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Production of Ethanol from Banana Waste Plantation by Using Cellulase of Fungal Species Isolated from Banana Plantations in and Around Mysuru District

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Abstract: ***Banana is widely used fruit in the world and India is the largest producer as per the FAO, Regarding ethanol production from banana waste biomass can be efficiently performed by the dissociation of the lignin-cellulose-hemicellulose complex and enable to attain of high yields of fermentable sugars. In the present study ethanol was produced by using cellulase of (Cellulase – enzyme which can convert cellulose to lower sugars) three fungi (Aspergillus, Curvularia, Fusarium) isolated from waste banana plantation dumping yard, were taken in concentration of 1 ml and inoculated to 100 ml fermentation medium containing banana waste plantation for 5 days, after saccharification is complete fermentation broth was inoculated with Saccharomyces sps. for 5 more days , presence of ethanol was identified by chromic acid test and confirmed by Gas chromatography. Among the three isolated enzymes, cellulase enzyme of Aspergillus species has produced 48.46%ethanol.***

Keywords: cellulase, fermentation, broth, Gas chromatography (GC)

1. Introduction

Ethanol (Bio ethanol) is a renewable energy source made by fermenting the carbohydrates of plant or its byproducts using yeast. It is also made from corn, potatoes, milk, rice, beetroot and recently grapes, banana and dates depending on the countries agricultural strength.

In India, molasses is the main source for ethanol production. But the less supply and increased cost is the problem for its use. The cellulose containing materials are much economical and abundant, but to convert cellulose to ethanol demands many steps which make it expensive. Thus need a conventional method to use cellulose as a substrate. Banana is one of major constitute food resources in the world and occupy the fourth world rank of the most significant foodstuff after rice, corn and milk (INIBAP, 2002). Most of the fruit peels/residues are dried, ground, pelletized, and sold to the feed manufacturers at a low price which is not considered a highly viable proposition (Mamma *et al.*, 2008). 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. Regarding ethanol production from banana waste biomass, the search for processes able to efficiently perform the dissociation of the lignin-cellulose-hemicellulose complex and enable the attainment of high yields in fermentable sugars in hydrolyzed liquor and most importantly, without generating elements that inhibit the fermentation process, is one of the great challenges for producing 2nd generation ethanol that is competitive with 1st generation ethanol from sugarcane, corn or sugar beet (Limayen and Ricke, 2012, Menon and Rao, 2012, Viikari et al., 2012, Mood et al., 2013). Routes most studied till now have been the use of chemical pretreatment with acids or bases, followed by enzymatic hydrolysis, of which the acid pretreatment is considered as one of the most important techniques and aims for high yields of sugars from lignocellulosic (Sarkar *et al.*, 2012).The pretreatment is done to break the matrix in order to reduce the degree of crystallinity of the cellulose and increase the fraction of amorphous cellulose, the most suitable form for enzymatic attack (Sanchezand Cardona, 2008). According to Souza et al. (2012) banana tree under natural conditions has 95.3% of moisture content, 44.0% of cellulose and 8.1% of lignin on a dry basis. This cellulose levels is higher than wheat straw (30%), grasses (25%-40%) and olive tree Wood (31.4%) and similar levels to pine Wood (46.4%). However, the high percentage of moisture content present in this biomass leads to the need to assess the influence of this variable on the bioethanol production.The present study aim to produce ethanol from banana waste plantation from the cellulase enzyme from the fungal isolated of banana fields and to find conventional enzymatic method for the banana biomass for scarification and fermentation with *Saccharomyces cerevisiae.*

2. Materials and Methods

1) Preparation of Banana plantation (fermentation medium)

Waste banana plant parts were collected from banana farms and finally chopped in to small pieces and grinded with blender using sterile water in 1:10 ratio and pH was set to 5.5 then stored in refrigerator for further use.

2) Isolation and Preparation of enzyme for inoculation

The crude Cellulase enzyme was isolated from three different species of fungi (*Aspergillus*, *Curvularia, Fusarium*) (13880 IJCR Lakshmi C.M) which were obtained from waste banana dumping yards and tested positive (Wood, T. M.) for the cellulase production. The isolated enzymes from three different fungi were taken in concentration of 1ml and then transferred to the fermentation medium.

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3) **Fermentation process**

I Phase (conversion of cellulose to lower sugars)

Cellulase enzyme inoculums of three fungi were prepared in the concentration of 1ml and transferred to the sterile 250ml conical flask containing 100 ml of fermentation medium and 1 ml of cellulose methyl cellulose and plugged with nonabsorbent cotton then incubated at 37C for four to five days in incubator and pH was maintained to 5.5, every 24 hr the flask was removed from the incubator and transferred to rotator shaker for 4 hr at 300 RMP at 37ºC then transferred to incubators same process was continued till 5 days. Conversion of cellulose to lower sugars was confirmed by qualitative analysis

Quantitative analysis for reducing sugars

Quantitative analysis for reducing sugars was carried out using standard DNS method, the amount of reducing sugar present in the sample was determined by adding 1ml of DNS reagent(3,5–dinitrosalicylic acid)and boiled for 10 min and cooled in water bath and 5 ml of distilled water was added. The optical density of each sample was taken by using ELICO photo colorimeter at 540 nm the concentrations of the samples were determined by using standard glucose curve.

II Phase (fermentation of sugars to Ethanol)

Once the sugar presence was confirmed 1 ml of 0.10 OD *Saccharomyces cerevisiae* was added to the conical flask and incubated at 37°C for 5 days, every 24 hr once the flask was removed from the incubator and transferred to shaker for 4 hr at 300 rpm at 37°C.

4) **Analysis of the sample**

10 ml of the sample was collected from each flask after 10 days of incubation was centrifuged at 450 rpm for 30 min to remove cells and the supernatant was filtered through Whatman filter paper No. 1 and the sample is used to determine the presence of ethanol further the sample is subjected to GC (Gas Chromatography) to find out the concentration of ethanol.

5) **Test for ethanol content in the sample**

a) **Chromic acid test –** to 1 ml of the sample 2 drops of acetone was added and 2 drops of freshly prepared chromic acid reagent (also called the Bordwell-Wellman Reagent) sample colour changes to blue green colour indicated the presence of ethanol in the sample

b) **Estimation of ethanol content by gas chromatograph**

A gas chromatograph (GC-1100, Mayura Analytical LLP Bangalore, Karnataka) equipped with a flame ionization detector (FID) and data acquire system with computer software was used to analyze the ethanol concentration. The installed column was a AB-624, 30 mtrs x 0.53 mm x 3 Microns Capillary column. During the analysis, the temperature was maintained at70°C. The injector and detector temperatures were maintained at 250°C. The flow rate for carrier gas (Nitrogen) was set at 3 ml/min. The injection sample volume was 0.2 Micro litre. The volume of standard ethanol used was 0.2 µl. The area of standard ethanol was found to be 954596 In each set of experiments, the data points were reported. The formula used for the calculation of Percentage of ethanol is given below.

$$\%\text{of Ethanol} = \frac{\text{Area of unknown sample}}{\text{Area of standard Ethanol}} \times 100$$

3. Result and Discussion

1) **Fermentation medium**

Waste banana plant parts were collected from banana farms and finally chopped in to small pieces and grinded with blender using sterile water in 1:10 ratio and pH was set to 5.5 then stored in refrigerator for further use.

Figure 1: Blended Fermentation Media

2) **Isolation and Preparation of enzyme for inoculation**

The crude Cellulase enzyme was isolated from three different species of fungi (Aspergillus niger, Curvularia and Fusarium) which were obtained from waste banana dumping yards and tested positive (Wood, T. M.) for the cellulase production. The isolated enzymes are then used for the fermentation process.

Figure 2: Crude enzymes from (*Fusarium, Curvularia, Aspergillus*)

3) **Fermentation Process**

I Phase (conversion of cellulose to lower sugars)

Fermentation media (100 ml) was inoculated with 1 ml of crude Cellulase enzyme from three fungi were transferred to the sterile 250ml conical flask and then incubated at 37ºC for four to five days in incubator and pH was maintained to

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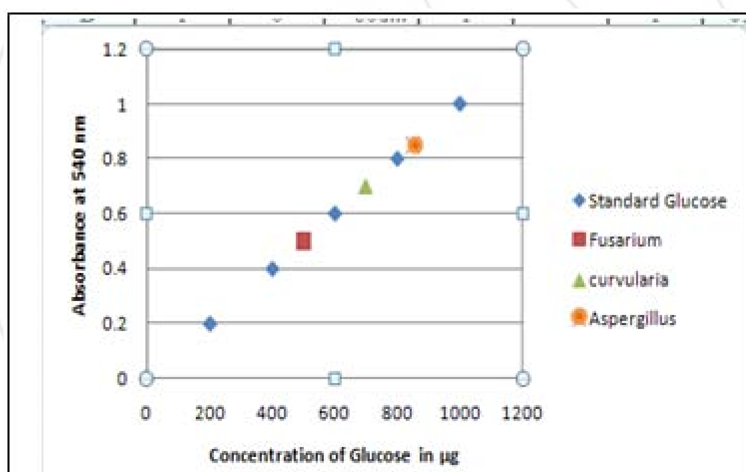
5.5, every 24 hr the flask was removed from the incubator and transferred to rotator shaker for 4 hr at 300 RMP at 37°C then transferred to incubators same process was continued till 4 days

S no	Cellulase enzyme	Quantity of crude enzyme
1	*Fusarium*	1ml
2	*Curvularia*	1ml
3	*Aspergillus*	1ml

Figure 3: At the time of incubation after 5 days of incubation



Graph 1: Showing standard glucose with sample

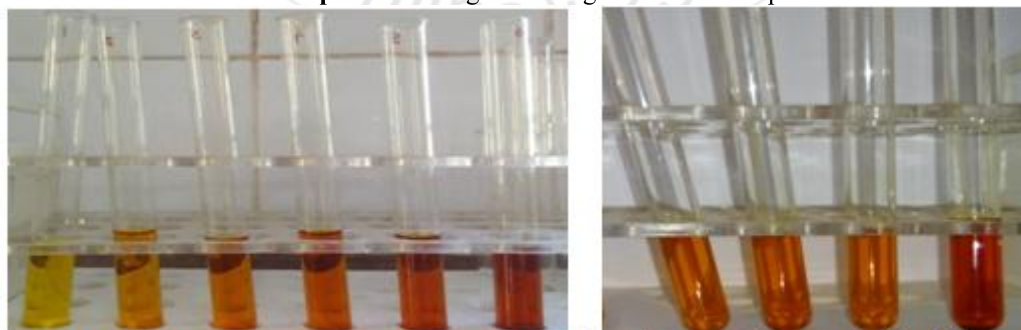


Figure 4: Standard glucose test tubes showing colours after adding DNS

Test tubes showing sample with DNS reagent

Phase II fermentations

Once the reducing sugar presence was confirmed 1 ml of 0.10 OD *Saccharomyces cerevisiae* was added to the conical flask and incubated at 37°C for 5 days, every 24 hr once the flask was removed from the incubator and transferred to shaker for 4 hr at 300 rpm at 37°C.

4) Quantitative analysis for reducing sugars

Quantitative analysis for reducing sugars was carried out using standard DNS method, the amount of reducing sugar present in the sample was determined by adding 1ml of DNS reagent(3,5–dinitrosalicylic acid)and boiled for 10 min and cooled in water bath and 5 ml of distilled water was added. The optical density of each sample was taken by using ELICO photo colorimeter at 540 nm, the concentration of reducing sugar produced by the 3 fugal isolated were determined by using standard glucose curve.

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Figure 5: After 4 days of incubation

5) Analysis of the sample

10 ml of the sample was collected from each flask after 10 days of incubation, sample was centrifuged at 450 rpm for 30 min to remove cells and the supernatant was filtered through what man's filter paper No. 1 and the sample is used to determine the presence of ethanol

a) Chromic acid test

All three samples turned bluish green colour thus the presence of ethanol is conformed.

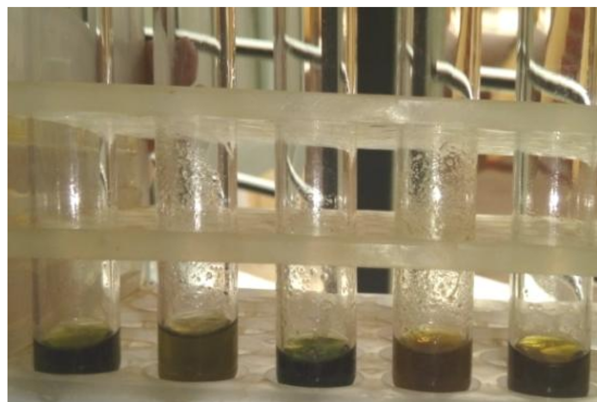


Figure 6: Test tubes showing positive chromic acid test

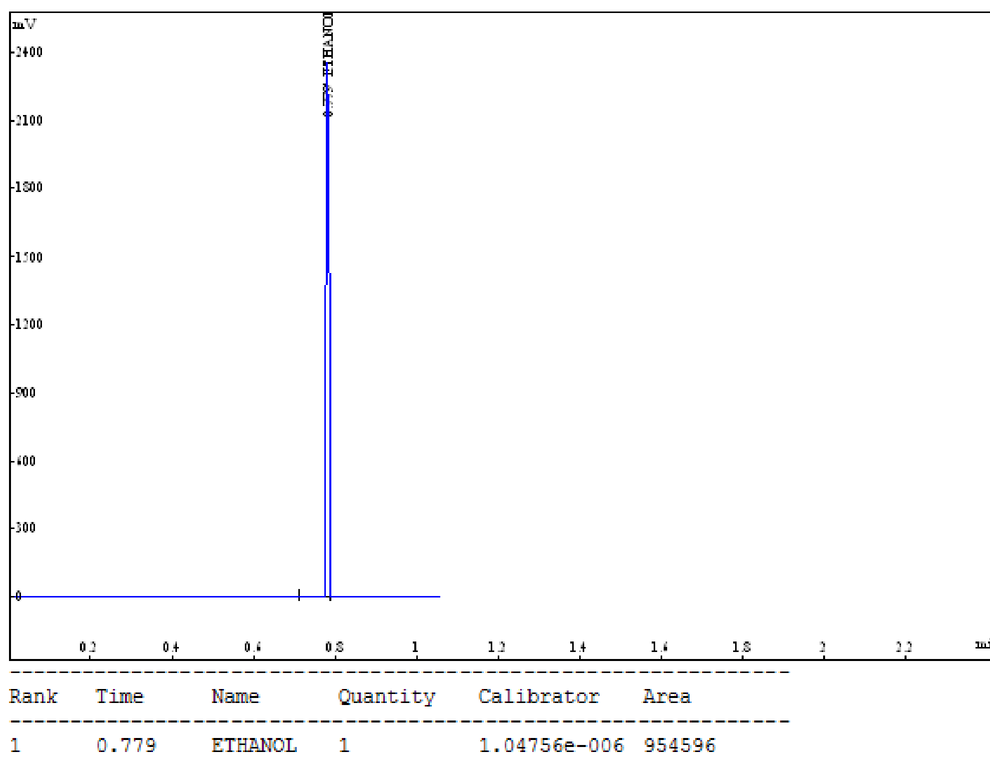
b) Conformation and Estimation of ethanol content by gas chromatograph

A gas chromatograph was carried out to confirm the presence of ethanol and its concentration. Area of standard ethanol was found to be 954596 in each set of experiments, the data points were reported. By using the formula Percentage of ethanol was identified shown in table 2.

Table 2: Results of GC

Sl.no	Particulars	Area of Gas Chromatography	Time taken In min	% ethanol
a	Ethanol standard	954596	0.779	99.9
b	*Aspergillus*	462666	0.777	48.46
c	*Curvularia*	420565	0.762	44.05
d	*Fusarium*	306145	0.777	32.07

c) G C of standard Ethanol



Rank	Time	Name	Quantity	Calibrator	Area
1	0.779	ETHANOL	1	1.04756e-006	954596

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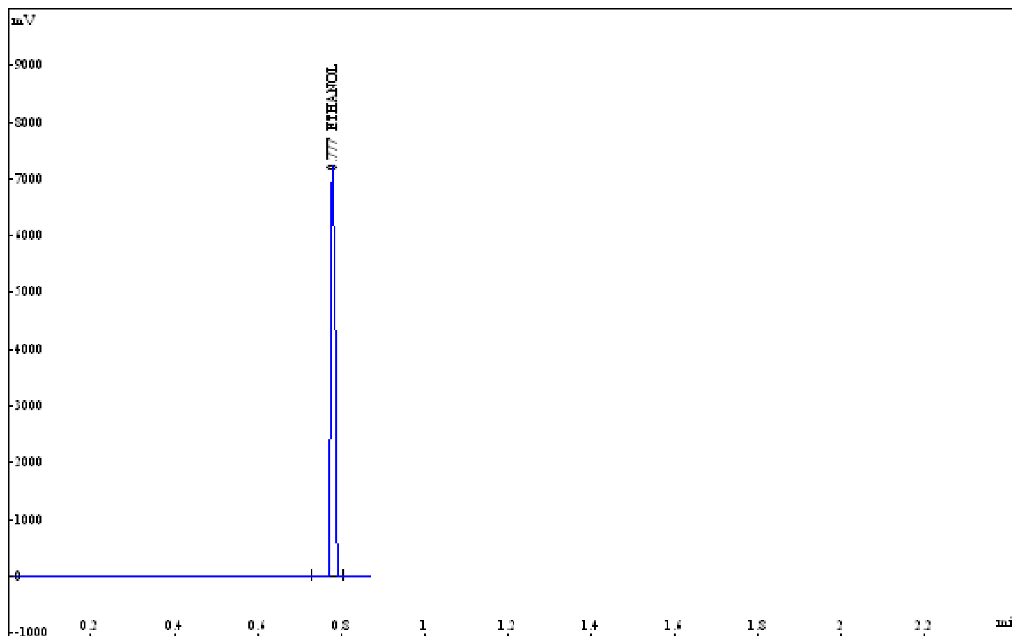
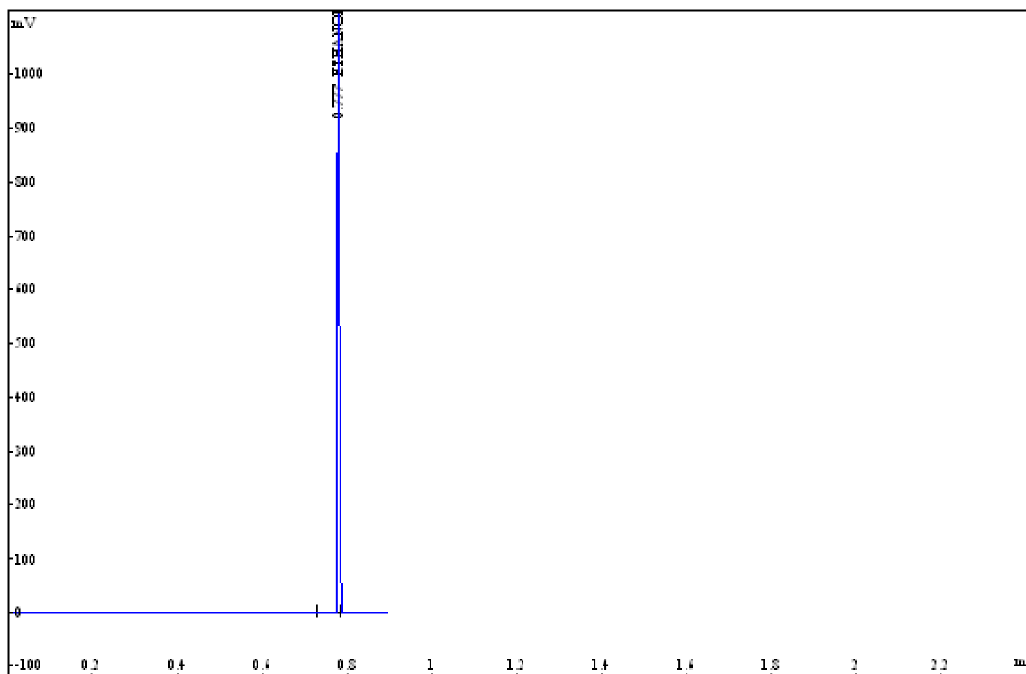
d) ***Aspergillus***



Rank	Time	Name	Quantity	Calibrator	Area
1	0.777	ETHANOL	4.847	1.04756e-006	4626660

e) ***Curvularia***



Rank	Time	Name	Quantity	Calibrator	Area
1	0.777	ETHANOL	0.3207	1.04756e-006	306145

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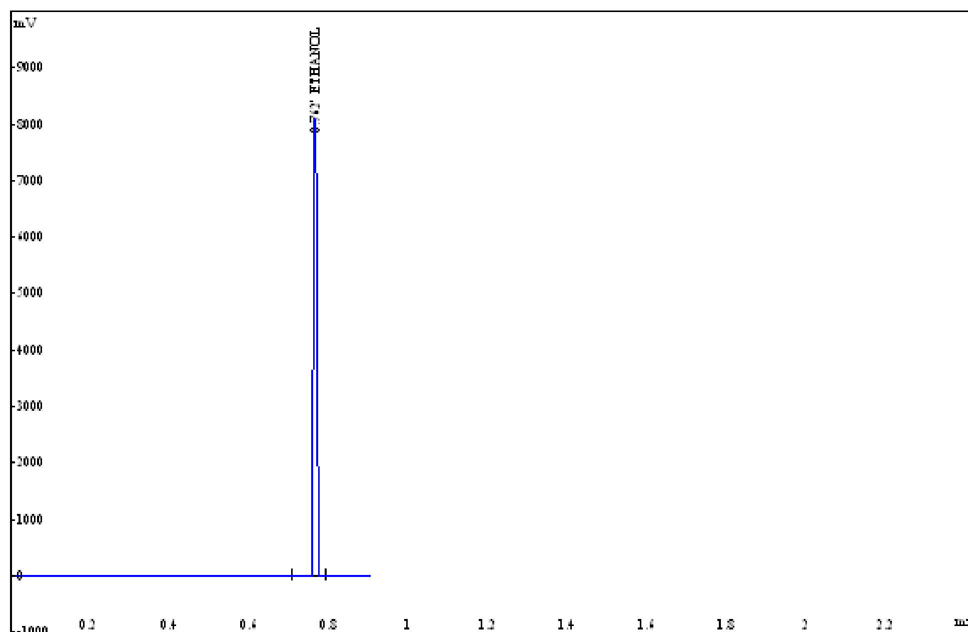
f) ***Fusarium***



Rank	Time	Name	Quantity	Calibrator	Area
1	0.762	ETHANOL	4.406	1.04756e-006	4205657

4. Conclusion

The waste banana waste plant parts (leaf, stem) was enzymatically treated with cellulase isolated from fungal stains according to the methods used in the study, the maximum yield was found out to be 48.46% with the cellulase concentration of 1ml for 100 ml of fermentation medium for 9-10days. Therefore the study suggests that waste banana plantation which is destroyed after the banana yield is obtained can be used for the production of ethanol. Further work to the present study includes standardization of the scarification process and testing the same procedure in the agricultural plot for bulk amount of banana waste plantations.

5. Acknowledgement

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