

Search on microbial bioconversion of molasses to ethanol exposed to coptisine

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Abstract : The effect of coptisine on microbial bioconversion of molasses to ethanol by *Saccharomyces cerevisiae* A - 25 has been examined. The compound coptisine is a natural protoberberine alkaloid found in plants like *Coptis chinensis* which has shown potential in various applications including fermentation technology. It has been observed that the alkaloid coptisine an isoquinoline alkaloid is stimulatory for alcoholic fermentation processes and upgraded the yield of ethanol to an extent of 21.300% higher in comparison to control, i.e, 6.15ml / 100ml under optimised parameters like pH, temperature, incubation period and concentration of molasses solution.

(Keywords : Coptisine, EtOH).

Introduction

Coptisine is an alkaloid found in chinese gold thread¹, greater celandine and opium² famous for the bitter taste that it produces. It is used in chinese herbal medicine along with the related compound berberine for digestive disorders caused by bacterial infections³. Similar to berberine and palmatine coptisine is an isoquinoline alkaloid desired from *Rhizoma coptidis* with many pharmacological activities including anti - inflammatory, antibacterial, anti - atherosclerosis and anti - tumor properties.

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 plant⁴. 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 alkaloids⁵⁻¹⁷ 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 extensively¹⁸. 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 alkaloids¹⁹⁻²⁵ 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. Caffeine has been reported as a mutagenic compound in various biological

processes and significant stimulating response has been observed by a number of workers. The prominent success of the microorganisms as a distinguished agent for the controlled hydroxylation 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 activities. Theophylline and theobromine both having structural similarities has been found useful in various biological fermentations. Theophylline, chloro-theophylline and theobromine all bears a structural similarities to the physiologically active²⁶⁻³⁶ organic compounds like alloxan, parabanic acid and barbituric acid. It was further, established by Margalith and Pagani³⁷, Margalith³⁸ and Baxter³⁹ 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 alkaloids⁴⁰⁻⁴⁸ 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* A-25 exposed to coptisine.

Experimental

The influence of coptisine on microbial bioconversion of molasses to ethanol by *Saccharomyces cerevisiae* A-25. The composition of the production medium for microbial bioconversion of molasses to ethanol by *Saccharomyces cerevisiae* A-25 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* A-25 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 coptisine 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 coptisine. The total volume in each fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of coptisine in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :

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

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 30.5°C and analysed colorimetrically after 58 hours for ethanol⁴⁹ formed and molasses sugars⁵⁰ left unfermented.

Results and Discussion

The results recorded in the table 1 shows that the alkaloid under trial, i.e coptisine has stimulatory effect at all concentrations used to study the effect of the alkaloid. It has been observed that the lower as well as higher concentrations of coptisine is most beneficial for

Table - 1
Studies on microbial conversion of molasses to ethanol exposed to coptisine.

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 58 hours in comparison to control.
Control	58	6.15	1.456	–
1.0 × 10 ⁻⁵ M	58	6.24	1.366	+1.463
2.0 × 10 ⁻⁵ M	58	6.35	1.256	+3.252
3.0 × 10 ⁻⁵ M	58	6.93	0.676	+12.682
4.0 × 10 ⁻⁵ M	58	7.10	0.510	+15.447
5.0 × 10 ⁻⁵ M	58	7.29	0.319	+18.536
6.0 × 10 ⁻⁵ M**	58	7.46***	0.148	+21.300
7.0 × 10 ⁻⁵ M	58	7.15	0.458	+16.260
8.0 × 10 ⁻⁵ M	58	6.72	0.888	+9.268
9.0 × 10 ⁻⁵ M	58	6.47	1.138	+5.203
10.0 × 10 ⁻⁵ M	58	6.25	1.360	+1.626

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

*** Optimum yield of ethanol in 58 hours. (+) Values indicate % increase in the yield of ethanol in comparison to control. Experimental deviation (+) 2.5–3.5%.

the production of ethanol. The effect of coptisine at molar concentration of 6.0 x 10⁻⁵M has been found most significant and effective for the optimum production of ethanol, i.e 21.300% higher in comparison to control, i.e 6.15ml / 100ml when all parameters were optimised, i.e 25% molasses solution (W/V) malt and yeast extract 0.3% each

, peptone 0.5%, pH 5.1 and incubation period 58 hours. On increasing the concentration of coptisine from 7.0 x 10⁻⁵M to 10 x 10⁻⁵ M it has been found in regular fall production of ethanol and finally it reached to 1.626% in comparison to control.

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