

## Bio-ethanol Production from Lignocellulosic Banana Waste Using Co-Culture Techniques

Rakesh Kumar Meena\*, Rajni Sahu, Prashant Shukla and Sanket Thakur

Aditya Biotech Lab & Research Pvt Ltd., Raipur, Chhattisgarh, India

\*For Correspondence - dbt.rakesh@gmail.com

### Abstract

Ethanol is the next energy molecule and its production from various biomasses is becoming the need of the hour. Plant based biomass is sustainable and available in large quantities for ethanol production. However, separation of cellulose from lignin is important for the production of ethanol. Banana pseudostem is one such source available in large quantities as cellulosic biomass. In this experiment, we have isolated three different strains of yeast which were found to be better for ethanol production. This study investigates the influence of biological pre-treatment method on sugar conversion and ethanol production from banana wastes rich in lignocelluloses that are thrown away. The two different cellulose degrading bacteria (A2 and A3) were used as biological pre-treatment for 24h, 48h and 72h. The three wastes i.e. banana peel (BP), banana dry pseudostem (DS) and banana wet pseudostem (WS), were taken and fermented with activated *S. cerevisiae* separately and taken in separate YPDA (yeast, peptone, dextrose, agar) slants as an adapted organism and labelled as *S. cerevisiae* (D), *S. cerevisiae* (P) and *S. cerevisiae* (W). It was found that the A2 strain efficiently degraded the banana wastes into its monomer in 72h. The total yield of ethanol was estimated by titration method. Among the three adapted organism, *S. cerevisiae* (D) was found as a good strain for ethanol production. The maximum yield by using dry pseudostem was 0.288 g/g of waste, while by using BP and WS produced 0.19 g/g and 0.2 g/g ethanol respectively.

**Key words:** Pseudostem, Cellulose, Co-culture, Biological pre-treatment, *S. cerevisiae*, cellulose degrading bacteria, ethanol, lignocellulose,

### Introduction

Bioethanol is the most dominant biofuel, considered as a good alternative for liquid transportation fuels with powerful economic, environmental and strategic attributes. Although the usable energy produced by burning ethanol is 68% lower than that of petroleum fuel, the combustion of ethanol is clean (because it contains oxygen). Worldwide production capacity of ethanol in 2005 and 2006 was about 45 and 49 billion litres per year respectively and the total projected demand in 2015 is over 115 billion litres (1). Liquid biofuels are being researched mainly to replace the conventional liquid fuels, such as diesel and petrol. The advantage of the second generation biofuels is the fact that they do not compete directly with the food market. It is possible to use entire above-ground biomass of a plant, thus enabling better efficiency and land use. Downside of the second generation biofuel production is the need for large investments and sophisticated processing equipment, compared to the first generation. In the future, the production of ethanol is expected to include both, traditional grain/sugar crops and lignocellulosic materials. Production of ethanol from lignocellulosic raw material and utilizing it as a substitute for petrol could help promote rural development, reduce greenhouse gases, and achieve independence from outside energy providers (2). Banana pseudostem can be used as raw material for the

production of bioethanol. Banana pseudostem is abundantly available agriculture residues in subtropical and tropical regions. India is the largest producer of banana, contributing to 27% of world's banana production. After harvesting, 60 – 80 t/ha of banana pseudostem is generated in the field. In India, presently after extraction of fibre from pseudostem the resulting biomass is dumped on roadside or burnt or left *in situ* causing detrimental impact on environment. High concentration of holocellulose (72%) with low lignin content (10%) and its easy availability makes banana pseudostem as a potential source of lignocellulosic biomass which could be used for the production of bioethanol. Innovation in the study is that use of co-culture bacteria and yeast for the production of ethanol from banana pseudostem. Large amount of lignocellulosic wastes are generated through forestry and agricultural practices, from sugar industry, pulp and paper industries, timber industries and many agro-industries, bagasse, rice straw, wheat straw, cotton straw, corn stover, groundnut shells, wood, grasses, paper pulp and many others (3).

### Materials and Methods

The harvested banana plants were collected from the fields of Aditya Biotech Lab & Research Pvt. Ltd, Chandandih, Nandanvan Road, Raipur, Chhattisgarh, India and Bakery yeast was procured from local market.

**Sample preparation:** After extraction of fibre from banana pseudostem, the rest of the biomass was categorised as follows. The pseudostem was taken as first pseudostem with juice i.e., wet biomass, second pseudostem without juice i.e., dried biomass and banana peel as third pseudostem.

**Inoculum preparation:** The bakery yeast was activated by suspending it in slightly warm autoclaved water under laminar air flow. Then activated yeast was taken on YPDA (yeast extract 10 g; peptone 20 g; dextrose 20 g and agar 15 g in per litre) slants by using inoculating loop. The activated yeast was inoculated in fermentation broth containing banana peel, banana dry

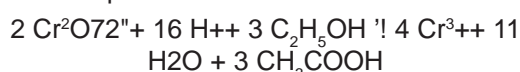
pseudostem and banana wet pseudostem separately and incubated at 30°C for 48h at 100 rpm. Then the adapted yeast was taken in separate YPDA slants from these fermented broths for further production of ethanol from banana wastes.

### Biological pre-treatment and fermentation:

After collection of samples and inoculum preparation, the fermentation process was carried out. The batch cultures were carried out in 150 mL Erlenmeyer flasks containing dry pseudostem, wet pseudostem and peel in 50 mL distilled water separately. They were then inoculated with cellulose degrading bacteria (identified in our Laboratory) in each flask, after 24h, 48h and 72h incubation of broth at 30°C at 100 rpm separately then inoculated activated bakery yeast and incubated at 30°C at 100 rpm. Then the production of ethanol was analysed after 24h and 48h by using potassium dichromate method.

### Quantitative analysis of bioethanol by potassium dichromate method:

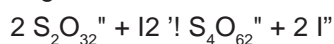
This method uses a redox titration to find the concentration of ethanol in an aqueous solution. The ethanol is oxidised to ethanoic acid by reacting it with an excess of potassium dichromate in acid.



The amount of unreacted dichromate is then determined by adding potassium iodide solution which is also oxidised by the potassium dichromate forming iodine.



The iodine is then titrated with a standard solution of sodium thiosulfate and the titration results are used to calculate the ethanol content of the original solution.



**Procedure:** Five mL of fermented broth was taken in 150 mL conical flask and made up the volume up to 125 mL. Then 20-20 mL of above aliquot was taken in three different 150 mL

conical flasks and 20 mL of 0.04 M potassium dichromate solution was added. Then ten mL of 40 % sulphuric acid was added, the conical flasks were sealed with aluminium foil. They were heated for 10 min in a boiling water bath at 50°C. The flasks were removed and 2 g of potassium iodide was added in each flask and titrated with burette filled with 0.1 M sodium thiosulfate till ting green colour appeared. One to two mL of 1% starch indicator was added and started titration till equivalence point appeared. The same process was repeated with references (distilled water was taken in the place of aliquot).

**Calculations:** Average volume of sodium thiosulfate used was determined for sample titration. Average volume of sodium thiosulfate used was also determined for the blank titration (reference). The volume of the sodium thiosulfate solution used for the sample titration was subtracted from the volume used for the blank titration. This volume of the sodium thiosulfate solution was used to determine the alcohol concentration. The number of moles of sodium thiosulfate in this volume was calculated. Using the equations, the relationship between the moles of sodium thiosulfate and the moles of ethanol was determined as 1 mol of  $S_2O_{32}^-$  is equivalent to 6 mol of  $Cr^{2}O_7^{2-}$  and 2 mol of  $Cr^{2}O_7^{2-}$  is equivalent to 3 mol of  $C_2H_5OH$ . Then 1 mol of  $S_2O_{32}^-$  has been found equivalent to 0.25 mol of  $C_2H_5OH$ . We used this ratio to calculate the moles of alcohol in the sample solution. Finally the dilution factor was multiplied with it.

## Results

The comparative analysis of bioethanol production from three different banana wastes by using two different cellulose degrading bacteria (A2 and A3) for pre-treatment method and fermented with adapted *Saccharomyces cerevisiae* (D, P and W) separately are given below

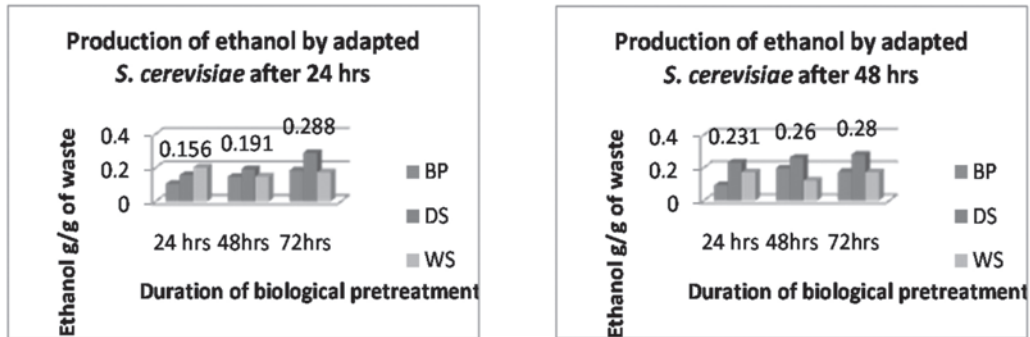
In the case of comparative study of bioethanol production by using adapted yeast with A2 cellulose degrading bacteria, maximum production was found in 72h pre-treated dry

pseudostem (DS). The yield of ethanol was 0.288 g per g of waste after 24h of incubation with yeast (Fig. 1) but it gradually decreased after 48 h where the yield was 0.280 g/g of ethanol. Good yield by using banana peel (BP) and wet pseudostem (WS) was found to be 0.19 g/g of ethanol and 0.2 g/g of ethanol respectively. Similarly, high production was found in dry waste fermented with yeast (P) (Fig. 2). The yield was 0.223 g/g of ethanol but it decreased after 48 h.

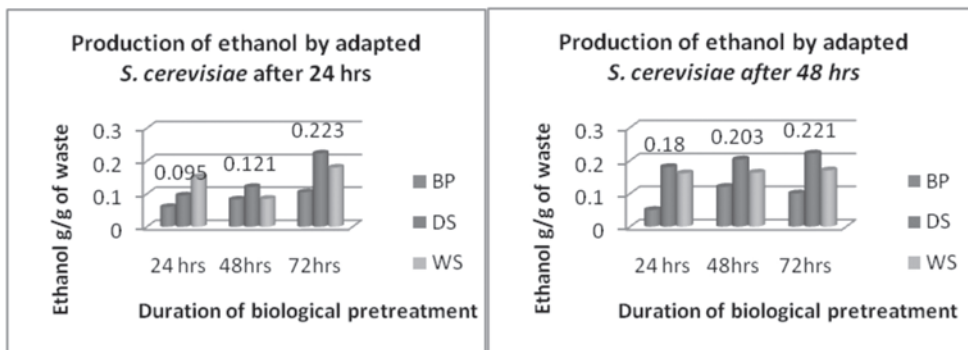
*Saccharomyces cerevisiae* (W) produced 0.161 g/g of ethanol in 24h, but after 48h of fermentation, it yielded 0.173 g/g of ethanol. By using other banana wastes, the yield of ethanol was reduced (Fig. 3). The production of bioethanol by using A3 cellulose degrading bacteria (Fig. 4) was increased after 48 h of incubation. The total yield was found to be 0.135 g/g of ethanol in dry waste while the maximum production was 0.14 g/g of ethanol in banana peel after 24 h incubation with yeast. Maximum production of ethanol was found to be 0.196 g/g from wet pseudostem in 24 h while the production was reduced after 48 h (Fig. 5). Maximum production of ethanol was noticed by using BP and DS and it was found to be 0.186 g/g and 0.196 g/g ethanol respectively. The yield of ethanol by using yeast (W) was found to be 0.156 g/g after 48 h fermentation of dry waste. But by using other wastes, the yield of ethanol was reduced (Fig. 5).

## Discussion

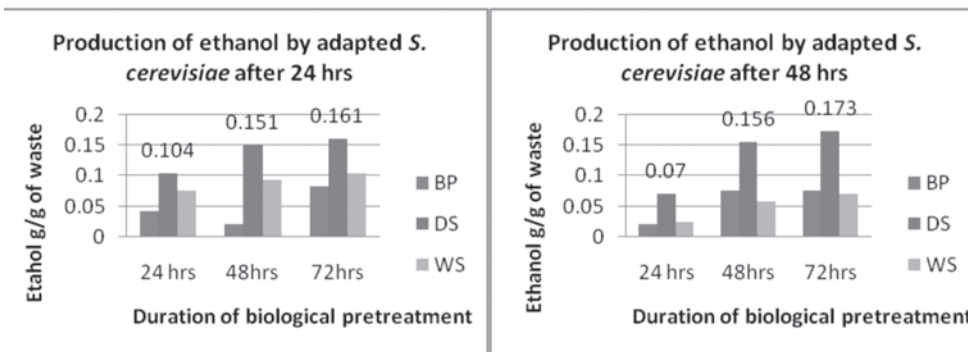
In this study, banana wastes were taken as a feedstock for the production of bioethanol. These wastes were banana peel (BP), dried pseudostem (DS) and wet pseudostem (WS). The wastes were pretreated separately with two different cellulose degrading bacteria (A2 and A3) for 24 h, 48h and 72h separately. After pretreatment, these wastes were fermented with adapted yeast. Higher yield was found in dry pseudostem with A2 cellulose degrading bacteria when compared with the other two wastes. Itelima (4) have used co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae* for ethanol



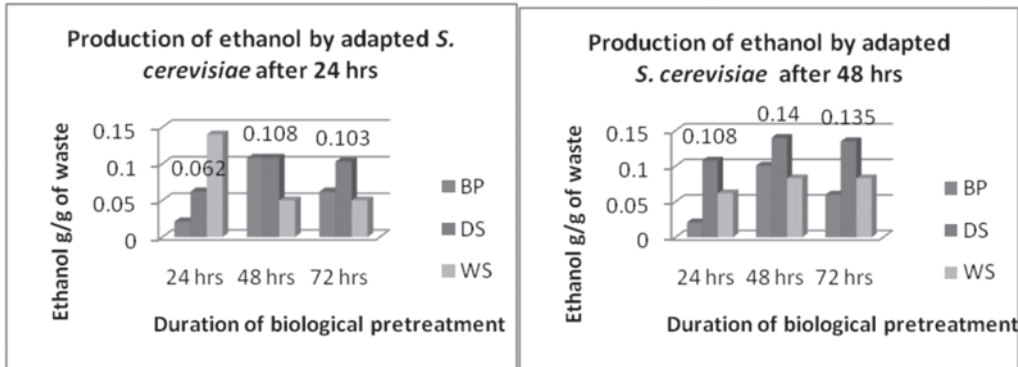
**Fig. 1.** Production of bioethanol by using A2 cellulose degrading bacteria with adapted *Saccharomyces cerevisiae* (Dry Mass)



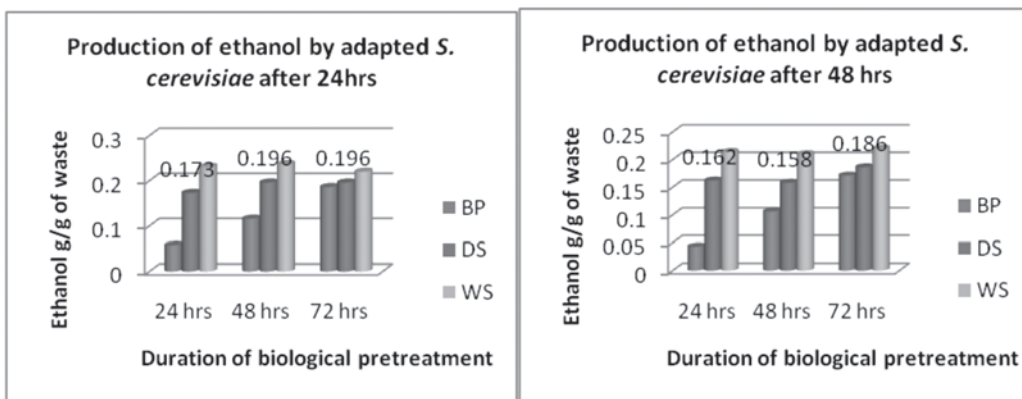
**Fig. 2.** Production of bioethanol by using A2 cellulose degrading bacteria with adapted *Saccharomyces cerevisiae* (Pulp)



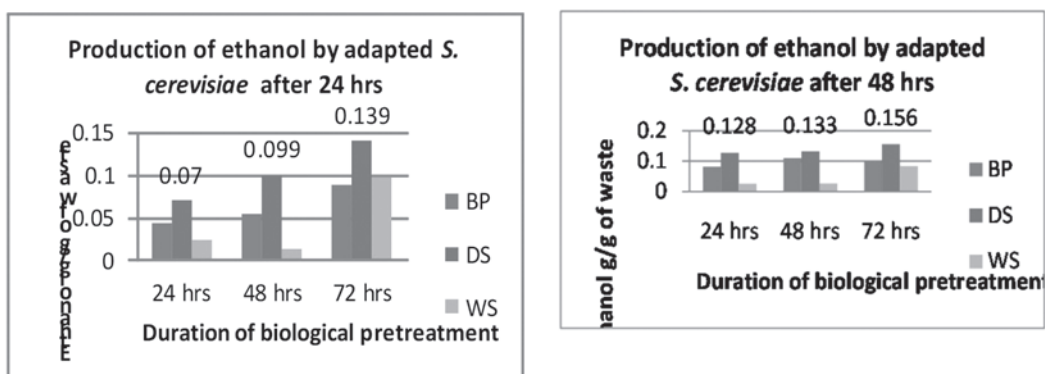
**Fig. 3.** Production of bioethanol by using A2 cellulose degrading bacteria with adapted *Saccharomyces cerevisiae* (Wet mass)



**Fig. 4.** Production of bioethanol by using A3 cellulose degrading bacteria with adapted *Saccharomyces cerevisiae* (Dry Mass)



**Fig. 5.** Production of bioethanol by using A3 cellulose degrading bacteria with adapted *Saccharomyces cerevisiae* (Pulp)



production from corn cobs. Svetlitchnyi (5) have successfully developed a technique for consolidated bioprocessing for converting lignocellulosic biomass into ethanol using thermophilic consortia of bacteria. Similarly, Park *et al.* (6) have described *Acremonium cellulolyticus* and *Saccharomyces cerevisiae* co-culture for ethanol production. *Pichia stipites* and *Zymomonas mobilis* co-culture was used for ethanol production by Laplace *et al.* (7). Lynd *et al.* (8) have described the use of consolidated bioprocessing of cellulosic biomass for ethanol production. Awan *et al.* (9) have described the effect of co-culture of *Xanthomonas axonopodis* sp. It has been observed that *Citri*, *Saccharomyces cerevisiae* and *Candida parapsilosis* have reduced the fermentation time significantly. A comparative study of ethanol production by using banana fruit, banana peel and banana pseudostem was reported earlier. In their study, they evaluated the acid hydrolysis of banana residues by using Aspen HYSYS(R) software for simulating the heating, hydrolysis, neutralization and cooling process. In the simulation of the hydrolysis of the pulp, peel, and pseudostem wastes, the energy consumed was found approximately 300.6 kJ/h for the pulp, 309.1 kJ/h for the peel, and 310.4 kJ/h for the pseudostem. The pseudostem was the most efficient glucose producer, producing approximately 0.87 g/h. For the analysis of the hydroxymethylfurfural formed, it was found that the highest production was for the pulp (mass fraction of  $2.86 \times 10^{-4}$ ), indicating that there is inhibition of production by this compound. The ratio of energy consumption to quantity sugars and glucose formed showed better results for the pseudostem, and this is the ideal waste product suited for hydrolysis because the highest quantity of sugar is formed in relation to the energy consumed (10).

#### Conclusions :

It can be concluded that the pseudostem is a good feedstock and A2 cellulose degrading bacteria efficiently degrade the dried pseudostem than the other pseudostem wastes in to its

monomers, which were utilized by *Saccharomyces cerevisiae* for the production of ethanol. This is a good alternative technology for the production of ethanol from banana pseudostem waste because the farmers dispose off the banana waste after harvesting the crop and these waste can be utilized by this process. It will also help in controlling soil pollution.

#### Acknowledgement

I sincerely acknowledge with thanks Mr. Shaliendra Jain (CEO) of Aditya Biotech Lab & Research Pvt Ltd, Raipur, Chhattisgarh, India, for his help and cooperation.

#### References

1. Chittibabu, S., Rajendran K. and Santhanmuthu M., (2011). Optimization of microwave assisted alkali pretreatment and enzymatic hydrolysis of banana pseudostem for bioethanol production, Vol. 6, 2nd International Conference on Environmental Science and Technology.
2. Tutt, M., KikasTimo and OltJuri, (2012). Comparison of different pretreatment methods on degradation of rye straw. Engineering for Rural Development. Jelgava, pp142-146
3. Hanh, V. and Nguyen, T. H. Minh (2012). Bioethanol production from marine algae biomass: prospect and troubles, J. Vietnamese Environ. Vol. 3: pp25-29.
4. Itelima, J., Ogbonna, A., Pandukur, S., Egbere, J. and Salami, A. (2013). Simultaneous saccharification and fermentation of corn cobs to bio-ethanol by co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae*. Int. J. Environment. Sci. Develop. Vol. 4, No. 2. Pp 329-242
5. Svetlitchnyi, V.A., Kensch, O., Falkenhan, D.A., Korseska, S.G., Lippert, N., Prinz, M., Sassi, J., Schickor, A. and Curvers, S. (2013). Single-step ethanol production from lignocellulose using novel extremely

- thermophilic bacteria. *Biotechnol Biofuels*, Vol. 6:31;pp2-15
6. Park, E.Y., Kazuya Naruse and Kato, T. (2012). One-pot bioethanol production from cellulose by co-culture of *Acremonium cellulolyticus* and *Saccharomyces cerevisiae*. *Biotechnology for Biofuels* Vol.5:64;pp 2-11
  7. Laplace, J.M., Delgenes, J.P., Moletta, R. and Navarro, J.M. (1993) Effects of culture conditions on the co-fermentation of glucose and xylose mixture to ethanol by a mutant of *Saccharomyces diastaticus* associated with *Pichia stipitis*. *Appl. Microbiol. Biotechnol.* 39: 760-763
  8. Lynd, L.R., van Zyl, W.H., McBride, J.E. and Laser, M. (2005). Consolidated bioprocessing of cellulosic biomass: an update. *Curr Opin Biotechnol.* Vol. 16: pp577–583
  9. Awan, A.T., Junko Tsukamotoa and Ljubica Tasic (2013). Orange waste as a biomass for 2G-ethanol production using low cost enzymes and co-culture fermentation. *RSC Adv.*, Vol. 3,pp 25071-25078 DOI: 10.1039/C3RA43722A
  10. De Souza, Priscila, Sellin, Noeli, Souza, Ozair and Marangoni (2013). Simulation of dilute acid hydrolysis of banana waste for ethanol production: Comparison between the use of fruits, peel and pseudostem, *The Italian Association of Chemical Engineering*, Vol. 32, PP: 1141-1146.