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Research Article

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Ethanol Fermentation from Molasses Using Free and Immobilized Cells of *Saccharomyces cerevisiae* [MTCC3090] - A Comparative Study

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ABSTRACT

Continuous depletion of fossil fuel reserves and consequent rise in the price demands some alternative technology to meet the global requirement of energy. According to the report published in the United Nations conference on trade and development, the demand of ethanol in India was projected for the year 2016-2017 as 965.30 million litres. The main objective of the present study was ethanol fermentation from molasses using Saccharomyces cerevisiae (MTCC3090) strain for biomass substrate conversion from both free and immobilized [sodium alginate] cells were made and their ethanol yield and corresponding sugar consumption was compared. During first few batches ethanol fermentation using immobilized cells was less in comparison to fermentation using free yeast cells, but if reused the immobilized cells for subsequent batches it showed gradual increase in sugar consumption and ethanol yield. Maximum ethanol production was given by immobilized yeast as 7.6% (in 3rd batch, 96 hrs each) and 10% from free cells. This study suggests that immobilized cells can be used to carry out sequential reuse cycles.

Keywords: Free yeast cells; Immobilization; Bioethanol; Molasses; Saccharomyces cerevisiae, Fermentation

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INTRODUCTION

In the era where every country is in a race to become super power and establish superiority over the world, proficient energy production and its utilization in various industries and transportation plays an important role. Since only few countries are sanctified with natural reserves of fossil fuels that too in limited amount. It is assumed that up till 2035 there will be one-third increase in the demand of such energy sources with India, the Middle East and China responsible for 60% of the increase at global level [3] and world will run out of fossil fuel by 2050-2075 if no more reserves are discovered. Continuous depletion of fossil fuel reserves and consequent rise in the price demands some alternative technology to meet the global requirement of liquid fuel. Demand of bioethanol in Indian economy is expected to grow at an yearly rate of 4.8% more than the next few decades [1]. Search for alternate source of clean and renewable source of energy ends with Bio-ethanol emerging as a clear winner among other sources. World shortly will attain its threshold value for energy disaster due to heavy increase use and demand of fossil fuels, because they are non-renewable. Biofuel predominantly bio-ethanol has achieved reputation in recent times due to a number of profits such as it reduces the emanation of pollutants, it is economically feasible, and it reduces the dependency non-renewable resources [4], it is used as chemical feed stock and solvent in large amounts in a variety of chemical industries[5]. Bio-ethanol exhibits low boiling point and produce negligible amount of any toxic compounds like carbon monoxides and nitrogen oxides on emission and is found to be ecofriendly [1]. It can be used as a fuel in vehicles with modified engines or by blending with petroleum for conventional engines. Due to all such positive aspects of Bio-ethanol, now its production has turn out to be a matter of research.

Bio-ethanol can be produced from various agricultural products like sugarcane, corn and waste products like molasses, wheat husk and rice husk. Cane molasses shows great potential for production of bio-ethanol at commercial level by conversion of sugar in presence of oxygen with strains of *Saccharomycess cerevisae. S.cerevisae* is most persuasive strain of yeast for the manufacture of ethanol [2].With the outlook to produce bio-ethanol and to achieve maximum productivity by using free and immobilised *S.cerevisae* cells from cheap raw material like cane molasses. Indian economy utilizes molasses as a major substrate for ethanol creation by fermentation. After Brazil, U.S. and China, India is fourth largest producer of ethanol, at an amount of 1,900 million litres having capacity for distillation as 2,900 million litres/annum [14]. Molasses is a by-product of sugar processing. Renovation of molasses to ethanol is possible alternative to maximize the use of molasses. Ethanol is comprehensively used as a motor fuel stabilizer. Carbon dioxide is an expected fermentation merchandise, but the off-gas can be sold as a high-quality raw material [16]. *S. cerevisiae* is mostly in use for ethanol production using renewable biomass such as sugar cane, sugar beet and molasses as carbon source because this strain exhibit attribute value for fermentation parameters, like its ability to ferment both in low sugar (5% of sugar) and in high sugar (30% of sugar). Among them, sugar-cane blackstrap molasses is a very useful raw material for that purpose, because it is not expensive and ample in the sugar industry [15].

MATERIALS AND METHODS

Feedstock

Molasses were purchased from local Sugar Mill [Phagwara, Punjab] and stored at −4°C until use.

Microorganism and maintenance

Strain of yeast *Saccharomyces cerevisiae* (MTCC3090) was used in this study. It can ferment increased amount of sugar in the medium and can tolerate high ethanol concentrations. [6] Yeast cultures were purely maintained on slants of yeast extract-peptone-dextrose (YPD) agar medium and incubated. Half of them was used as free cells and half were immobilized [9, 20].

Preparation of free yeast cells

Aseptically 1ml of yeast cell suspension was added to 50ml YPD and incubated at 160 rpm at 30°C for 24 hrs. Centrifuged (6000rpm, 15min) and Separated out the cells as pellet. The cells were washed with saline water. Re centrifuged for 5min to obtain final pellet. Air dried the pellet. Free cells were obtained.

Preparation of immobilized yeast cells

0.1M $CaCl_2$ solution was prepared and was kept for chilling at 4°C. 3% of sodium alginate solution was prepared using magnetic stirrer. To this slurry free yeast cells were added in 1:1 with constantly for even dispersion. For their hardening beads were stored at 4°C for 1 to 2 hrs [17]. The obtained diameter of beads was 4.4mm [19].

Pretreatment of molasses

After weighing molasses 0.5% v/v H_2SO_4 was added and kept at 80°C for 30 min then left overnight. Two layers were shaped, upper shiny black layer and lower yellowish brown (due to precipitation of trace metals). Upper shiny black layer was collected and autoclaved. It was used in fermentation medium with 15% sugar content [9, 18].

Fermentation procedure

Pretreated molasses (15% sugar) were used as a source of carbon, 1% peptone as nitrogen source for fermentation. It was streamed at 90°C in water bath for 15-20min. It was conducted in triplet for both free and immobilized cells. 6gm of immobilized beads were added in first set and 6gm free cells in second. Incubated at 30°C for 96 hrs. After every 24 hrs sugar consumption and ethanol yield was calculated and analyzed

Analytical methods:

Reducing sugar estimation assay: Amount of reducing sugar was estimated using Dinitrosalicylic acid [DNS] method [7]. The DNS reagent was prepared by dissolving 5 g of dinitrosalicylic acid in 250 ml of distilled water at 80ºC. At room temperature, 100 ml of NaOH 2 N and 150 g of potassium sodium tartarate-4-hydrate were added and the volume was completed with distilled water to 500 ml [8]. Sugar contents in the supernatant were determined by taking 1.0 ml of supernatant to which 2.0 ml of DNS reagent was added and control contained 1.0 ml distilled water and 2.0 ml of DNS. After cooling, 8 ml of distilled water was added in each test tube and absorbance was

noted at 546nm using spectrophotometer. Sugar concentration was determined from the standard curve of glucose. [9, 21]

Sugar consumption estimation: An empty flask was weighed then it was filled with water up to desired level and weight was noted. Further same flask was filled with sample up to desired level and weighted. Then subtracted the weight of empty flask from both the above to get absolute weight of water and sample. Then explored www.brewers.com/brix-converter/ to calculate the brix of sugar present in the sample, 1° of brix means 1gm of sugar present in 100ml of sample.

Ethanol concentration estimation: In the distillation apparatus vapoured the fermented sample at 70°C. Cooled the distilled alcohol and measured the volume percentage of alcohol using calibrated alcoholmeter.

RESULT

Pre-treatment efficiency

For the estimation of the efficiency of pre-treatment concentration of reducing sugar is calculated both after and before pre-treatment. Glucose concentration was found to be 0.09g/ml, further calculated as 9g/100ml. The ratio of glucose and fructose in molasses is 1:1. The sugar concentration obtained after performing DNS test was 18%, further maintained at 15% by dilution.

Rate of ethanol production from free and immobilized yeast cells

Ethanol production from both free and immobilized *Saccharomyces cerevisiae* [MTCC3090] was investigated after every 24 hours of first inoculation. For immobilized cells sugar consumption was found to be 3.5% providing a very low ethanol yield i.e. 0.3%, in first batch and in case of free cells after 24 hour ethanol yield was 1.3%, consuming sugar as 4.1% [figure 4]. For immobilized cells in first batch of 96 hours the final ethanol yield was found to be 4.6% with the sugar consumption of 7.3%. [Figure 1]

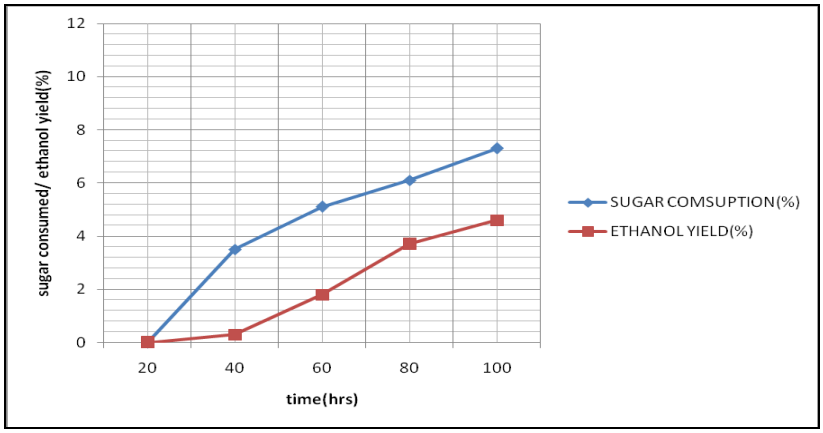

Figure 1: Graph of sugar consumption and ethanol yield for 1st batch of immobilized yeast cells

Reprocessing of immobilized yeast cells for ethanol production by free and immobilized yeast cells

The rate of ethanol production by immobilized *S. cerevisiae* cells was studied by reusing the cells up to three consecutive batches each of 96 hours. Consumption of sugar was more in second batch then first batch i.e. 9.3% in second and 7.3% in first batch, providing an ethanol yield as 5.8% in second batch [Figure 2]. For the third batch also same beads of yeast cells were used and highest ethanol yield was obtained i.e. 7.6%, with sugar consumption as 12.4% [Figure 3] [Table 1].

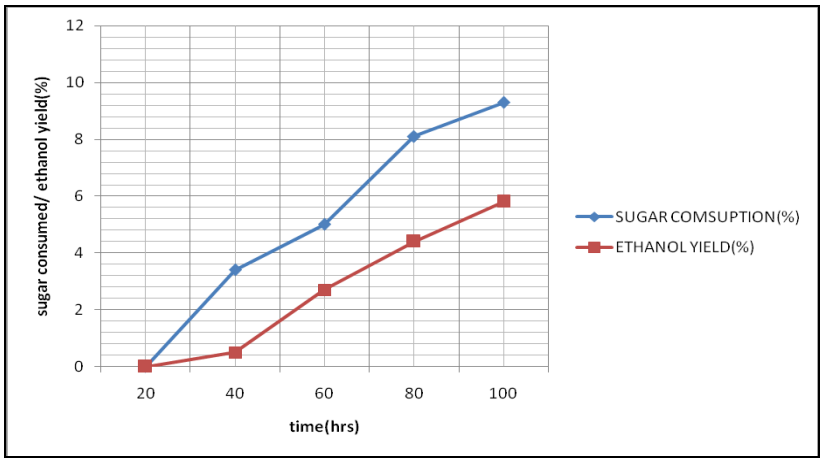

Figure 2: Graph of sugar consumption and ethanol yield for 2nd batch of immobilized yeast cells

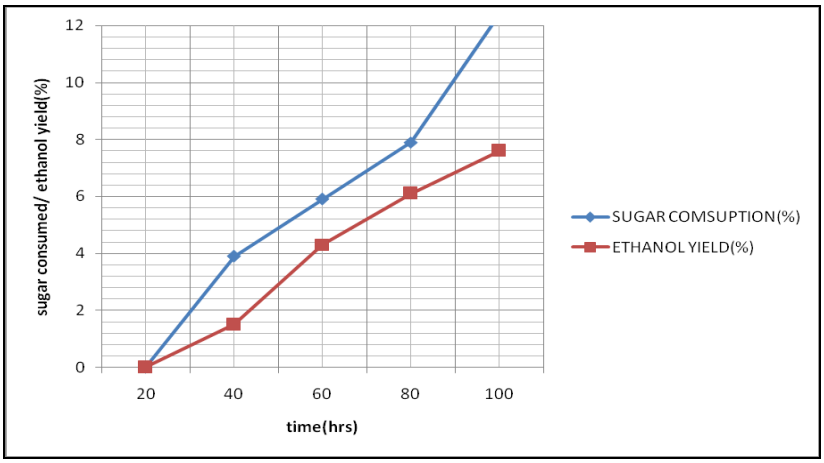

Figure 3: Graph of sugar consumption and ethanol yield for 3rd batch of immobilized yeast cells

Table 1: Comparison between 3 batches of immobilized yeast cells (reused in consecutive batches) for sugar consumption and ethanol yield rate

Batch No.	Time [hrs]/paramters [%]	0	24	48	72	96
1	Ethanol yield	0	0.3	1.8	3.7	4.6
	Sugar consumption	0	3.5	5.1	6.1	7.3
2	Ethanol yield	0	0.5	2.7	4.4	5.8
	Sugar consumption	0	3.4	5	8.1	9.3
3	Ethanol yield	0	1.5	4.3	6.1	7.6
	Sugar consumption	0	3.9	5.9	7.9	12.4

Rate of ethanol production from free yeast cells

In case of free yeast cells ethanol yield continuously increased after every 24 hours i.e. after 48 hours of incubation 3.1% of ethanol was obtained with 6.1% sugar consumption. After 72 hours sugar consumption was found as 7.3% providing 5.1% ethanol yield and on the final day after 96 hours ethanol yield was found as 10% with 9.5% sugar consumption. [Figure 4]

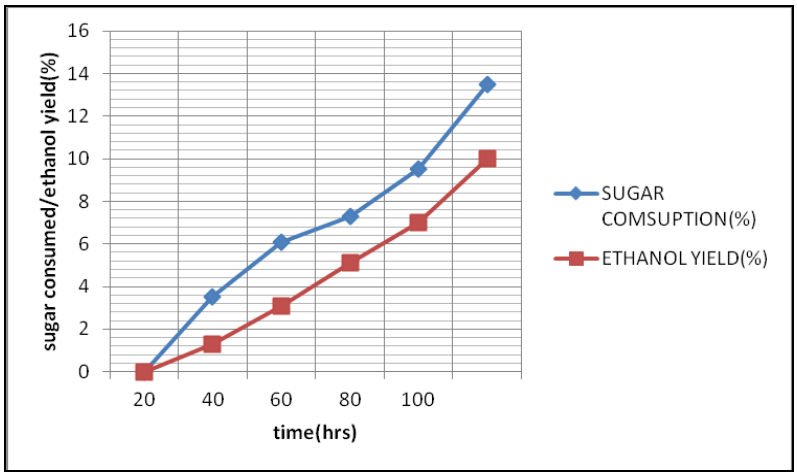

Figure 4: Graph of sugar consumption and ethanol yield for free yeast cells

DISCUSSION

In this study, *Saccharomyces cerevisiae* (MTCC3090) was used for the fermentation of molasses. The major parameter used during the line of study was recycling of immobilized yeast cells to observe high ethanol production rate. Further few other factors were taken under consideration such as incubation period with free and immobilized *S. cerevisiae*, pH, temperature of incubation, initial sugar concentration, initial volume of fermentation medium. [10]. In the present study, cane molasses were used as a carbon source for fermentation. Previous researches also reported that cane molasses provides consistent and enhanced yield of ethanol [11,12]. The main objective of using *Saccharomyces cerevisiae* for the fermentation as it is widely used for bio-ethanol production in industrial processes. If these yeast cells are given suitable environment and all essential nutrients in adequate amounts are provided, they can ferment amplified amount of sugars in the medium. It is also reported that under appropriate environment and nutritional condition, *S. cerevisiae* could produce and tolerate high ethanol concentrations. [13]

CONCLUSION

In conclusion, the present investigation utilizes molasses as carbon source adding advantages such as it is not only cheap instead in a industrial by product and has no environmental hazards also importance of some fermentation parameters in improving the alcoholic fermentation technology were investigated. In this study ethanol yield and corresponding sugar consumption was compared. When free cells of S. cerevisiae were utilized and incubation period of 96h at 30°C gave optimal fermentation efficiency in the first use of yeast. Free cells gave ethanol yield 10%. In case of immobilized cells, during first few batches ethanol fermentation using immobilized cells was less in comparison to fermentation using free yeast cells. When the same beads of yeast were reused for next few subsequent batches it showed gradual increase in sugar consumption as well as in ethanol yield, providing highest yield as 7.6% in third batch.

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