

Studies on alcoholic fermentation from sugar industry by product molasses exposed to ephedrine

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Abstract : The efficacy of ephedrine on microbial bioconversion of molasses to ethanol by *Saccharomyces cerevisiae* J-16 has been assessed. It has been found that the alkaloid under observation, i.e., ephedrine at 5×10^{-5} M molar concentration enhances the microbial bioconversion of molasses to ethanol by *Saccharomyces cerevisiae* J-16 to an extent of 18.9368% higher in comparison to control fermentor flasks, i.e., 6.02 ml/100mL in 55 hrs of incubation period, 5.1 pH and 30.5°C temperature with 25% (w/v) molasses solution.

(Keywords: Molasses, ethanol, ephedrine *Saccharomyces cerevisiae* J-16)

Introduction

The biological roles of alkaloids in the plants that constitute them are unknown, and it has been suggested that they performs no vital metabolic function, being merely by products of other more important pathways. Nevertheless, several examples are known in which they are of ecological importance, providing same survival value to the plant.¹ A survey of the literature reveals that a very few attempts have been made to study the influence of different alkaloids on the enzyme systems participating in the process of yeast fermentation. Although alkaloids are not essentially growth factor for the yeast yet certain alkaloids²⁻¹⁴ are utilized by the microbes for their stimulation. Also, since some alkaloids are known to be produced during some fermentation processes, it is obvious that such alkaloids are not toxic to the organisms involved. Although a few workers have tried to explore the influence of some alkaloids on fermentation processes, but

there is no definite opinion regarding the influence of alkaloids on enzyme systems and growth of microbes.

The influence of alkaloids on growth of microbes and stimulation of their enzymes has not been studied extensively¹⁵. They have not been used extensively in microbial processes due to, probably, the Toxic nature, but on the other hand alkaloids have been extensively used in the preparation of different life saving medicines in very small quantities, and a few alkaloids¹⁶⁻²² are also known to be produced during some fermentations. Thus, it is obvious that alkaloids are associated with biological systems and may not be poisonous or toxic always to the microorganisms.

Kligher³³ found that caffeine had a slight inhibitory action on several intestinal microbes and it is slightly more active at pH values 7.4 then it is at 6.2. Caffeine has been reported as a mutagenic compound in various biological processes and significant stimulating response has been observed by a number of workers^{2-6, 24, 25}.

The prominent success of the microorganisms as a distinguished agent for the controlled hydroxylation²⁶ of the steroid nucleus has stimulated research into the possibility of performing similar transformation in other complex molasses such as an alkaloids. In addition to the fundamental interest of such type of studies, it is expected that hydroxyl groups substituted alkaloids may possess desirable physiological activities²⁷⁻³⁴.

Table - 1
Studies on alcoholic fermentation from sugar industry byproduct
molasses exposed to ephedrine

Concentration of alkaloid used A X 10 ^{-x} M	Incubation Period in hours	Yield of ethanol* in ml/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in yield of ethanol after 55 hours in comparison to control.
Control				
(-) Alkaloid	55	6.02	1.475	—
1.0 × 10 ⁻⁵ M	55	6.10	1.395	+ 1.3289
(+) Alkaloid	55	6.39	1.105	+ 6.1461
3.0 × 10 ⁻⁵ M	55	6.70	0.796	+ 11.2956
(+) Alkaloid	55	6.90	0.593	+ 14.6179
5.0 × 10 ⁻⁵ M**	55	7.16***	0.338	+ 18.9368
(+) Alkaloid	55	7.10	0.393	+ 17.9401
7.0 × 10 ⁻⁵ M	55	6.95	0.545	+ 15.4485
(+) Alkaloid	55	6.20	1.295	+ 2.9900
9.0 × 10 ⁻⁵ M	55	5.80	1.695	— 3.6544
(+) Alkaloid	55	5.40	2.095	— 10.2990

* Each value represents mean of three trials. ** Optimum concentration of the alkaloid used.

*** Optimum yield of ethanol in 55 hours. (+) Values indicate % increase in the yield of ethanol in comparison to control. Experimental deviation (±) 2.5–3.5%.

Theophylline and theobromine both having structural similarities has been found useful in various biological fermentations⁷⁻⁸. Theophylline, chloro-theophylline and theobromine all bears a structural similarities to the physiologically active organic compounds like alloxan, parabanic acid and barbituric acid. It was further, established by Margalith and Pagani³⁵, Margalith³⁶ and Baxter³⁷ that alkaloids of general skeleton as in barbiturates were the most effective in industrial fermentation process.

The literature is inadequate on data pertinent to the participation of alkaloids on fermentation process and poisonous nature of the alkaloids. Since some alkaloids³⁸⁻⁴⁵ are known to be produced during some fermentation process it is obvious that such alkaloids being toxic are not toxic to the organisms involved in the fermentation bioprocess. The present study was undertaken to assess and analyse the microbial bioconversion of molasses to ethanol by *Saccharomyces cerevisiae* J-16 exposed to ephedrine.

Experimental

The influence of ephedrine on microbial bioconversion of molasses to ethanol by *Saccharomyces cerevisiae* J-16. The composition of the production medium for microbial bioconversion of molasses to ethanol by *Saccharomyces cerevisiae* J-16 is prepared as follows : Molasses : 25% (w/v), Malt-Extract : 0.3% , Yeast-Extract : 0.3%, Peptone : 0.5% Distilled water : To make up 100 ml, pH : 5.1, Distilled water was added to make up the volume up to '100 ml'. The pH of the medium was adjusted to 5.1 by adding requisite amount of lactic acid.

Now, the same production medium for ethanol production by *Saccharomyces cerevisiae* J-16 was prepared for 99 fermentor-flasks, i.e., each containing 100 ml of production medium. These fermentor-flasks were then arranged in 10 sets each comprising 9 fermentor-flasks. The remaining 9 fermentor-flasks out of 99 fermentor-flasks were kept as control and these were also rearranged in 3 subsets each consisting of 3 fermentor flasks.

Now, M/1000 solution of ephedrine was prepared and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 ml of this solution was added to the fermentor-flasks of first 10 sets respectively. The control fermentor-flask contained no ephedrine. The total volume in each fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of ephedrine in first, second, third, fourth, fifth, sixth, seventh,

eighth, ninth and tenth subsets were approximately as given below :

$A \times 10^{-x} \text{M}$, $1.0 \times 10^{-5} \text{M}$ to $10.0 \times 10^{-5} \text{M}$ respectively. Where, A= amount of ephedrine in ml, ie; from 1.0 ml to 10.0 ml.

x = molarity of the ephedrine solution.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 30.5°C and analysed colorimetrically after 33, 55, and 75 hours for ethanol⁴⁷ formed and molasses sugars⁴⁸ left unfermented.

Results and Discussion

The influence of ephedrine

The results recorded in the table-1 shows that the alkaloid ephedrine has stimulating effect at lower concentrations i.e., between $1.0 \times 10^{-5} \text{M}$ to $8.0 \times 10^{-5} \text{M}$ however, optimum concentration for maximum yield of alcohol was observed at $5.0 \times 10^{-5} \text{M}$. On increasing the concentration of ephedrine from $5.0 \times 10^{-5} \text{M}$ to $8.0 \times 10^{-5} \text{M}$ there was gradual fall in the production of alcohol but the yields obtained was comparatively higher than control. The concentrations of ephedrine between $9.0 \times 10^{-5} \text{M}$ to $10.0 \times 10^{-5} \text{M}$ causes comparatively more fall in the production of alcohol and was recorded to be 3.65% and 10.29% less comparatively in comparison to control flask in 55 hours of optimum incubation period. The maximum yield of alcohol in the presence of ephedrine was observed at $5.0 \times 10^{-5} \text{M}$ in 7.16 ml/100 ml in 55 hours of optimum incubation period which was 18.9368% higher in comparison to control 6.02 ml/100 ml.

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