A Study on Bio-Ethanol Production from Sugarcane Molasses by using Yeast Strain, *Saccharomyces cerevisiae*

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Abstract—*A renewable and environmentally friendly version of bio-ethanol (Bio-fuel) was obtained from sugarcane, a renewable resource.The objective of this study was to produce bio-ethanol from sugarcane molasses using yeast strain, Saccharomyces cerevisiae. Molasses was propagated by harvesting sugarcane in hot summer season. The molasses mash was then fermented for 20 hours under optimum temperature for obtaining ethanol. To determine the quantity of bio-ethanol the measurement of ethanol content was done with ebulliometer . This Bio-ethanol can be used in reducing dependence on foreign produced oil and in increasing the country's energy security as this was produced from domestically available energy sources.*

Keywords*: Bio-ethanol, Sugarcane, Molasses, Saccharomyces cerevisiae.*

1. INTRODUCTION

The never ending constant consumption of fossil fuels over time has led to severe depletion of reserves which in turn has led to increased cost of these fuels and this has also fuelled research into alternate sources and technologies which could replace or reduce the dependence on fossil fuels. A lot of research has been done for investigating the role of methane, hydrogen & ethanol as a possible alternate to fossil fuels. Among them ethanol has been found to be the most promising as a substitute for fossil fuels because it is sourced from renewable cultivable sources like sugarcane juice, molasses, cassava etc. Molasses are by-products of the industrial process which converts sugarcane & sugar beet into sugar, frequently employed in alcohol distilleries because of presence of large amounts of fermentative sugars like glucose, sucrose and fructose which are a good source of carbon for the metabolic requirement of the various microbes involved in the process [1]. Usually ethanol is blended with gasoline to give-Gasohol . A lot of in depth research has been conducted on various aspects of ethanol production [2]. This includes identification of efficient fermentative organisms, cost effective fermentation, substrates and the best environmental condition for fermentation to take place [3]. The cost of producing fuel grade ethanol is at present the biggest obstacle towards using ethanol as a commercial fuel. In this context the research into fermentation processes using economical carbon sources is absolutely vital [4].

2. METHODOLOGY

The study was carried out at Manav Rachna International University (Department of Biotechnology) in hot summer season of 2015.

2.1 Sugarcane Molasses

Sugarcane molasses were obtained from local sugar factory at Haryana and these acted as the carbon source for ethanol production.

2.2 The Clarification of Molasses

The clarification of molasses was done by adding 3 ml of concentrated sulphuric acid to to a kg of molasses mixed with a litre of distilled water to achieve a final pH of 4.5. After heating the mixture in a water bath to boiling for half an hour it was completed to 2.5 L then stored in a refrigerator overnight. After that the mixture was centrifuged and then sterilized at a temperature of 121 degree Celsius for 15 min duration. The concentration of sugar in the mixture was 23 % [5].

2.3. Yeast strain

The yeast strain of *S.cerevisiae* was collected from Microbiology laboratory, Manav Rachna International University.

2.4. Preparation of Inoculums

To prepare the inoculums conical flasks of 500 ml capacity were autoclaved and 200 ml of a medium containing (g/L) malt extract, yeast extract, peptone and sucrose was added. This was then steam sterilized at 121^{o} C for 15 minutes and

then cooled to room temperature. A loop of yeast strain *S.cerevisiae* was then inoculated and this preparation was incubated statically at 35^{o} C for 24hrs. After that it was transferred to flat round 1000 ml bottom flasks which contained 700 ml of sterilized molasses which were diluted to 3-4% (w/v) sugar content and supplemented with 0.1% yeast extract. The preparation was incubated overnight statically at 35^{o} C. Cultures of yeast were readied separately in seed fermenter. Molasses were diluted to 3-4% (w/v). The pH of the medium was adjusted using diluted sodium hydroxide and diluted sulphuric acid. This preparation was steam sterilized and then cooled to 30^{o} C ±2. Subsequently two flat round bottom flasks from this inoculum preparation were added and the seed fermenter was aerated to support the growth of yeasts [6].

2.5. Process for Fermentation

To provide the best environment for fermentation a batch culture system was used for the yeast strain which was shifted to fermenter. Initially a bed of 20% volume was made by yeast culture at the bottom of fermenter in molasses medium contained 5-6% sugars, supplemented with the parameters to be optimized, but afterwards feeding of diluted molasses gave final concentration 10% (w/v) The molasses were converted into ethanol by yeast fermentation using sugars (anerobic respiration). To optimise the conversion of molasses into ethanol the fermentation was continued for a period of 20 hours with the amount of molasses regulated such that fermenter vessels were filled to 80% of their capacity. The samples obtained after the fermentation was completed and were evaluated for ethanol content [6].

2.6. Estimation of Sugar Concentration

The concentration of sugar was arrived at by using the formula : Sugar Concentration (gm/L) = [(Dilution factor × Fehling factor)/Titrate value] × 100. Five ml of fermented sample was dissolved in 100mL of distilled water and mixed with 5mL of conc. HCL acid and was heated at 65^{o} C for a period of 15 min. The obtained sample was neutralized by adding NaOH and it was prepared to 1000 ml and taken into burette solution. The 5mL of Fehling A and 5mL of Fehling B were taken and mixed with 15mL of distilled water in a conical flask and methylene blue indicator was added. The conical flask solution was titrated with burette solution when it was boiled until disappearance of the indicator color.

2.7. Measurement of Ethanol Content

The content of bio-ethanol from the fermented samples was measured with ebulliometer.

3. RESULT AND DISCUSSION

The chemical composition of the yeast on basis of dry weight was protein 35.4% and mash 9.6%. Yeasts are a good source of vitamins and proteins. Being rich in protein yeast have been used to augment protein content in fish diet [7]. The most common yeast species used in the feed industry is *Saccharomyces cerevisiae*. It is typically fed in dairy cattle rations to alter rumen fermentation in an attempt to improve nutrient digestion and reduce the risk of rumen acidosis and improve animal performance [8]. And this is also a cheap nutritional supplement because they are produced industrially with little effort [9]. Rumen pH regulation is a key determinant in the maintenance of an optimal rumen function. Stabilization of rumen pH in the presence of live yeast has been reported [10]. The main advantages of the fermentation process are: High yield and Faster fermentation. It can be a batch or a continuous process.

In this study the yield of bio-ethanol was affected by sugar concentration, pH, temperature and the use of fermenters. An increase or decrease in any of this parameters affected productivity. The solution was transferred from conical flasks to fermenters because they provided optimum conditions for fermentation. It is also easier to control parameters like temperature which affect the growth of the culture in fermenter as compared to a flask. At every step optimum conditions had to ensure maximum yield [11].

A study done at TIET Patiala did in depth research into parameters affecting bio-ethanol yield and change in yield with change in parameters. our study is mostly in agreement with optimum conditions define in this study [12].

Skotnicki *et al* (1981) took 11 strains of *Zymomonas* and compared the rates of production of ethanol. Some strains were more tolerant to high temperature and high sugar than others. *Zymomonas mobilis* is a very good ethanol producing bacteria and this bacteria is also suitable for making palm wines to make palm wines [13].

Renu Bansal and R.S. Singh (2003) performed a study on ethanol production from molasses by comparing *Saccharomyces cerevisiae* and *Zymomonas mobilis* In their study they found that yeast was more ethanol tolerant rather than bacteria [14].

Our study showed that *S. cerevisiae* is a very potent agent for bio-ethanol production using molasses and that sugar concentration in these molasses affected the speed of the process. The ideal initial sugar concentration for ethanol production and fermentation efficiency was found to be 23% sugar in treated molasses and the optimum incubation temperature for this process was found to be 35^{o} C and the pH was 4.5. The ethanol concentration that was obtained in this study was 8.0% (v/v).

REFERENCES

[1] Jimenez, A. M. , Borja, R. and Martin, A. "A comparative kinetic evaluation of the anaerobic digestion of untreated molasses and molasses previously fermented with *Penicillium decumbens* in batch reactors", *Biochemical Engneering J*ournal, 18, 2004, pp. 121-132.

[2] Staniszewski, M., Kujawski, W. and Lewandowska, M., "Ethanol production from whey in bioreactor with Coimmobilized enzyme and yeast cells followed by preparative recovery of product-kinetic model predictions", *Journal of Food Eng*neering 82, 2007, pp. 618-625.

[3] Cysewski, G. R., Wilke, C. R. and Wilke, C. F., "Process design and economic studies of alternative fermentation methods for the production of ethanol", *Biotechnology and Bioenneering*, 20, 1978, pp. 1421-1427.

[4] Cazetta, M. L., Celligoi, M. A., Buzato, J. B. and Scarmino, I. S., " Fermentation of molasses by Zymomonas mobilis effects of temperature and sugar concentration on ethanol production", Bioresources Technology, 98, 2007, pp. 2824-2828.

[5] Amin, G. F. "Further studies on Bakers yeast" *M. Sc. Thesis*, Faculty of Agriculture, Cairo University, 1978.

[6] Fadel, M., Keera, A. A., Mouafi, F. E. and Kahil, T., "High Level Ethanol from Sugar Cane Molasses by A New Thermotolerant *Saccharomyces cerevisiae* Strain in Industrial Scale", *Biotechnology Research International, 2013*.

[7] Ballerini, D. and Thcmon, C., *Proceedings of OAPEC Symposium on Petroprotein, Organization of Arab Petroleum Exporting Countries (OAPEC)*, Safat, Kuwait, 1980.

[8] Gohl, B., "Tropical Feeds", *FAO/Oxford Computer Journals LTD*, Version 1–7, 1991.

[9] Otero, M. A., Guerrero, I. and Wagner, J. R., “Yeast and its derivatives as ingredients in the food industry", *Biotecnologia Aplicada*, 28, 4, 2011, pp. 272–275.

[10] Abd ELHalim, M. E., Omar, A., Nour, A. M. and Abd Ellatif, M. G., “Utilization yeast (*Saccharomyces cerevisiae*) in fish feeds,” in *Proceedings of the 2nd Alexandria Conference of Food Science and Technology*, 1995.

[11] Plevako, E. A. and Bakoshinskaya, O. A., "Microbiology, Chemistry and Technology control of yeast production", Food Industry, Moscow, Cited from Amin, 1964.

[12] Kadambini, G., Process Optimization for the Production of Ethanol via Fermentation, *M.Sc. Thesis*, Department of Biotechnology and Env. Sciences, Thapar Institute of Engg. & Technology, Patiala, 2006.

[13] Skotnicki, M.L., Lee, K. J.,Tribe, D. E. and Rogers, P. L. "Comparison of ethanol production by different *Zymomonas* strains", *Applied and Environmental Microbiol*, 1981, pp. 889-893.

[14] Bansal, R. and Singh, R. S., "A comparative study on ethanol production from molasses using *Saccharomyces cerevisiae* and *Zymomonas mobilis*," *Indian Journal Microbiology*, 43, 2003, pp.261- 264.