## Research & Reviews: A Journal of Life Sciences



ISSN: 2249-8656 (Online), ISSN: 2348-9545 (Print) Volume 7, Issue 3 www.stmjournals.com

# Efficiency of Waste Banana Peels in Bio-ethanol Production

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#### Abstract

Aim: To check the efficiency of waste banana peels in bio-ethanol production. Methods and Results: Waste from banana fruit peel powder is subjected to the simultaneous saccharification and fermentation process for 7–10 days of co-culture of Aspergillus niger and Saccharomyces cerevisiae and also with a co-culture of Bacillus subtilis and Saccharomyces cerevisiae, in two different batch processes. The extracted  $\beta$ -amylase enzyme from the biomass of Aspergillus niger is also used along with Saccharomyces cerevisiae, as a third batch of fermentation process. The ethanol produced, can be obtained by the fractional distillation process and total ethanol yield is determined by the titrimetric ethanol assay. The ethanol obtained by saccharification and fermentation process gives 6.34, 4.6 and 11.73% yield respectively, for all the three batches of fermentation. Conclusion: The findings of this study suggest that wastes from fruits that contain fermentable sugars can no longer be discarded into our environment, but should be converted to useful products like bio-ethanol that can serve as alternative energy source. Significance and Impact of the study: Rapid increase in the generation of huge quantity of waste is one of the major environmental crises of today. Large quantities of waste materials are generated annually from agricultural activities and processing of agricultural products. Due to their abundant availability and renewable nature, ethanol production from such substrates appears to have immense commercial potential. A different category of waste from the food processing industry is also under investigation as the potential feedstock for value-added products such as bio-ethanol. Out of the total fruit production worldwide, banana (Musa sapientum) constitutes of 16% share in it and Maharashtra being second largest world fruit producers consisting of 21.4% of total share in India.

**Keywords:** Bio-ethanol production, Aspergillus niger, Saccharomyces cerevisiae, simultaneous saccharification and fermentation (SSF)

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### INTRODUCTION

Bio-ethanol is a renewable energy source, completely composed of biological products and made by fermenting the sugar and starch components of plant by-products, mainly sugarcane, using yeast. It is also made from corn, potatoes, rice, beetroot and recently from grapes, banana dates etc. depending on the countries' agricultural strength. The world ethanol production has reached about 51,000 million liters being the USA and Brazil the first producers and India stands fourth among the top fuel ethanol producers. Main feed bio-ethanol for production sugarcane (in Brazil) and corn grains (in

USA), while many other agricultural raw materials are also used worldwide. In India, as well ethanol is mainly produced from molasses but its alternative uses, low availability, and a continuous increase in its demand has led to a search for other suitable feed stocks like fruit residues [1].

As per the FAO statistics, India is the largest producer of banana in the world and accounts for nearly 30% of the total world production of banana [2]. It is most widely grown tropical fruit, cultivated over 130 countries, including other major producer countries like China, Columbia, Costa rica, Mexico and Phillipines [4].

Banana is the most important fruit crop, accounting 31.7% of the total fruit production. Out of over 50 varieties of banana cultivated across India, 20 are commonly grown. It is an herbaceous plant of the family Musaceae. The three common species of the family; Musa cavendishii. Musa paradisiaca, sapientum are widely grown in the world. The advantage of this fruit is its availability round the year. It can be easily maintained and grown on less fertile land, especially on land that has been degraded by farming [3]. Though banana peel is a fruit residue, it accounts for 30-40% of the total fruit weight and contains carbohydrates. proteins, and fiber in significant amounts and serves as a supplementary source for production of commercially important products like ethanol, enzymes, organic acids, vitamins, and biogas [4].

Assuming, that banana peel accounts for 30% of the total fruit weight, and has 20% dry matter, India is likely to produce more than 1.6 million metric tons of dry banana peels every year which do not find any commercial application. In India, such fruit residues are generally disposed of in municipal bins due to unavailability of proper infrastructure to handle them.

It has been reported that India would need about 1.5 billion liters ethanol every year, if 10% ethanol is blended with gasoline [5]. Thus, ethanol production from banana peels could partially supplement the ethanol needs of India for use as bio-fuel. Apart from that, to meet the increasing demand of the ethanol for various industrial purposes like industrial solvents in the manufacturing of varnishes and perfumes, cleansing agent, preservatives for biological specimens, in the preparation of essences and flavoring, in many medicines and drugs as disinfectant and tinctures (e.g. tincture of iodine) etc., increased production of ethanol is necessitated. Therefore, in most developing countries producing conversion of such residues into commercially important products will help mitigate environmental pollution problems.

# **Current Management of Food Processing Waste**

Solid waste from food processing is currently managed in various ways. Many factories simply dump the waste close to the plant.

Since these wastes have high nutrient levels and water content and can support bacterial growth and fermentation, these may cause odors and other environmental problems. In some cases, wastes may be transported to the landfill site for disposal, which could lead to the additional costs as some countries may charge landfill disposal fees. A further means of disposal of food waste is through its utilization as animal feed, mostly for cattle feed. The waste may be dried and formed into pellets prior to sale as animal feed. However, most food wastes have low protein content and are therefore not ideal for animal feed. High lignin content in some wastes, for example sugarcane bagasse, also limits utilization as animal feed as it makes the waste difficult to digest. Whereas, wastes dried prior to being used as animal feed, additional costs may be incurred, which are rarely recovered from the cost of sales.

Food waste can also be utilized as a soil conditioner or fertilizer. This can be done through spreading of the untreated food waste on the soil, thereby increasing the organic content and microbial biomass of the soil. In some cases, composting can be undertaken prior to utilization as a soil amendment. In view of the above, present study aims to assess the ethanol production potential from banana peels.

### MATERIALS AND METHODS Pre-treatment of Banana Peels

The waste banana peels were procured from the local market, Dombivli, Maharashtra, India. Before processing, ripe waste banana peels were cleaned, hand-chopped (3–5 cm) and disinfected with 70% ethanol, which were then kept in sunlight for 7 days and ground to fine powder.

### **Isolation of Micro-organisms**

Micro-organisms required for bio-ethanol production, Aspergillus niger and Bacillus subtilis were isolated from soil by spread-plate technique on Sabouraud's agar and nutrient agar medium respectively. Thus, the pure cultures obtained were maintained on Sabouraud's agar slants at 4°C. Yeast strain Saccharomyces cerevisiae (Baker's yeast) was obtained from the local market. It was maintained on the Sabouraud's Agar slants at 4°C.

ISSN: 2249-8656 (Online), ISSN: 2348-9545 (Print)



# **Determination of Amylolytic Activity of Organisms**

The amylolytic activity of isolated organisms Aspergillus niger and Bacillus subtilis were determined by starch agar plate method. The broth cultures were inoculated into starch agar medium. After incubation period of 24 h, diameter formed after the addition of iodine solution was measured to represent the amylolytic activity of organisms.

# Production and Extraction of $\beta$ -Amylase Enzyme

The amylase production was carried out for 50 ml medium formulated using soluble starch (5 gm/l), yeast extract (5 gm/l), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (2.5 gm/l), MgSO<sub>4</sub>.7H<sub>2</sub>O (0.2 gm/l), KH<sub>2</sub>PO<sub>4</sub> (3 gm/l) and CaCl<sub>2</sub>.2H<sub>2</sub>O (0.25 gm/l). The medium was then incubated at 50°C under shaking conditions (200 rpm) and inoculated with 2.5% of 24 h old culture of *Aspergillus niger*. After incubation, the production medium was centrifuged at 6000 rpm for 30 min to separate the cells. The supernatant was collected as it contained the crude enzyme and stored at 4°C till further use.

#### **Preparation of Growth Medium**

The growth medium prepared for all the three batches of ethanol production consists of 5.0 gm of banana peel powder in 250 ml of conical flasks containing 200 ml distilled water. The flask was then incubated at 121°C for 20 min.

#### **Saccharification of the Growth Medium**

The growth medium prepared above was inoculated with the broth cultures of Aspergillus niger (10% v/v), Bacillus subtilis (10% v/v) and extracted β-amylase enzyme (10 ml) respectively into the three different batches of saccharification. The growth media were then incubated at room temperature under rigorous aerobic condition for 7 days. After 7 days, the maximum total sugar content can be obtained by DNSA method.

#### **Fermentation of the Growth Medium**

The medium was then inoculated with the broth culture of *Saccharomyces cerevisiae* (10% v/v) and maintained in static condition for 4 days at 28°C. After 4 days, the ethanol produced was obtained by fractional

distillation process and then estimated quantitatively using titrimetric ethanol assay.

#### **Ethanol Estimation**

10 ml of sample solution obtained after the distillation process was treated with acidified potassium dichromate solution and kept in a water bath for 10-15 min and then added with 100 ml distilled water and 10 ml of potassium iodide solution which turns the color of the solution brown. This solution was titrated against 0.03 M sodium thiosulfate solution till the color changes to yellow. Then add 1% starch solution and titrate again with sodium thiosulfate solution till the color changes from blue to colorless. Note the burette readings for samples and blank. Using the equations, the relationship between the moles of sodium thiosulfate and the moles of ethanol can be determined i.e. 1 mol of S<sub>2</sub>O<sub>3</sub><sup>2</sup>- is equivalent to  $0.25 \text{ mol of } C_2H_5OH.$ 

# RESULTS DNSA Method

Reducing sugars produced in the banana peel substrate during the saccharification process can be estimated using this method. Presence of reducing sugar in the substrate indicates the conversion of starch or carbohydrates from the banana peels to the reducing sugars, which is the simplest form of sugar (Figure 1). This conversion takes place due to the enzyme Bamylase produced by the organisms Aspergillus niger and Bacillus subtilis.

Banana peel powder, when subjected to the simultaneous saccharification and fermentation process, it gives percent ethanol concentration which can be estimated by titrimetric ethanol assay. The % ethanol yield can be given by the formula as follows:

% Yield of Ethanol  $= \frac{\text{Amount of alcohol (gm/100 ml)} \times 100}{\text{Amount of substrate used (gm)}}$ 

Table 1 gives percentage ethanol yield obtained during the process. Maximum ethanol yield was obtained by using  $\beta$ -amylase enzyme.

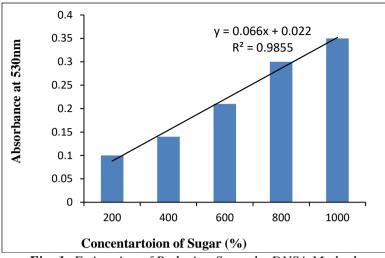


Fig. 1: Estimation of Reducing Sugar by DNSA Method.

**Table 1:** Percent Ethanol Yield Obtained Using Different Organisms.

Sr. No.	Organisms Used	Titrimetric Ethanol Assay
1.	Aspergillus niger	6.34%
2.	Bacillus subtilis	4.6%
3.	β-amylase enzyme	11.73%

#### **DISCUSSION**

The conversion of corn and other food crop residues (sugarcane and beet molasses) into ethanol by fermentation is a well-known and established technology. However, utilization of waste, generated from the food crops like banana peels can also be used for bio-ethanol production.

Rather than using costly techniques for conversion of starch to reducing sugars from the banana peels, microorganisms like Aspergillus niger and Bacillus subtilis can be used effectively. The microorganisms act on the banana peels, converting the starch into reducing sugars like maltose and glucose, with the help of enzymatic reactions. Enzyme βamylase is produced by both, Aspergillus niger and Bacillus subtilis which helps in this conversion. In fermentation process, Saccharomyces cerevisiae converts these reducing sugars into the ethanol and carbon dioxide.

In the present study, extracted  $\beta$ -amylase enzyme gives higher ethanol yield that is 11.73%, which is higher than the yield given by the *Bacillus subtilis*. The maximum yield given by the enzyme also proves that instead

of using Aspergillus niger, its enzyme extract can be effectively used for bio-ethanol production. Extracted enzyme will directly act on starch present in the substrate. This can reduce the time required for the growth of Aspergillus or other starch hydrolyzing microorganisms.

The estimated production of fruits and vegetables in India is 150 million tones and the total waste generated is 50 million tons (30%) per annum [6]. Around 25 to 30% of fruits and vegetables India which get wasted due to the lack of basic as well as specialized infrastructure can be converted to bioethanol; which will facilitate to resolve an issue of agricultural waste as well as clean alternative fuel production.

#### CONFLICT OF INTEREST

The authors of this paper have no conflict of interest in terms of financial relationship, membership of a company board of directors, membership of an advisory board or committee for a company or any stock ownership or patenting.

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ISSN: 2249-8656 (Online), ISSN: 2348-9545 (Print)



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Apurva Barve, Kishori Tarfe. Efficiency of Waste Banana Peels in Bio-ethanol Production. *Research & Reviews: A Journal of Life Sciences*. 2017; 7(3): 28–32p.