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Bioethanol Production from Agave Leaves Using *Saccharomyces cerevisiae* (MTCC 173) and *Zymomonas mobilis* (MTCC 2427)

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Abstract: Ethanol is a liquid biofuel produced from sugar rich biomass and ethanol can be blended upto 20% with diesel or petrol. In the present investigation the production of ethanol by *Saccharomyces cerevisiae* and *Zymomonas mobilis* using Agave leaves was compared. The ethanol was produced from agricultural wastes by using two enzymes namely amylase and cellulase obtained from *Aspergillus niger* and *Trichoderma viridae* respectively, to hydrolyze the starch and cellulose present in the raw materials. The hydrolyzed and filtered extracts were fermented using *Saccharomyces cerevisiae* and *Zymomonas mobilis*. The fermented product was purified by distillation process and the presence of ethanol was determined by alcoholmeter method. Results indicated that the *Agave* leaves containing *Zymomonas mobilis* yielded maximum ethanol (5%) where as minimum ethanol (4%) was recorded with *Agave* leaves containing *Saccharomyces cerevisiae.*

Key words: Bioethanol · Enzymes · *Saccharomyces cerevisiae* · *Zymomonas mobilis* · Fermentation

INTRODUCTION

Bioethanol as an alternative source of energy has received special attention world wide due to depletion of fossil fuels. According to United States department of energy, for every unit of energy put towards ethanol production 1.3 units are returned [1]. In India sugarcane molasses is the main raw material for ethanol production but now the short supply and increased cost is the main hindrance for its use. The sugary substrates available are comparatively expensive than molasses but can be easily used for ethanol production with some modification in the process. On the other hand cellulosic materials are cheaper and available in plenty but their conversion to ethanol involves many steps and is expensive. In recent years because of increase in price of molasses and its limited availability, ethanol production has been greatly affected in 285 molasses based Indian distilleries [2]. Under such circumstances a novel approach is essential to use easily available renewable substrates such as starch and abundantly available ligno cellulosic substrates. Alcoholic fermentation is the main activity of yeasts, while *Saccharomyces cerevisiae* is the major species used in wine making. It utilizes sucrose, glucose, fructose, maltose and malto trioses carbon sources to produce alcohol under anaerobic conditions. *Zymomonas mobilis,* Gram negative anaerobic bacterium, is another suitable organism for ethanol production [3]. *Zymomonas mobilis* is one of the most promising microorganisms for genetic engineering envisaging the development of strains for lignocellulose fermentation [4]. *Zymomonas mobilis* presents some advantages when compared with yeasts: 98% efficiency in ethanol production and a specific rate twice bigger [5].

Common names of this substrate are True Aloe, Medicine Plant, Burn Plant, Barbados Aloe and its scientific name is *Aloevera*, also known as *Aloebarbadensis*. *Aloevera* is one of about 250 species of Aloes. Agave leaves, for example, have 3-15% lignin by dry weight and up to 68% cellulose [6]. In the Agave, the sugars are present in a proportion of about 9:1 fructose: glucose [7]. The sugars are fermented primarily by yeasts which produce ethanol and acetic acid, compounds toxic to microorganisms [8].

Hence in our present study Agave leaves were used as raw material for the production of bioethanol using *Saccharomyces cerevisiae* and *Zymomonas mobilis*.

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MATERIALS AND METHODS

Collection of Substrate and Pretreatment: The substrate (*Aloe barbadensis*) was collected from agricultural land, Periyamathampalayam, Coimbatore. The pretreatment of the substrate was done by washing peeled agave (1 Kg) and cooking in a pressure cooker in one liter water containing 0.5% potassium metabisulphite. Boiled agave was crushed and it was used for ethanol production.

Source of Organisms: *Saccharomyces cerevisiae* (MTCC 173), *Trichoderma viridae* (MTCC 800) and *Zymomonas mobilis* (MTCC 2427) were obtained from IMTECH, Chandigarh. *Aspergillus niger* was isolated from spoiled bread sample and identified. The stock cultures of *Saccharomyces cerevisiae*, *Trichoderma viridae and Zymomonas mobilis were* maintained on Yeast Extract Peptone Dextrose Agar (YEPD), Malt Extract Agar (MA) and Rich medium (RM) respectively. Isolation and identification of the organisms were done on the basis of morphological, cultural and biochemical characteristics [9].

Enzyme Production and Assay: *Aspergillus niger* and *Trichoderma viridae* were inoculated in Potato dextrose broth and incubated at room temperature for 7 days. After incubation, fermented media was filtered by Whatmann No-1 filter paper and centrifuged and the supernatant was collected for enzyme assay. Amylase and cellulase enzymes were determined by Starch agar plate method [9] and Dinitro Salicylic acid (DNS) method [10,11].

Enzymatic Hydrolysis: Twenty g of Agave leaves were crushed and dissolved in 200ml of distilled water. The content was boiled and filtered through Whatmann No-1 filter paper. Then extract was sterilized and after sterilization 5% of enzymes were added to the extracts and incubated at 37°C for 3 hours for hydrolysis process [12].

Determination of Total and Residual Sugars: The total sugar content of Agave leaves was determined by Phenol sulphuric acid method [13] and the residual sugar content of Agave leaves was determined by Nelson Somogyi [14] method with glucose as standard. The Reducing sugar (sugar utilized by the organism during fermentation) percentage was calculated by subtracting the residual sugar% from total sugar%.

Fermentation: The hydrolyzed and filtered extracts were fermented using *Saccharomyces cerevisiae* and *Zymomonas mobilis* for 7 days of incubation at room temperature under anaerobic condition. After fermentation it was filtered and distillation was carried out in round bottom flask at 80°C [12]. After that percentage of ethanol was estimated by using Alcoholmeter.

RESULTS

In the present study raw materials such as Agave leaves were collected in and around Coimbatore, India. The enzymes amylase and cellulase produced by *Aspergillus niger* and *Trichoderma viridae* were determined. Assay of amylase was done in starch agar plate method, results in zone of inhibition which indicates presence of amylase and cellulase activity was determined by DNS method.

In the present investigation, total sugar estimation was carried out by Phenol sulphuric acid method using untreated Agave leaves. The maximum total sugar percentage of *Agave* leaves was found to be 3.14%. The Residual sugar estimation was carried out by the method of Nelson somogyi using fermented agave leaves. The Reducing sugar percentage of amylase and cellulase treated agave leaves were found to be 2.23 and 2.59% respectively (Table 1 and 2).

In the cellulase treated (*Zymomonas mobilis*) Agave leaves, the ethanol yields were noticed as 2, 2.5, 3.5, 4.0 and 5.0 %, where as in the amylase treated (*Saccharomyces cerevisiae*) agave leaves

Table 1: Ethanol production from amylase treated Agave leaves using *Saccharomyces cerevisiae*

S. No.	Amount of Agave Juice (ml)	Volume of Inoculum(ml)	Total number of cells in the sample (Cells/ml)	Inoculum %	Reducing sugar %	Ethanol%
1	100	1	$32352x10^7$ cells/ml	1	1.03	1
2	100	2	$55204x10^7$ cells/ml	2	1.21	1.5
3	100	3	$6962x10^8$ cells/ml	3	1.63	2.5
4	100	4	$8154x10^8$ cells /ml	4	1.95	3.0
5	100	5	$9188x10^8$ cells/ml	5	2.23	4.0

Table 2: Ethanol production from cellulase treated Agave leaves using *Zymomonas mobilis*

S. No.	Amount of Agave Juice (ml)	Volume of Inoculum(ml)	Total number of cells in the sample (Cells/ml)	Inoculum %	Reducing Sugar%	Ethanol%
1	100	1	36176x10^7 cells/ml	1	1.26	2
2	100	2	59272x10^7 cells/ml	2	1.43	2.5
3	100	3	74184x10^7 cells/ml	3	1.82	3.5
4	100	4	85616x10^7 cells /ml	4	2.21	4.0
5	100	5	98052x10^7 cells/ml	5	2.59	5.0

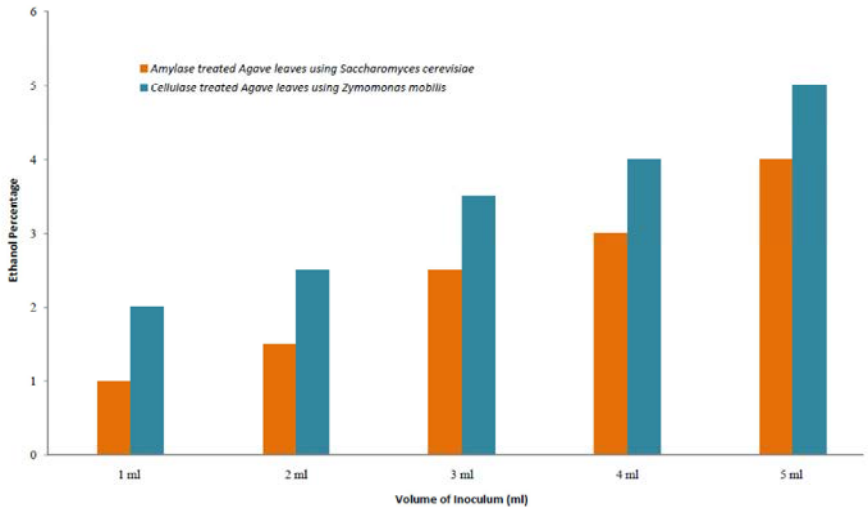

Fig. 1: Comparison of ethanol production from Agave leaves using *Saccharomyces cerevisiae* and *Zymomonas mobilis*

was found to be 1, 1.5, 2.5, 3.0 and 4.0%. The percentage of ethanol was found to be reduced in the amylase treated substrates when compared with cellulase treated substrates (Table 1 and 2 and Fig. 1).

DISCUSSION

In the recent years, production of ethanol using fermentation on a large scale has attained considerable interest. The economic feasibility, however always has been focused towards high yield of ethanol that is full use of raw material associated with high productivities so as to reduce the cost of production. The crude enzymes like amylase and cellulase were used for the enzymatic hydrolysis of biomass. In the hydrolysis process, few extract of biomass and whole biomass were treated with 5% of the crude enzymes and incubated at 37°C for 3 hrs and reaction was arrested by incubating at 4°C for 15 minutes. Purification of the crude enzymes and optimization parameters may give better result for degradation of starch or hemicelluloses and cellulose present in biomass [12].

The Maximum ethanol percentage of Amylase treated (*Saccharomyces cerevisiae*) agave leaves and cellulase treated (*Zymomonas mobilis*) agave leaves were found to be 4.0 and 5.0% respectively. This is due to the fact that *Zymomonas mobilis* organism has several advantages over yeast like higher rates of glucose uptake and ethanol production, higher ethanol yields and ethanol tolerance [15, 16]. Some of the reported advantages of *Zymomonas mobilis* over yeast are high sugar uptake and ethanol productivities on cell recycle systems, no oxygen requirement and genetic manipulation potential [17].

Bioethanol was produced from Agave leaves (*Aloe barbadensis*) using an enzymatic hydrolysis process, viz.enzyme hydrolysis (amylase and cellulase) followed by fermentation. Results showed that the Agave leaves containing *Zymomonas mobilis* yield maximum ethanol where as minimum ethanol is recorded with Agave leaves containing *Saccharomyces cerevisiae*. Further studies by optimizing certain parameters and by proceeding secondary distillation to produce pure form of ethanol using cheap raw materials and other sources are needed.

REFERENCES

1. Hill, J., E. Nelson, D. Tilman, S. Polasky and D. Tiffancy, 2006. Environmental, economic and energetic costs and benefits of biodiesel and ethanol biofuels. In the Proceedings of the Nat. Academy of Sci. USA, 103: 11206-11210.
2. Verma, G., P. Nigam, D. Singh and K. Chaudary, 2000. Bioconversion of starch to ethanol in a single step process by coculture of amylolytic yeast and S. cerevisiae. Bioresource Technology, 72: 261-266.
3. Quiseri, N. and G.J. Manderson, 1995. Bioconversion of renewable resources into ethanol: an economic evaluation of selected hydrolysis, fermentation and membrane technologies, Energy Sources, 17: 241-265.
4. Zaldivar, J., J.E. Nielsen and L. Olsson, 2001. Fuel ethanol production from lignocelluloses: a challenge for metabolic engineering and process integration, Applied Microbiology Biotechnology, 56: 17-34.
5. Mullan, O., P. Szakacs-Dobozi and D.E. Eveleigh, 1991. Identification of Saccharolytic enzymes of Zymomonas mobilis CP4. Biotechnology letters, 13: 137-142.
6. Garcia Reyes, B.R. and J.R. Rangel Mendez, 2009. Contribution of Agro waste material main components (hemicelluloses, cellulose and lignin) to the removal of chromium (III) from aqueous solution. Journal of Chemical Technology and Biotechnology, A1533-1538.
7. Lopez, M., M.N. Mancilla and G. Mendoza Diaz, 2003. Molecular Structures of Fructans from Agave tequilana Weber var.azul. J. Agri. Food Chem., 51: 7835-7840.
8. Cabrenes, C., J. Moreno and J.J. Mangas, 1990. Dynamics of yeast populations during cider fermentation in the Asturian region of Spain. Appl. Environ. Microbiol., 56: 3881-3884.
9. Aneja, K.R., 2002. Experiments in Microbiology, Plant pathology, Tissue culture and Mushroom production technology, New Age Publishers, New Delhi.
10. Varalakshmi, K.N., B.S. Kumudini, B.N. Nandini, J. Solomon, R. Suhas, B. Mahesh and A.P. Kavitha, 2009. Production and Characterization of α-amylase from Aspergillus niger JGI 24 Isolated in Bangalore. Polish Journal of Microbiology, 58: 29-36.
11. Miller, G.L., 1959. Use of dinitro salicylic acid reagent for determination of reducing sugars. Anal. Chem., 31: 426-429.
12. Prasad, M.P., Rekha sethi, M. Tamilarasan and K.S. Subha, 2009. Production of bioethanol using various Agricultural Raw Materials by two step Enzymatic Process. Advanced Biotechnology, pp: 41-43.
13. Buysse, J. and R. Merckx., 1993. An improved colorimetric method to quantify sugar content of plant tissue. J. Exp. Bot, 44: 1627-1629.
14. Somogyi, M., 1945. A New reagent for the determination of sugar. Journal of Biological Chemistry, 160: 61-68.
15. Lee, K.J., D.E. Tribe and P.L. Rogers, 1979. Ethanol production by Zymomonas mobilis in continuous culture at high glucose concentrations. Biotechnol. Letters, 1: 421-426.
16. Rogers, P.L., K.J. Lee and D.E. Tribe, 1980. High productivity ethanol fermentations with Zymomonas mobilis . Process Biochem., 15: 7-11.
17. Lavers, B.H., P. Pang, C.R. Mackenzie, G.R. Lawford, J. Pik and H.G. Lawford, 1980. Industrial alcohol production by high performance bacterial fermentation. In the proceedings of the 6th International Fermentation symposium, pp: 81.