerevisiae* was used for bioethanol production due to its high ethanol yield and high tolerance. The bioethanol concentration was estimated by potassium dichromate $K_2Cr_2O_7$ method. Comparative studies were also carried out on bioethanol production from different fruits. The main purpose of the present study was to search for relatively cheap sources for the bioethanol production and the production efficiency of bioethanol produced by different fruits was also determined. This study also revealed the optimum conditions like pH, temperature for biofuel production.

MATERIAL AND METHODS

Sample Collection

For the present experimental work waste spoiled fruits such as apple, pomegranate and Banana (Fig:1) were collected from the local fruit market of Shahada region and yeast cells of *Saccharomyces cerevisiae*were collected from bakery situated in Shahada. Collected fruit samples were washed with 5 % Potassium permanganate (KMnO4) solution & rinsed twice well in distilled water.

Figure1: Spoiled fruits apple, pomegranate and banana

Preparation of fruits sample as a substrate for fermentation process

The spoiled fruits pomegranate, banana and apple were washed initially with 5% Potassium permanganate (KMnO4) solution, then washed with distilled water for two to three times. Then 100 g of fruit samples were weighed separately in different beakers, chopped into smaller pieces together with their spoiled skin and then blended in the mixer. All the blended samples were filtered by using minute pores size sieve and used as a fruit juice for fermentation process.

Preparation of fermentation medium

100 ml of each fruit juice was inoculated with 0.5 g of glucose, dipotassium hydrogen phosphate (K_2HPO_4) 0.5g, potassium dihydrogen phosphate (KH_2PO_4) 0.5g and urea 0.1g. pH of the fermentation broth was adjusted to 5.5.The juice was heated at 120°C for10 minutes under autoclaved. All flasks were cooled at room temperature and aseptically 0.1 g pellet suspension of *Saccharomyces cerevisiae* culture prepared in warm saline was added in each flask. The flask containing fruit juice medium were maintained at 37°C after covering with dark papers. After the 72 hours of incubation the fermented samples were centrifuged at 10000 rpm for 10 minutes. The pellet was discarded and cell free debris supernatant was collected. The collected supernatant was used for further distillation process.

The Distillation process

For the process of distillation supernatant obtained was inoculated into the round-bottom flask as shown in Fig 2. The flask containing sample was boiled for each different fruit wastes. The mixture was maintained at a constant temperature until the boiling has ceased. At this point, distillation process was completed. The continuous circulation of cold water around the condenser pipe assisted in cooling the alcohol rich vapors back to the liquid state. The condensed liquid enters the still receiver and is then collected as a distilled. Collected distilled bioethanol was store at low temperature in closed glass container.

Figure 2: Distillation process in distillation assembly

Alcohol estimation by Potassium Dichromate method

Alcohol content in the distillate obtained after the process of distillation was estimated by the Potassium Dichromate method (13;14]. Potassium dichromate (Cr_2O_7, Cr(VI)) is yellowish in color and the reduced chromic product (Cr+++, Cr(III)) is intensely green in color which can be measured spectophotometrically (15).

Preparation of standard reference curve of alcohol (%) was carried out using (80%) standard stock of ethanol as 0.1, 0.2, 0.3, and 0.4 up to 1ml of alcohol. 1 ml ethanol sample collected by distillation of the fermented broth was used as test sample for each different fruit sample. In each tube 25ml of chromic acid reagent was added. And tubes were incubated in water bath at 70ºC for 15 min. After 15 min in all the tubes 24 ml of distilled water was added to stop the reaction. Absorbance was measured at 600 nm and absorption values of distillate obtained from each fruit sample were compared with standard graph. Percentage of ethanol was calculated.

Estimation of sugar content in fruit juices before and after fermentation by DNSA method

Fruits contain naturally sugar so it is necessary to determined initial sugar content in each waste fruit sample. Bioethanol production depends upon initial sugar concentration hence it is one of the influencing parameter in ethanol production. Sugar estimation was carried out by DNSA method [16].

3, 5-Dinitrosalicylic acid (DNSA) is commonly used for the estimation of reducing sugars. It detects the presence of free carbonyl group (C=O) of reducing sugars. This involves the oxidation of the aldehyde functional group (in glucose) and the ketone functional group (in fructose). During this reaction DNSA is reduced to 3-amino, 5-nitrosalicylic acid (ANSA) which under alkaline conditions is converted to a reddish brown colored complex which has an absorbance maximum of 540 nm. Standard reference curve of glucose was prepared by using stock of 1000 μg/ml and 3 ml of DNSA reagent was added in all test tubes and all tubes were kept in boiling water bath for 15 minutes. After cooling to room temperature, absorbance was recorded on spectrophotometer at 540 nm [17].

Influence of various parameters on bioethanol production

Effect of various parameters like temperature and pH were investigated affecting fermentation process and production yield of bioethanol.

Effect of temperature

Temperature is one of the most important parameter that determines the ethanol production [18].To optimize the fermentation temperature yeast cells were inoculated in fermentation media prepared from each fruit sample. Fermentation process was carried out for 72 hours at 20, 30 and 35ºC. The amount of ethanol content was determined by dichromate method.

Effect of pH

pH value also has significant influence on bioethanol fermentation [18]Like temperature, pH also plays an important role in any fermentation process. Effect of different pH values on bioethanol production was determined by changing the pH of fermentation broth from 5.5 to 4.0 and 6.0 fermentation processes was carried out up to 72 hours.

Statistical analysis

All the experiments were conducted in triplicate results were expressed as mean ± standard error. The statistical analysis of data was carried out to determine the significance among different fruit samples and the value of $p > 0.05$ was considered as significant.

RESULTS AND DISCUSSION

This study revealed that different damaged fruits waste, not utilized for consumption can be used as substrates for bioethanol production. The comparative study has been carried out to check the efficiency of bioethanol produced from these fruit wastes. Various parameters influencing bioethanol production were also determined. Fruits are rich source of carbohydrates and organism *Saccharomyces cerevisiae* is able to convert sugars into ethanol by fermentation process. Hence this study was conducted on conversion of waste substrates into useful product.

Bioethanol production was determined from in each distillate obtained from waste fruit sample, bioethanol concentration was found to be highest (fig:4) in distillate of waste pomegranate fruit as 59.1% followed by in waste apple fruit as 32% and in banana as 23.2%. The results of bioethanol production revealed that there is significant difference between pomegranate and other two fruits. Bioethanol production was found to be maximum ($P>0.05$) in waste fruit with its peel in case of pomegranate as compared to apple and banana. Results also revealed that there is insignificant difference ($P< 0.05$) in banana and apple waste fruits distillate sample.

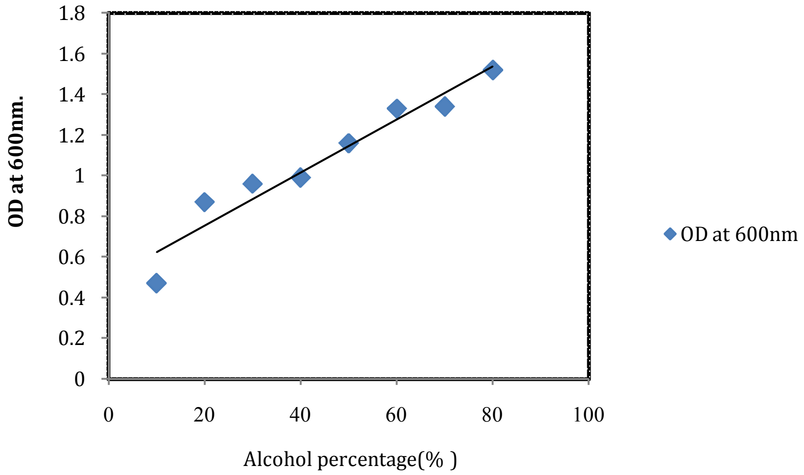

Figure 3: Standard curve of ethanol

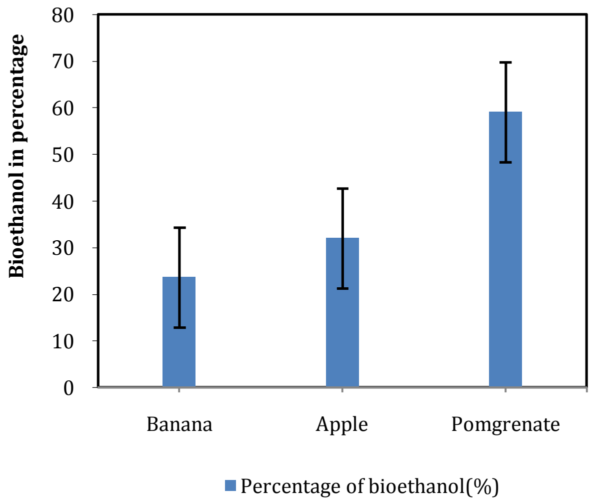

Figure 4: Percentage of bioethanol yield from different fruit waste samples. Bar indicates ± SE.

Estimation of sugar content in fruit juices before fermentation

The amount of sugar was found to be different in different fruit juice samples. It was observed that sugar content was found to be 930 ug/ml in banana fruit sample and in to apple and pomegranate fruits amount of sugar was found to be more than 1000ug/ml.

Estimation of sugar in fruit juices after fermentation

After the alcoholic fermentation process sugar estimation was again carried out to determine the residual sugar present in each fermentation broth. The content of sugar was found to be decreases in amount in all waste fruits fermented broths. In waste banana fruit broth, before fermentation process sugar was 930µg/ml which after fermentation process was found to be 300 µg/ml while in apple and pomegranate sugar concentration 400 and 200µg/ml was determined respectively.

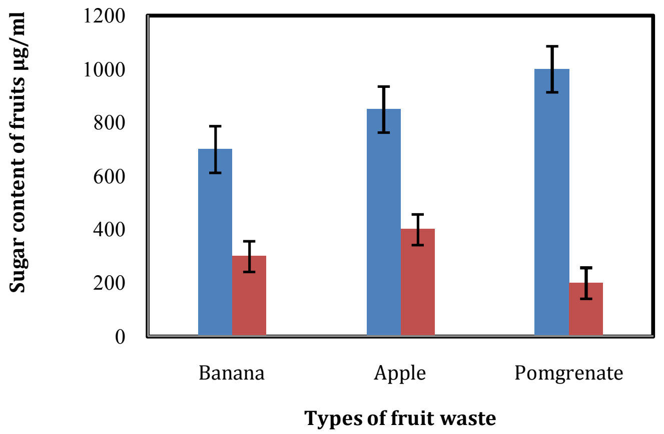

Fig.5. Amount of sugar content in fruit samples before fermentation and after fermentation. Values are ± SE.

Effect of temperature

Temperature is one of the most important parameter that determines the ethanol production. When effect of temperature on ethanol production was observed, highest amount of bioethanol was found to be produced at temperature 35°C as compared to 25°C and 30°C. Maximum amount of bioethanol was found in fermentation broth containing pomegranate fruit (62.0 %) at temperature 35°C as compared to apple (53.0 %). While in banana fruit sample maximum amount was produced at temperature 30°C (36.0 %) as compared to 35°C. From the above results it is revealed that optimum temperature for bioethanol production varies with type of fruit sample. As the fermentation process is exothermic reaction bioethanol production take place in temperature between 25-35°C.

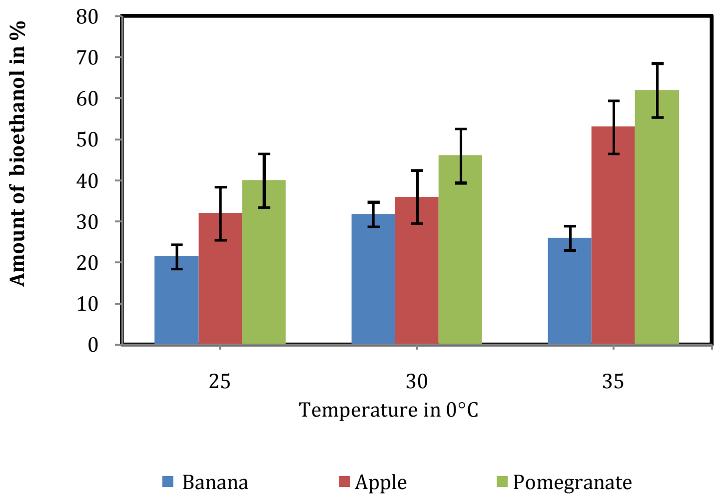

Figure 6: Effect of temperature on bioethanol production

Effect of pH

pH value also has significant influence on bioethanol fermentation [18]. pH of bioethanol produced from different fruit wastes were determined by changing the pH values of fermentation media ranges from 4 to 6. Yeast cells better grow and survive in a slightly acidic environment with pH between 4 to 6. The percentage of bioethanol obtained at different pH values were determined which revealed that maximum ethanol was found to be obtained at pH 5.5 in apple as well as pomegranate as compared to banana. At pH value 4.0 and 6.0 bioethanol yield was low as compared to pH 5.5.

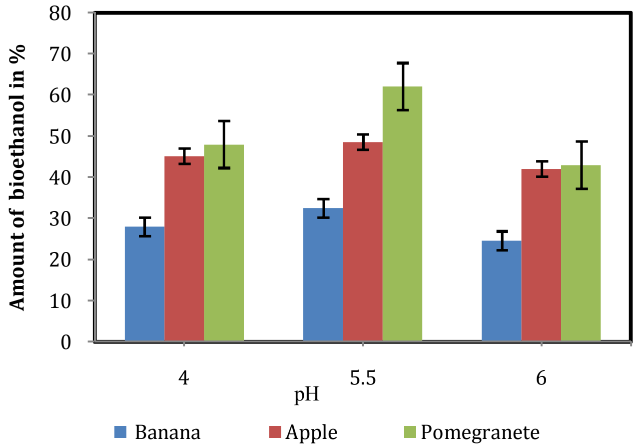

Figure 7: Effect of pH on bioethanol production

DISCUSSION

From the above results it can be revealed that different fruit wastes like banana, apple and pomegranate can be used as alternative substrates for the production of bioethanol. Comparative studies on the ethanol production from different fruits showed that maximum yield obtained from pomegranate fruit at 35^0C at pH 5.5 was 62%. As compared to apple and banana, pomegranate has higher efficiency of fermentation and more yields were obtained. Significant difference was observed in bioethanol production from different fruit samples. Bioethanol production from fruit waste is cost effective and environment friendly process.

CONCLUSION

Conventional technology of bioethanol production uses molasses generated after sugar production. In the present study bioethanol was produced using different fruit wastes. Spoiled, non consumable fruits can serves as raw material for bioethanol production. Fruits contain large amount of carbohydrates, if not preserved properly, they cannot remain fresh for long period of time. The waste part of fruit is easily available at large amount and they obtained at free of cost. Production of bioethanol from waste fruit samples help to reduce the cost of bioethanol production. It is the best green solution for biofuel production.

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