Optimum Initial Sugar Concentration for Efficient Mollasses Fermentation by *Saccharomyces cerevisiae*

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ABSTRACT: Molasses fermentation at elevated sugar concentrations has damaging effects on the yeast cell. Thus the identification of optimum concentration of sugar for efficient fermentation is essential. In the present study, two experiments were conducted using different molasses dilutions. Sugar concentrations used were 10, 15, 20, 25 and 30% (w/v), and 10, 12, 14, 16 and 18% (w/v) in the experiments. These solutions were inoculated with a pure strain of Saccharomyces cerevisiae and fermented for 96 hours. Alcohol percentage and sugar concentration were measured at 24 hrs. intervals.

In the first experiment sugar concentration of 20% and higher, reduced the fermentation efficiency. In the second experiment 14% sugar concentration had the lowest percentage of unfermented sugar (7.5% of the initial sugar content) and the highest fermentation recovery value (0.62 ml/g). Thus the molasses dilution containing 14% sugar would be the optimum for the strain of Saccharomyces cerevisiae used in the present study.

INTRODUCTION

Molasses is a by-product in the sugar industry. It is a treacle like syrup which is mainly used for ethanol production. The preparation of molasses for alcoholic fermentation basically consists of dilution with water to an appropriate sugar concentration, the adjustment of pH with sulphuric acid to 4.5 - 5.0 and a heat treatment to pasteurise the medium prior to its inoculation with yeast.

The formation of ethanol occurs by a number of well-documented metabolic pathways which varies according to the microorganism involved. Properly cultured and adopted strain of *Saccharomyces cerevisiae*, which has genetic stability and, rapid and relevant sugar fermentation ability is commonly used for molasses fermentation. Their ability to tolerate high

levels of ethanol, elevated incubation temperatures and concentrated sugar solutions (osmotic tolerance) is very important in efficient ethanol production (Stewart *et al.*, 1984).

Fermentation produces a dilute alcoholic solution, usually containing 7 – 10% v/v ethanol and lesser quantities of higher alcohols, esters, aldehydes *etc*. The ethanol is concentrated and purified by the process of distillation in alcohol distilleries (Adams *et al.*, 1982).

MATERIALS AND METHODS

Yeast culture

A pure culture of *Saccharomyces cerevisiae* isolated from baker's yeast was used for molasses fermentation. It was prepared from a single yeast colony using the streak plate method.

Preparation of pure culture by streak plate method

Selection of a single yeast colony from a master culture of *Saccharomyces cerevisiae* was accomplished by dipping a sterilised platinum needle point into the culture flask and streaking it over the surface of a sterile agar medium in a petridish. After several series of streaks are made, the point finally reached a level where only single cells are dislodged. Following incubation for about 48 hrs. at 25 C, colonies of cells were visible to the naked eye.

After microscopic and macroscopic examinations, four or five of the best isolated colonies which did not touch the adjacent colonies were selected, so as to ensure that selected colonies grew from a single cell. The selected colonies were used to inoculate fresh media in test tubes. After incubation for 1 – 2 days the best tube culture was selected and used to inoculate fresh tubes for use as the starting point in the production of pure cultures. Yeast propagation was done using molasses culture medium until the required volume obtained. The pH was maintained at 4.8 for both solid and liquid molasses culture media.

Fermentation study

Molasses obtained from the Sevanagala Sugar Development Project, Sevanagala was used in this study. The total sugar percentage of molasses was found to be approximately 62 by Lane and Eynon Method (AOAC 1979) and the specific gravity was 1.45. Two separate experiments replicated thrice were conducted.

In the first experiment, molasses dilutions were prepared with water so as to obtain five different sugar concentrations, namely, 10, 15, 20, 25 and 30%. Three replicates were used for each concentration and the replicate volume was 800 ml. Sterilized samples were inculated with pure culture of *Saccharomyces cerevisiae*. Cell concentration of the yeast culture used for inoculation of the fermentation mash was 2×10^8 cells/ml approximately and the inoculum was 1/10 of the fermentation mash.

Ammonium sulphate (0.5 g per litre) was used as a nitrogen source for molasses dilutions. The pH was adjusted to 4.8 using concentrated sulphuric acid.

Fermentation was carried out for a period of 96 hours at room temperature (30 C). Samples were taken at 24 hour intervals and alcohol percentages and remaining sugar concentrations were measured. The results of experiment 1 indicated that the optimum sugar concentration for efficient molasses fermentation to be between 10 and 20%. Hence in experiment 2, molasses was diluted, to obtain five different sugar concentrations, namely, 10, 12, 14, 16 and 18% (w/v). the experimental procedure was similar to that of experiment 1.

Analytical methods

Lane and Eynon method (AOAC – 1975) was used for sugar analysis. The volume of molasses solution required to completely reduce 10 ml mixed Fehling's solution was determined using methylene blue as the redox indicator for assessing the end point. Hydrochloric acid (6.34 N) was used for the inversion of sucrose before the titration. Alcohol percentage of the fermented mash were measured by the ebulliometer which is based on the boiling point of the sample (Joslyn, 1950).

RESULTS AND DISCUSSION

The changes in the alcohol percentage and sugar concentrations during 96 hours fermentation of the different molasses dilutions in experiment 1 are presented in Table 1. The data show that sugar concentrations of 20% and higher reduced the fermentation rate, as indicated by higher amounts of remaining unfermented sugars after 96 hr fermentation. The percentage of sugar remaining unfermented increased drastically above 20% initial sugar concentration. The values for 10, 15, 20, 25 and 30% sugar concentrations were 7.3, 7.3, 18.9, 26.5 and 45.8%, respectively. The results show that the use of sugar concentrations of more than 20% (w/v) in single batch fermentation is less efficient.

Results of experiment 2 show that the percentage of unfermented sugar was high in the molasses with 18% initial sugar concentration (Table 2). Almost 13% of the initial sugar content remained unfermented at this concentration. This confirms the results of experiment 1 and suggests that the use of high sugar concentrations lead to sugar wastage in distilleries where single-batch fermentation is employed.

The unfermented sugar percentages in molasses dilutions containing initial sugar concentrations of 14 and 16% were 7.5 and 10.5, respectively. But this loss may be acceptable when considered along with alcohol production. When the recovery of fermentation after 96 hrs. was considered, sugar concentration of more than 14% had the highest values (Table 3). Thus it is concluded that the molasses dilution containing 14% sugar concentration would be the optimum for the strain of *Saccharomyces cerevisiae* used for alcohol production in the present study.

Fermentation rate was very low after 72 hours (Figure 1 and 2). Thus in large scale industrial fermentation for more than 72 hours will be inappropriate.

Table 1. Changes of sugar concentration and alcohol percentage during fermentation in different molasses dilutions (experiment 1).

Initial Sugar Concentration (w/w) molasses dilutions	Time of Fermentation							
	24 hr.		48 hr.		72 hr.		96 hr.	
	Alcohol %	Total %	Alcohol %	Total %	Alcohol %	Total %	Alcohol %	Total %
10%	2.80	5.20	5.40	0.88	5.50	0.73	5.50	0.73
15%	3.50	9.12	6.90	3.41	8.10	1.68	8.40	1.10
20%	3.40	14.30	7.40	7.51	9.10	4.64	9.60	3.78
25%	2.70	20.5	7.30	12.7	9.90	8.51	11.00	6.63
30%	0.70	28.4	5.10	21.3	8.10	16.55	9.80	13.75

Table 2. Changes of sugar concentration and alcohol percentage during fermentation in different molasses dilutions (experiment 2).

Total Sugar Concentration (w/w)	Time of Fermentation							
	24 hr.		48 hr.		72 hr.		96 hr.	
	Alcohol %	Total %	Alcohol %	Total %	Alcohol %	Total %	Alcohol %	Total %
10%	2.93	4.26	4.73	1.71	5.36	0.85	5.51	0.65
12%	3.59	5.44	5.49	2.64	6.50	1.23	6.79	0.82
14%	3.76	7.40	6.36	3.50	7.59	1.78	8.03	1.05
16%	3.75	9.30	6.81	5.01	8.25	2.74	8.85	1.68
18%	3.75	11.44	7.15	6.30	8.81	3.84	9.65	2.41

Table 3. Fermentation recovery in different molasses dilutions after 4 days fermentation.

Initial Sugar Percentage (w/v)	Alcohol % (v/v)	Fermented Sugar% (w/v)	Fermentation Recovery (v/w).
10	5.51	9.35	0.589
12	6.79	11.18	0.607
14	8.03	12.95	0.620
16	8.85	14.32	0.618
18	9.65	15.59	0.618

a – volume of alcohol/wt. of sugar used.

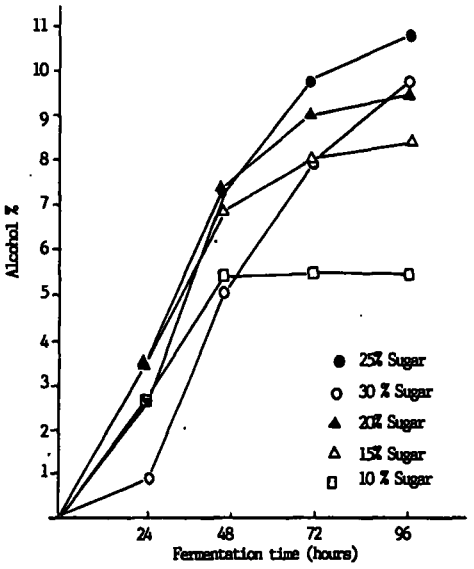

Fig. 1. Production of ethanol by Saccharomyces cerevisiae in molasses dilutions containing different sugar concentrations (experiment 1).

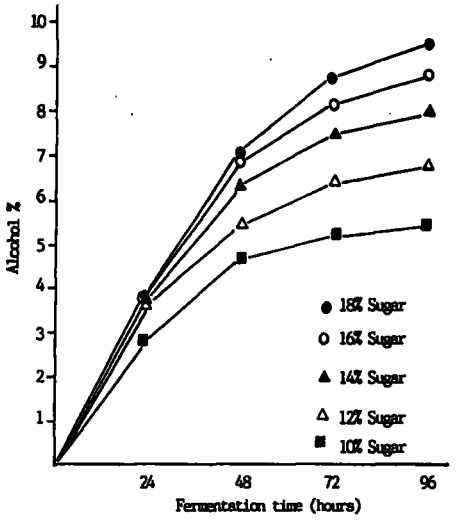

Fig. 2. Production of ethanol by Saccharomyces cerevisiae in molasses dilutions containing different sugar concentrations (experiment 2).

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