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Bioconversion of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM -1154 exposed to cetyl (hexadecyl) trimethyl ammonium bromide (CTAB)

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Abstract: The efficacy of micelle, i.e., cetyl (hexadecyl) trimethyl ammonium bromide (CTAB), on bioconversion of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM -1154 has been assessed. It has been found that the cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) under trial has stimulatory effect on bioproduction of ethanol and enhances the yield of ethanol to an extent of 8.695% higher in comparison to control fermentor flasks, i.e., 8.05 ml/100ml in 55 hours of optimum incubation period, 4.2 pH and 33^{0}C temperature with 22% (w/v) molasses solution.

(**Key words :** Molasses, ethanol fermentation, micelles, cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) and *Saccharomyces cerevisiae* NCIM -1154)

Introduction

A micelle or micella is an aggregate of surfactant molecules dispersed in a liquid colloid. The molecules (surfactants) are amphiphilic, which means having spatially distinct polar and nonpolar parts. The micelles are in rapid equilibrium with single molecules (or ions), commonly referred to as monomers.[1-10] Micelles either accelerates or retards the organic reactions depending on its nature[11-20]. It is assumed that micelles are moderators of enzyme actions in some biological systems[21-23]. There are several known micelles, but a very few micelles have been used in submerged fermentation processes[24-39]. Since micellar effect on fermentation studies especially alcoholic fermentation is relatively new and almost unexplored, it needs careful and specific experimentations. In the present investigation the authoress has made an attempt to study the effect of cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) on ethanol fermentation by *Saccharomyces cerevisiae* NCIM -1154.

Experimental

The influence of cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) on bioproduction of ethanol by *Saccharomyces cerevisiae* NCIM -1154.

Saccharomyces cerevisiae

The composition of the production medium for bioproduction of ethanol by *Saccharomyces cerevisiae* NCIM -1154 has been prepared as follows :
Molasses : 22% (w/v), Malt extract :0.40%, Yeast extract 0.40%, Peptone : 0.60%, $(NH_4)_2HPO_4$: 0.30%, pH : 4.2

The pH of the production medium was adjusted to 4.2 by adding requisite amount of lactic acid and this pH was also ascertained by a pH meter. The above composition medium represents

volume of a fermentor flask, i.e., "100ml" production medium for bioproduction of ethanol by *Saccharomyces cerevisiae* NCIM -1154. Now, the same production medium for bioproduction of ethanol by *Saccharomyces cerevisiae* NCIM -1154 was prepared for 99-fermentor flask, i. e; each contained '100ml' of production medium.

The above 99-fermentor flasks were then arranged to 11-sets each comprising of 9-fermentor flasks. Each set was then rearranged in 3-subsets, each consisting of 3-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.

After preparing the above sets of fermentor flasks M/1000 solution of cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) was prepared and from the above cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) solution 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 ml was added to the fermentation flasks of above 1st to 10th sets respectively. The control fermentor flasks contained no cetyl (hexadecyl) trimethyl ammonium bromide (CTAB).

Now, the total volume in each fermentor flasks was made upto 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

A x 10^{-x} M, i.e.,

1.0×10^{-5}M	6.0×10^{-5}M
2.0×10^{-5}M	7.0×10^{-5}M
3.0×10^{-5}M	8.0×10^{-5}M
4.0×10^{-5}M	9.0×10^{-5}M
5.0×10^{-5}M	10.0×10^{-5}M

Table - 1
Bioproduction of ethanol exposed to cetyl (hexadecyl) trimethyl ammonium bromide (CTAB)

Concentration of cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) used a x 10^{-5}M	Incubation period in hours	Yield of ethanol* in ml/100ml	Molasses* left unfermented in g/100 ml	% difference in the yield of ethanol in 55 hours
Control	55	8.05	2.305	–
1.0 x 10^{-5} M	55	8.17	2.998	+1.490
2.0 x 10^{-5} M	55	8.27	2.900	+2.732
3.0 x 10^{-5} M	55	8.43	2.818	+4.720
4.0 x 10^{-5} M	55	8.51	2.800	+5.714
5.0 x 10^{-5} M	55	8.59	2.790	+6.708
6.0 x 10^{-5} M**	55	8.75***	2.750	+8.695
7.0 x 10^{-5} M	55	8.60	2.785	+6.832
8.0 x 10^{-5} M	55	8.42	2.790	+4.596
9.0 x 10^{-5} M	55	****	–	–
10.0 x 10^{-5} M	55	****	–	–

* Mean of three observations; ** Optimum concentration of cetyl (hexadecyl) trimethyl ammonium bromide (CTAB)
*** Optimum yield of citric acid; (+) ve values indicate % increase in the yield of citric acid
Experimental deviation (±) 1.5 to 3.5%; **** Insignificant values

A = amount of cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) , in ml, i.e., 1.0 ml ..... to 10 ml.

x= Molarity of the cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) solution

The above fermentor flasks were then sterilized, cooled inoculated and incubated at 33^{0}C and analysed after50, 55 and 60 hours for ethanol[40] formed and molasses[42] left unfermented.

Results and Discussion

The influence of cetyl (hexadecyl) trimethyl ammonium bromide (CTAB),

The table -1 shows that the cetyl (hexadecyl) trimethyl ammonium bromide (CTAB), has been found significantly stimulating for bioenergetic conversion of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM -1154. The maximum yield of alcohol in the presence of cetyltrimethylammonium bromide [CTAB] was observed at 6.0 x 10^{-5}M (8.75ml/100 ml) in 55 hrs of optimum incubation period which was found to be 8.695% higher in comparison to control flasks in the same time course of fermentation processes. The higher concentration of cetyltrimethyl-ammonium bromide [CTAB] were not much favourable for bioenergetic conversion of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM -1154 So, the gradual addition by cetyltrimethylammonium bromide [CTAB] after the concentration of 7.0 x 10^{-5} M gradually slow down the bioenergetic conversion of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM -1154. However, at all the concentrations of cetyltrimethylammonium bromide [CTAB] used, the bioenergetic conversion of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM -1154 has been found slight higher in comparison to the control fermentor-flasks.

The micelles are long chain molecules containing twelve and more member of carbon atoms in the hydrocarbon tail with a head group. The surfactant cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) is surface active and lowest the surface tension of water when dissolved in it. Surfactants undergo self-assembly thus cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) also undergo self assembly, which depends on the chemical structure and nature of the media specially production medium containing different bioingredients. Opposite forces of repulsion between polar head groups (charged) and attraction between the hydrocarbon chains of the surfactants especially cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) are responsible for self-assembly and may also influence the enzyme activity of the reaction. Due to a balance between these hydrophilic and hydrophobic interactions, a specific structure stabilises.

However, micellar mechanism of fermentative enzyme action is not known and a little work has been done regarding the influence of micelles on fermentation technology. The use of micellar systems for enzymes-catalysed reactions specially in fermentation technology may vigorously stimulate the enzyme action and thereby enhances the yield of different fermentative production. The structure of micellar system is grossly similar to that of protein and their chemical composition is infinitely less complex. However, micelles may be taken for trial for different microbial synthesis as it may be proved to be an important biological tool in the field of fermentation technology specially in alcoholic fermentation.

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