

Ethyl Alcohol Production by Fermentation of Diluted Sugarcane Molasses using *Saccharomyces Cerevisae* and its Optimization

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ABSTRACT

“India is a leading country in sugarcane production and hence has many sugar industries. Molasses is a by-product of these sugarcane industries. A large portion of Ethanol produced is obtained from these molasses. Main concern is to get the maximum yield of ethanol with an economical process. Purpose of this research was to find the optimum condition for maximum yield of ethanol by fermenting type B and type C molasses using a yeast strain- *Saccharomyces cerevisae*. Experiments were conducted where parameters like dilution ratio, temperature, pH of medium and fermentation period were varied at a time. We found that with specific gravity in the range of 50-55%(W/V) of molasses, temperature of around 30^o-33^oC, pH of 4.6 and fermentation period of 48 hours the yield of the ethanol was maximum.”

Keywords: Ethanol, Molasses, Fermentation, *Saccharomyces Cerevisae*.

1. Introduction

In the current scenario, ethanol is emerging as the best alternative to fossil fuels. It is used in automobiles as an alternative fuel. In present state of energy crises, efforts are being made to reduce the dependence upon non-renewable energy sources, one of which is fuel alcohol produced by fermentation of agricultural/agro industrial wastes and by-products. An efficient ethanol production requires four components: fermentable carbohydrates, an efficient yeast strain, a few nutrients and simple culture conditions.

The properties of ethanol stem primarily from the presence of its hydroxyl group and the shortness of its carbon chain. Ethanol's hydroxyl group is able to participate in hydrogen bonding, rendering it more viscous and less volatile than less polar organic compounds of similar molecular weight. Ethanol has slightly more refractive than water with a refractive index of 1.36242 (at $\lambda=589.3$ nm and 18.35°C) ^[1] Approximately 80% of world supply of alcohol is produced by fermentation of sugar and starch containing crops or by-products from

industries based on such crops. Among the widely used substrates for ethanol production are the molasses of sugarcane and sugar beet ^[2]. This is because they are ready for conversion with limited pre-treatments as compared with starchy or cellulosic materials. In India at present there are more than 285 distilleries which produces ethanol by fermentation process but the distillation to recover the ethanol is uneconomical. Optimisation of the process is important to produce ethanol at low costs. Of the important parameters that could affect ethyl alcohol fermentation are: type of molasses, dilution ratio, temperature and improvement in fermentation technology. As India is one of the largest sugarcane producing countries, molasses, a by-product of sugarcane industry available in plenty at cheap rate is mostly used as a raw material for fermentation. This study deals in basic factors for increasing the fermentation yield and productivity.

Many studies has been carried out for increasing the yield of ethanol. The supplementation of chitin in cane molasses (0.2%) fermentation containing 16% reducing sugars showed an enhanced rate of ethanol production. It is further observed that ethanol increased by 50%: more as compared to that in control, without any supplement or with the yeast extract supplement, at 37°C after a period of 36 hours ^[3]. Attempts are always made to improve the yield of ethanol by selecting the yeast strains that will give high yields of ethanol and work at moderate temperatures of 37°C-40°C at high substrate levels of sugars of around 25%. Andreason and Stieff ^[4] have increased the cell mass by the addition of ergo sterol in a defined medium, while Saigal and Viswanathan ^[5] have improved the rate of ethanol production by supplementation with vegetable oils and fatty acids.

2. Materials and Methods:

2.1 Materials

Molasses samples were obtained from a local sugar factory (Kumbhi Kasari Sugar Industry, Kolhapur). Yeast strain of *Saccharomyces Cerevisae* ^[6], sulphuric acid, glucose, peptone.

2.2 Evaluation of Molasses

Molasses used for study was blackstrap sugarcane molasses (Type C) having 83° brix containing approx 50% sugar concentration which we found out using brixometer. The pH of the sample was 5.

2.3 Molasses preparation for fermentation

3 samples of 100ml molasses was weighed and kept in clean washed beakers. Three samples of water having 400 ml, 500ml and 600ml were taken and were added to the molasses sample making dilution ratio of 1:4, 1:5 and 1:6 respectively. Sulphuric acid was added to maintain the pH at 4.6.

2.4 Preparation Of Yeast Culture

We prepared yeast culture in aerobic. We added 15ml distilled water and 5gm of *Saccharomyces Cerevisae* yeast strain along with 5gm of glucose and 2gm of peptone in a open flat dish. The growth was rapid and we obtained the yeast culture within 1 hour.



Fig. 1: Yeast Cultivation

2.5 Fermentation

We transferred the yeast culture to the prepared molasses sample for fermentation in a 1lit. conical flask. Various time period and temperature conditions were facilitated to different sample. Different samples were kept for different time period (1, 2 and 4 days).

2.6 Distillation

After the allotted time period the sample was filtered and tested for ethanol presence. Then the samples were transferred to the distillation setup where the ethanol was separated out.

2.7 Evaluation Of Product

After the basic confirmatory test for ethanol (like specific gravity and boiling point) we sent the sample for qualitative and quantitative analysis by Gas Chromatography (GC) method to Mumbai University Subcenter, Ratnagiri.

3. Results and Discussion

The main factors in the ethanol production that affects the yield of ethanol are dilution ratio, temperature, pH, fermentation period.

3.1 Effect of Dilution Ratio

100ml samples of molasses having 83° brix were diluted in the ratio of 1:4, 1:5 and 1:6 and other parameters were kept constant. The results obtained are given in the table below.

Molasses volume (ml)	Water added (ml)	Sugar content after dilution (°Brix)	pH	Temp. (°C)	Fermentation time (hours)	Yield% (w/v)
100	400	16.6	4.6	32	48	8.69
100	500	13.83	4.6	32	48	11.23
100	600	11.85	4.6	32	48	10.03

Table 1.1 Effect of Dilution Ratio

It was clear that when the sugar content in the molasses is between 13-14°brix, the yield obtained was maximum.

3.2 Effect of Temperature

Taking the parameters from above table which gave maximum yield and keeping the temperature constant this time the result obtained was, at 32°C the yield obtained is 11.19 that is maximum.

Molasses volume (ml)	Water added (ml)	Sugar content after dilution (°Brix)	pH	Temp. (°C)	Fermentation time (hours)	Yield% (w/v)
100	500	13.83	4.6	27	48	11.01
100	500	13.83	4.6	32	48	11.19
100	500	13.83	4.6	36	48	10.98

Table 1.2 Effect of Temperature

3.3 Effect of pH

The next parameter we changed was the pH of the fermentation medium. The medium was kept acidic but the pH were varied and the results obtained are tabulated below.

Molasses volume (ml)	Water added (ml)	Sugar content after dilution (°Brix)	pH	Temp. (°C)	Fermentation time (hours)	Yield% (w/v)
100	500	13.83	6.2	32	48	8.9
100	500	13.83	4.6	32	48	11.3
100	500	13.83	4	32	48	10.87

Table 1.3 Effect of pH

In this run we found that the yield was maximum when the pH was kept around 4.6 while keeping other parameters constant.

3.4 Effect of fermentation period

In this run, all the above parameters were kept constant varying only the time of fermentation and it was found that the complete fermentation of the sample requires minimum 48 hours (i.e.) 2 days. Increasing the further period for fermentation is unnecessary as it has no significant effect on the yield.

Molasses volume (ml)	Water added (ml)	Sugar content after dilution (°Brix)	pH	Temp (°C)	Fermentation time (hours)	Yield% (w/v)
100	500	13.83	4.6	32	24	10.12
100	500	13.83	4.6	32	48	11.26
100	500	13.83	4.6	32	96	11.17

Table 1.4 Effect of fermentation period

4 Conclusion

We conclude from this study that the optimum conditions and parameters for the maximum yield of ethyl alcohol are

- 4 Dilution ratio should be kept as 1:5.
- 5 Temperature should be kept at 32°C.
- 6 pH should be kept at 4.6.
- 7 Fermentation time should be 48 hours.

The maximum yield of ethanol obtained from above conditions is 11.3% (w/v) which was determined by Gas Chromatography^[7].

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