



## Chemo-enzymatic conversion of biomass into bio-ethanol

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Received 06-Dec-13

### ABSTRACT

The energy crisis and environmental pollution from use of fossil fuels have become more serious globally which insisted us to explore the use of bio-fuels as cheaper and cleaner alternatives to fossil fuels. The higher production costs of alcohol, however, are an obstacle to the production of this energy source. Selection of biomass with higher yields and higher sugar content and at lower cost is, therefore, essential to cut down costs of ethanol production. In the present research we have taken fruit peels (orange and banana) as a substrate for the production of ethanol by some chemo-biological methods. *Saccharomyces cerevisiae* was taken for the fermentation and then the distillation we could recover 10-11% ethanol concentration. It has various advantages over the traditional fuel. It is biodegradable, low in toxicity and causes little environmental pollution if spilt. Bio-ethanol has higher octane number, broader flammability limit, higher flame speed and higher heat of vaporization than gasoline. It is much cleaner and releases no toxic gases.

*Keywords: Bio-ethanol, Biomass, cellulose, fermentation, Saccharomyces cerevisiae*

### INTRODUCTION

Ethanol is a clear liquid alcohol that is made by the fermentation of different biological materials. This alcohol is known to have many uses, but one in particular is becoming more popular. Ethanol, the most widely used bio fuel, is made in a process similar to brewing beer. The ethanol in the end is blended with gasoline to improve vehicle performance and reduce air pollution. Ethanol fermented from renewable sources for fuel or fuel additives are known as bio-ethanol. Bio-fuels, are being touted as a partial solution both to the world's mushrooming energy demands and to the challenge of reducing greenhouse gas emissions from fossil fuels.<sup>1</sup> Production of ethanol from biomass (bio-ethanol)<sup>2</sup> is one way to reduce both consumption of crude oil and environmental degradation. Bio-ethanol is the most common bio-fuel, accounting for more than 90% of total bio-fuel usage.<sup>3</sup> The world's largest producers of bio-ethanol are

Brazil (sugar-cane ethanol) and the United States (corn ethanol). Ethanol, unlike gasoline, is an oxygenated fuel that contains 35% oxygen, which reduces particulate and NO<sub>x</sub> emissions from combustion. Important feedstocks for ethanol production are Sugar,<sup>4</sup> starch and Cellulose.<sup>5</sup> Biomass materials containing high levels of glucose or precursors to glucose are the easiest to convert to ethanol. other potential ethanol feedstocks are starchy materials which can be fermented after breaking starch molecules into simple glucose molecules cereal grains, potato, sweet potato, and cassava. Fruit peels and dried fruit peels are rich in cellulose, hemicelluloses, proteins and pectin, the fat content is however low.<sup>6</sup> Being abundant and outside the human food chain makes cellulosic materials relatively inexpensive feed stocks for ethanol production.<sup>7</sup> Some microorganisms especially *Acetobacter aceti*, *Clostridium butyrium*, *Bacillus spp*, *Saccharomyces spp* and *Micrococcus*; some fungal microbes *Aspergillus flavus*, *A. niger*, *Penicillium chrysogenum*, and *fusarium species* were best for fermentation process<sup>8</sup> and gives the nutritive values. Lignocellulosic biomass<sup>9</sup> is envisaged to provide a significant portion of the feedstocks for bioethanol production in the medium and long form due to their low cost and high availability. The banana and orange fruit peels are attractive resources for economical production of ethanol. Therefore, efforts are to be intensified to produce ethanol efficiently through improved fermentation technologies. Reduction in the cost of celluloses can be achieved by use of cheaper raw materials and economically viable fermentation strategies. In continuation to these requirements and cosmopolitan challenges, we have done

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Cite as: *J. Integr. Sci. Technol.*, 2014, 2(1), 34-36.

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the present work using waste biomass (cellulosic material) i.e. peels of different fruits and by bio-chemical conversion methods we produce bio-ethanol, the results were found quite promising with the required parameters of ethanol.

## MATERIALS AND METHODS

Ethanol can be produced in two different ways, either chemically, by hydration of ethylene, or by fermentation of sugar containing feeds, starchy feed materials or lignocellulosic materials. Biochemical conversion process was used to produce ethanol from cellulosic feedstock. There are mainly three processes involved in the conversion lignocellulosic to bioethanol, which are pretreatment to remove lignin or delignification, hydrolysis of cellulose in the lignocellulosic biomass to produce reducing sugars by chemical or enzymatic process, and fermentation of the sugars to ethanol by yeast. Some of the important reasons for the pretreatment step are to (i) break the lignin-hemicellulose-pectin complex, (ii) disrupt/loosen-up the crystalline structure of cellulose and (iii) increase the porosity of the biomass. These changes in lignocellulosic materials make it easier for enzymatic saccharification (hydrolysis), results in higher fermentable sugars levels and will have a significant impact on the overall process.

### COLLECTION OF MATERIALS

For this purpose we had collected 1kg of each of fresh peels of Orange & banana peels from juice corners present at local areas at Chandigarh.

### PREPARATION OF SAMPLE

Waste peel of orange and banana, each 1kg was used for the sample preparation. They were cut by knife into pieces of about 3-5 cm length for drying and grinding. Sample drying was carried out in oven (60°C for 72-120 hrs) to obtain easily crushable material. After drying, each of the samples was milled separately. The maximum particle sizes of the ground mixed sample were 2 mm. The sample of larger particle size than 2 mm was ground over and over again until all particle size became 2 mm. The sample was kept at low temperature until the next stage of experiment.

### PRETREATMENT OF SAMPLE

The fruit peel powders were treated and it was feed as batches, every batch contains 50 g of screened fruit peel powder with 10:1(v/w) ratio of water to the sample. The temperature was applied at 121°C; then released the pressure until the pressure became 0 bars. The retention time for every batch was 15 min. Finally the samples was kept in autoclave for the given pretreatment time and temperature and allowed to cool. The sample was separated into soluble and non-soluble parts.

### HYDROLYSIS OF SAMPLE

Non-soluble component was mixed with 500 ml of 1% (v/v) dilute sulphuric acid and soaked for 24 h. Then the sample was hydrolyzed for 25 minutes at 100°C. The hydrolyzed sample was then neutralized with 10 M NaOH until pH become around 7. The sample was centrifuged to

separate solid from liquid portion. Then the liquid portion was boiled for 20 minutes. Finally, the liquid portion was mixed with the soluble component from the pretreatment step.

## PREPARATION OF INOCULUMS

Yeast *Saccharomyces cerevisiae*, MTCC no. 36, was collected from IMTECH, Chandigarh. 500 µl of this *S. cerevisiae* was mixed with 100 ml of nutrient broth prepared in a 250 ml flask. The flask containing the YPD media and the yeast was properly sealed with cotton plug and covered with aluminium foil. Then the flask was kept for 24 h incubation at 37°C in a shaker.

## FERMENTATION

The prepared sample was mixed with the media using sterilized pipette. The parameters of fermentation i.e. fermentation time, yeast concentration (yeast proportion) and fermentation temperature were set to be at 72 hour, 10% (with the proportion of 1:10 that is the prepared media and sample respectively) and 30°C respectively. And after 72 hours of fermentation, the samples were taken out and distilled.

### pH

The changes of pH in all fermentations were determined by pH meter. The pH was checked before and after the fermentation process.

### GLUCOSE ESTIMATION

Glucose content was determined by DNS method invented by Miller's method.<sup>10</sup> Standard sugar solution in the range of 0-3 ml in 7 test-tubes was taken and volume was made up to 3 ml with dist water. 1 ml DNS reagent was added to all test-tubes, mixed and boiled for 5 minutes. The tubes were allowed to cool and OD was read at 540 nm.

## RESULTS AND DISCUSSION

After 72 hrs of incubation a total volume of 250 ml of 10.79% ethanol from 480 ml of orange substrate and 180 ml

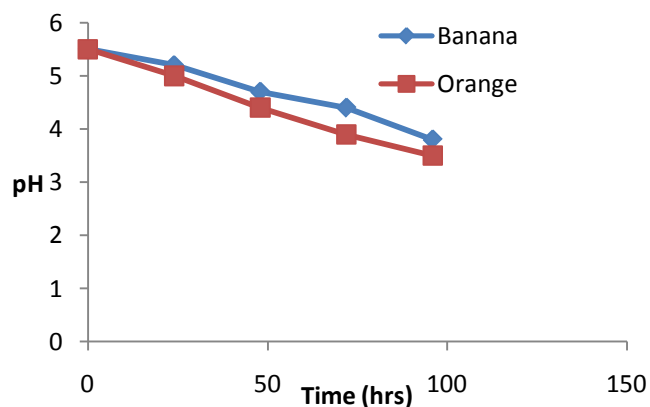
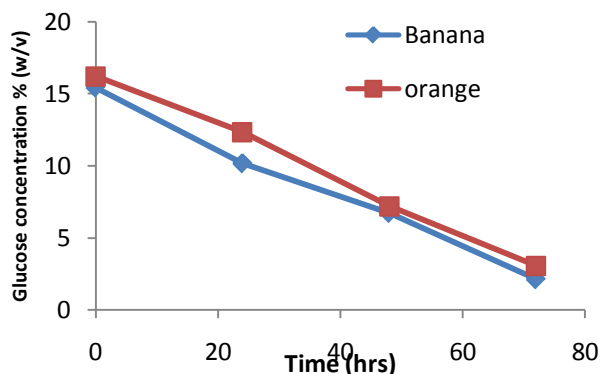


Figure 1. Comparison of pH change during fermentation of orange peels and banana peels.

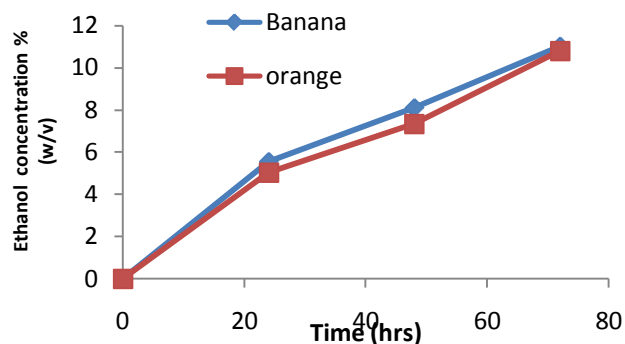
of 11.01% ethanol from 420 ml of banana substrate was obtained after distillation. Using higher grade distillation assembly a more concentrated product can be recovered by re-distillation. There was a decline in the pH from 5.5 to 3.8 for banana peels and for orange peels from 5.5 to 3.5 as shown in figure 1.

In orange and banana peels the glucose content decreased<sup>11</sup> throughout the fermentation process as shown in figure 2.



**Figure 2.** Comparison of change in glucose concentration during fermentation of orange and banana peels.

After the complete process of fermentation and distillation, the total ethanol produced for orange peels was found to be 10.79% (w/v) and for banana peels, it was 11.01% (w/v).



**Figure 3.** Comparison of ethanol production of orange peels and banana peels during fermentation.

## CONCLUSION

In this present study, efforts were made to identify the fruit wastes as potential raw material for bio-ethanol production and the results showed that fruit peels of banana and orange treated with steam, dilute acid and microbial

enzymes of *Saccharomyces cerevisiae* showed a potential production of 10-11% ethanol. The high non structural carbohydrates, reserve starch content and low fiber contents showed the potentiality of bananas and orange peels as a good feedstock for ethanol production. Utilization of these wastes could solve the disposal problem and reduce the cost of waste treatment.<sup>12-14</sup> Thus it brings no wastes that are dangerous to the environment and health. The by-product is also biodegradable and we can use it for the production of fertilizers afterwards. This provides alternative uses for plant products besides medicinal<sup>15</sup> and other applications.

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