J. Mycopathol, Res, **48(2)** : 365—369 (2010)
© Indian Mycological Society, Department of Botany,
University of Calcutta, Kolkata 700 019, India

Production of bio-alcohol utilizing jackfruit wastes

DEBAJYOTI BOSE[1]; SRIJA BASU[2] AND HIRANMAY GANGOPADHYAY[3*]
[1,3] Department of Food Technology & Biochemical Engineering, Jadavpur University, Kolkata-700032
[2] Life Science Department, Reva Institute of Science and Management, Kattigenahalli, Bangalore 560024,
*E-MAIL: debajyotibose@yahoo.co.in, srija_basu2003@yahoo.com and hganguly2@yahoo.com**

Recent developments in both price and availability of crude oil have motivated a worldwide search for cheap alternatives. Starch is an alternative source of energy because it is both renewable and available throughout the globe in large quantities. There are a variety of products that can be obtained from starch biomass via hydrolysis. Alcohol is one of the largest volumes of products that can be produced from biomass. Recently there has been active research aimed at attaining an increase in ethanol productivity by fermentation. Jackfruit is a starch rich material, besides acting as a delecious seasonal fruit. Jackfruit (*Artocarpus heterophyllus*) generates large amount of wastes which creates disposal problem along with environmental pollution by generation of non-ecofriendly gases due to natural fermentation. This large amount of jackfruit wastes produced every year in India, after saccharification can be used for production of alcohol which may generate employment potential apart from saving good amount of foreign exchange. Jackfruit has tremendous potentiality to obtain alcohol as fermented product and very few reports are available on this account. No such systematic work has been reported in India on alcohol production from jackfruit wastes. Therefore, a systematic study were carried out on production of bio-alochol utilizing different waste parts of jackfruit with different treatments. The bio-alcohol produced was maximum (88% on dry weight basis and 3.81% on wet weight basis) when the core of jackfruit (considered as waste) was subjected to saccharification with *Aspergillus oryzae* (NCIM No. 645) followed by fermentation with *Saccharomyces cerevisiae* (NCIM 3314).The other waste parts of the jackfruit also yielded some amount of alcohal which will be reported.

Key words : Jackfruit wastes, *Aspergillus oryzae, Saccharomyces cerevisiae*, bio-alcohol

INTRODUCTION

The demand for ethanol has been increased tremendously in recent years not only for its use as feedstock of chemical manufacturing but also it has been considered as a potential alternative source of liquid fuel for automobiles. Ethanol can be produced easily by fermentation from various renewable resources, however, the major disadvantage of this process is its high production cost as reported by Pramanik *et al.* (2005). To make the fermentation method a cost competitive and to meet the great demand for ethanol in the present situation of energy crisis, research study has been directed in two areas recently, namely, the production of ethanol from comparatively cheaper source of raw materials and to study the new microorganism or yeast strains efficient for ethanol fermentation as reported by Favela-Torres *et al.* (1986). In this respect, inexpensive raw materials like by-products or wastes, such as, molasses, agricultural wastes, cellulose wastes, fruit wastes, vegetable wastes, municipal and industrial wastes can be utilized to produce ethanol cheaply as reported by Park *et al.* (199l), Joshi *et al.*(1991), Green *et al.*(1989), Schugerl (1994), Muttara *et al.*(1982), Shiyuan *et al.* (1987) and Singh *et al.* (1995).

Jackfruit (*Atrocarpus heterophyllus* Lam.) is a monoecious evergreen tree that is popular in several tropical countries other than India, like Malaysia, Philippines, Thailand, Cambodia, Laos and Vietnam. It is an excellent example of a food prized in some areas of the world and allowed to go to waste in others as reported by Babitha *et al.*(2006). The ripe fruit contains well flavored yellow sweet bulbs and seeds (embedded in the bulb). The edible bulbs of ripe jackfruit are consumed fresh or processed into

canned products.But, the major part is considered as waste. In India, West Bengal produces a huge quantity of jackfruit every year in summer season, which in turn generates a large quantity of wastes.This jackfruit wastes gets contaminated by environmental bacteria and thus forms a foul mass beside creating disposal problem. However, this fruit waste contains high quantity of sugar which can be fermented to produce ethanol and high value products. Moreover, efforts must be given to improve the alcohol fermentation of this inexpensive raw material for ethanol production by the exploitation of effective microbial strains. Considering the above facts, a systematic study has been carried out on production of bio-alochol utilizing different waste parts of jackfruit with different treatments.

MATERIALS AND METHODS

Microorganisms

Aspergillus oryzae (NCIM No. 645) collected from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune(India) was maintained on Czapek Dox agar medium consisting of Glucose 5%, $NaNO_3$ 0.2%, KC1 0.05%, $MgS0_4.7H_2O$ 0.05%, $FeS0_4$. $7H_2O$ 0. 001%, KH_2PO_4 0.1%, Agar 3% with a pH of 5 and stored under refrigerated condition at 4°C.

Saccharomyces cerevisiae (NCIM 3314) collected from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune(India) was maintained on YPD agar slants containing glucose l%, peptone 0.5%, yeast extract 0.3%, malt extract 0.3 % and agar 2 % at a pH of 4.5. After growth at 30°C for 48 hrs on agar slants, the culture was stored at 4°C.

Viable spore count

The total viable spore number on a Czapek Dox agar slant was determined by colony count technique. The spores were suspended in 10 ml of distilled water using a sterile transfer needle and diluted serially. One ml of spore suspension was poured onto sterile Petri-plates, containing sterile Czapek Dox agar medium and spread uniformly. The inoculated Petri-plates were incubated at 30°C for 48-72 hrs. A plate that developed between 7 to 200 colonies was selected for counting. The spore density was calculated as the count multiplied by the dilution factor.

Viable cell count

The total viable cell number on YPD agar slant was determined by colony count technique. The cells were suspended in 10 ml of distilled water using a sterile transfer needle and diluted serially. One ml of cell suspension was poured onto sterile Petri-plates, containing sterile YPD agar medium and spread uniformly. The inoculated Petri-plates were incubated at 30°C for 48 hrs. A plate that developed between 7 to 200 colonies was selected for counting. The cell density was calculated as the count multiplied by the dilution factor.

Substrate used

The waste parts of jackfruit such as core and the interior fibrous(pulp) part were used as substrate.

Moisture content of the substrate

Per cent (%) moisture of the core and the interior fibrous part was determined by keeping the tared materials at 90°C for 24 hrs. and then after cooling the weight was again taken. The difference in initial and final weight was taken as moisture content.

Preparation of inoculum

(i) A suspension of the mold i.e. one loopfull in 10 ml of sterile water blank was used as the inoculum for each conical flask in our present study. A constant ratio of 4:1 (w/v) of waste to inoculum was maintained throughout the study, (ii) A suspension of the yeast i.e. one loopfull in 10 ml of sterile water blank was used as the inoculum for each conical flask in our present study. A constant ratio of 4:1 (w/v) of waste to inoculum was maintained throughout the study.

Production of alcohol

Production of alcohol was carried out in four sets of 500 ml conical flasks which contained substrate and enzyme/inoclulam as mentioned below,

A. ***Jackfruit core treated with diastase*** : 100 g of dried core of jackfruit was cut and ground and

was prepared into slurry by adding water in the ratio of 1:3 .The slurry was then treated with diastase in ratio of 1:300 (w/v) after sterilization and incubated overnight at 30°C.The medium was centrifuged and the supernatant thus collected was sterilized. This sterilized medium was then inoculated with *S.cerevisiae* NCIM 3314 and incubated for 2 days at 30°C following the method of Ghosh *et al.*(2003,2005).The resulting medium was then filtered and the filtrate was distilled. The percentage of alcohol thus produced was determined by A.O.A.C. method using specific gravity bottle.

Test for amount of reducing sugar was performed using dinitrosalicylic acid (spectrophotometric method) by following the method of Bose *et al.*(2006) and Miller (1959) on untreated slurry, slurry after treatment with diastase and lastly after fermentation. The pH of the medium was measured before and after fermentation.

B. Fibrous part of jackfruit treated with diastase : 300 g fibrous part of jackfruit considered as waste was cut, dried and ground and was prepared into slurry by adding water in the ratio of 1:2.The slurry was then treated with diastase in 1:300 ratio (w/v) after sterilization and incubated overnight at 30°C.The medium was centrifuged and the supernatant thus collected was sterilized. This sterilized medium was then inoculated with *S.cerevisiae* and incubated for 2 days at 30°C.The resulting medium was then filtered and the filtrate was distilled. The percentage of alcohol thus produced was determined by A.O.A.C. method using specific gravity bottle.

Test for amount of reducing sugar was performed using dinitrosalicylic acid (spectrophotometric method) on untreated slurry, slurry after treatment with diastase and lastly after fermentation. The pH of the medium was measured before and after fermentation.

C. *Jackfruit core treated with mold* : 150 g of core of jackfruit was cut, dried and ground and was prepared into slurry by adding water in the ratio of 1:3.The slurry was then treated with *Aspergillus oryzae* after sterilization and incubated for 5 days at 30°C.The medium was centrifuged and the supernatant thus collected was sterilized. This sterilized medium was then inoculated with *S.cerevisiae* and incubated for 2 days at 30°C.The resulting medium was then filtered and the filtrate was distilled. The percentage of alcohol thus produced was determined by A.O.A.C. method using specific gravity bottle.

Test for amount of reducing sugar was performed using dinitrosalicylic acid (spectrophotometric method) on untreated slurry, slurry after treatment with *A.oryzae* and lastly after fermentation. The pH of the medium was measured before and after fermentation.

D. *Fibrous part of jackfruit treated with mold*: 200 g fibrous part of jackfruit considered as waste was cut, dried and ground and was prepared into slurry by adding water in the ratio of 1:2.The slurry was then treated with *A.oryzae* after sterilization and incubated overnight at 30°C.The medium was centrifuged and the supernatant thus collected was sterilized. This sterilized medium was then inoculated with *S. cerevisiae* NCIM 3314 and incubated for 2 days at 30°C.The resulting medium was then filtered and the filtrate was distilled. The percentage of alcohol thus produced was determined by A.O.A.C. method using specific gravity bottle.

Test for amount of reducing sugar was performed using dinitrosalicylic acid (spectrophotometric method) on untreated slurry, slurry after treatment with *A.oryzae* and lastly after fermentation. The pH of the medium was measured before and after fermentation.

RESULTS AND DISCUSSIONS

The alcohol production was found to be 88% (Table 1) when the core of jackfruit waste was treated with *A.oryzae* and 86.5% (Table 2) when the interior fibrous part of jackfruit waste was treated with diastase. The core of jackfruit waste on treatment with diastase gave 19.9% alcohol (Table 2). However, the interior part of jackfruit waste on treatment with *A.oryzae*, produces no alcohol (Table 1). The medium on double fermentation does not produce any alcohol because the saccharification by the mold was incomplete and the little amount of sugar produced by the mold was initially utilized by the yeast for its own metabolism. The production of high percentage of alcohol may be due to maximum saccharification of the jackfruit waste (Table 3).

Table. 1 : Production of alcohol by *S. cerevisiae* after treatment with *A. oryzae*

Jackfruit waste	% moisture	Reducing suger (mg/ml)		% Alcohol produced	
		Before fermentation	After fermentation	Wet weight	Dry weight
Core	95.7	1	0.6	3.81	88
Fibrous part	90.4	1.13	1.12	Not produced	Not produced

Table. 2 : Production of alcohol by *S. cerevisiae* after treatment with diastase

Jackfruit waste	% moisture	Reducing suger (mg/ml)		% Alcohol produced	
		Before fermentation	After fermentation	Wet weight	Dry weight
Core	95.7	1.1	0.83	0.85	19.9
Fibrous part	90.4	1.12	1	8.3	86.5

Table. 3 : Comparison between diastase treated and mold treated jackfruit waste

Treatment with	Jackfruit waste	% moisture	Reducing sugar (mg/ml)	
			Before fermentation	After fermentation
Diastase	1. Core	95.7	0.9	1.1
	2. Fibrous part	90.4	1.1	1.2
Mold	1. Core	95.7	0.9	1.0
	2. Fibrous part	90.4	1.1	1.13

From previous studies, it was observed that treatment of starchy medium with mold for 5 days produce good result and the optimum temperature was also referred from previous studies made by Bose *et al.*(2009) and Ohata *et al.*(1993). To optimize the yield, further investigation is required.

CONCLUSION

The results obtained in this study indicate that various parts of jackfruit waste could be utilized for production of alcohol using *Saccharomyces cerevisiae* (NCIM 3314) after saccharification with diastase or *Aspergillus oryzae* (NCIM No. 645) at 30°C. Saccharification was maximum when core of the jackfruit (generally cosidered as waste) was treated with *A.oryzae*, which in turn supported the maximum production of alcohol by *S.cerevisiae*.

ACKNOWLEDGEMENT

Authors gratefully acknowledge the financial assistance obtained from Department of Food Processing Industries & Horticulture (DFP1 &H), Govt, of West Bengal, India for carrying out the study.

REFERENCES

Babitha, S.; Soccol, C.R. and Pandey, A. 2006Jackfruit Seed - A novel Substrate for the production of *Monascus* pigments through Solid-State Fermentation. *Food Technol. Biotechnol.* **44** (4): 465-471.

Bose, D.; Ghosh, U. and Gangopadhyay, H. 2006. Effect of different salts of metals on production of fungal alpha amylase by solid state fermentation utilizing agricultural wastes. *J.MycopatholRes.* **44**(2): 225-229

Bose, D.; Ghosh, U. and Gangopadhyay, H. Production of extracellular alpha amylase from *Aspergillus oryzae* by

solid state fermentation utilizing agricultural wastes. *J. Mycopathol. Res.*

Favela-Torres, E.; Allais, J. J. and Baratti, J. 1986 .Kinetics of Batch Fermentations for Ethanol Production with *Zymomonas mobilis* growing on Jerusalem Artichoke Juice. *J.Biotecnol. Bioengg.* **28**: 850.

Ghosh, U.; Bose, D. and Gangopadhyay, H. 2003. Production of Ethanol from potato. 4th edition, All India Peoples Technology Congress, Organised by Forum of Scientists. Engineers & Technologists, FOSET. pp. 39-41.

Ghosh, U.; Bose, D. and Gangopadhyay, H. 2005. Studies on Production of Bio-Alcohol from Potato & its wastes using *Saccharomyces cerevisiae*. 5th edition, All India People's Technology Congress, Organised by Forum of Scientists, Engineers & Technologists, FOSET. **AB - 30**:72-75.

Green, M. and Shelef, G. 1989.Ethanol Fermentation of Acid Hydrolysate of Municipal Solid Waste. *J. Chem. Engg.* **40**: B-25.

Joshi, S. S.; Dhopeshwarkar, R.; Jadav, U.; Jadav, R.; D.souza, L. and Jayaprakash, D. 2001.Continuous Ethanol Production by Fermentation of Waste Banana Peels using Flocculating Yeast. *Ind. J. Chem. Tech.* **8**:153.

Miller, G. G. 1559. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Anal Chem.* **31**: 426-428.

Muttara, S. and Nirmala, D. J. 1982.Production of Alcohol and Acetic Acid from Pineapple Waste. *J. Water Quality Bull.* **7(2)**: 76.

Ohata, K.; Hamada, S. and Nakamura, T. 1993. Production of High Concentrations of Ethanol from Inuiin by Simultaneous Saccharification and Fermentation using *Aspergillus niger* and *Saccharomyces cerevisiae. Appl. Em. Microbiol.* **59**(3): 729-733.

Park, S. C. and Baratti, J. 1991 .Kinetics of Sugar Beet Molasses by *Zmobilis. J.Biotecnol. Bioengg.* **38**:304.

Pramanik, K. and Rao, D.E. 2005 .Kinetic Study on Ethanol Fermentation of Grape Waste using *Saccharomyces cerevisiae* Yeast Isolated from Toddy. *IE (I) Journal.CH.* **85:** 53-58.

Schugerl, K. 1994. Agricultural Wastes: A Source of Bulk Products. *J. Chem. Engg.Technol.* **17**: 291.

Shiyuan, Y.; Morris, W. and Sarad, K. P. 1987 .Fermentation of Ethanol of Pentose Containing Spent Sulphite Liquor. *J.Biotecnol. Bioengg.* **29:** 1144.

Singh, A. and Jain, V. K. 1995.Batch Fermentation of Cane Molasses for Ethanol Production by *Zymomonas mobilis. J.Ind.Chem. Engg.* **37:** A-94.

(Accepted for publication June 15, 2010)