

Ethanol bioproduction by yeast *Saccharomyces cerevisiae* NCIM-2232 exposed to ethanesulfonylchloride

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Abstract: Alcoholic fermentation is a complex biochemical process during which yeasts convert sugars to ethanol, carbon dioxide, and other metabolic byproducts that contribute to the chemical composition and sensorial properties of the fermented foodstuffs. Alcoholic fermentation is the basis for the manufacturing of alcoholic beverages such as wine and beer. The effect of ethanesulfonylchloride on bioproduction of ethanol by yeast *Saccharomyces cerevisiae* NCIM-2232 has been assessed. It has been found that the mutagen, i.e., ethanesulfonylchloride under observation has stimulatory effect on bioproduction of ethanol by yeast *Saccharomyces cerevisiae* NCIM-2232 and enhances the yield of ethanol to an extent of 6.76328% higher in comparison to control flat bottom fermentor flasks, i.e., 6.21 ml/100mL in 50 hrs of incubation period, 4.25 pH and 30°C temperature with 18% (w/v) molasses solution.

(Keywords: Molasses, ethanol, mutagens, ethanesulfonylchloride, *Saccharomyces cerevisiae* NCIM-2232)

Introduction

Alcoholic fermentation is a biotechnological process accomplished by yeast, some kinds of bacteria, or a few other microorganisms to convert sugars into ethyl alcohol and carbon dioxide. In this fermentation process, yeast is mostly used as a bio-culture and aqueous solution of monosaccharide (raw materials) as the culture media for the production of beverages. In the alcoholic fermentation process, yeast generally carries out the aerobic fermentation process, but it may also ferment the raw materials under anaerobic conditions.

Chemical mutagens are standard tools for mutagenesis in a variety of organisms, and they are a primary means of creating mutations in phenotype-based screens in most genetic systems.¹⁻⁶ Early studies by Ames showed around 90% of known carcinogens which can be identified in Ames test as mutagenic and 80% of the mutagens identified through Ames test may also be carcinogens.⁷ Mutagens are not necessarily carcinogens, and vice versa. Sodium Azide for example may be mutagenic (and highly toxic), but it has not been shown to be carcinogenic.⁸⁻¹⁴

Literature survey reveals that a very little work has been done on ethanol bioproduction by yeast *Saccharomyces cerevisiae* NCIM-2232 exposed to chemical mutagens, therefore, the author has employed ethanesulfonylchloride on ethanol bioproduction by yeast *Saccharomyces cerevisiae* NCIM-2232.

Experimental

The composition of production medium for the ethanol bioproduction by yeast *Saccharomyces cerevisiae* NCIM-2232 is prepared as follows :

Molasses : 18 % (w/v), Malt-Extract : 1.25%
Yeast-Extract : 1.25%, Peptone: 1.25%, Distilled water : To make up 100 ml pH: 4.25.

The pH of the medium was adjusted to 4.25 by adding requisite amount of lactic acid.

The same production medium for ethanol

Table - 1
Ethanol production by yeast *Saccharomyces cerevisiae* NCIM-2232
exposed to ethanesulfonylchloride

Concentration of mutagen 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 in comparison to control after 50 hours
Control	45	5.67	3.21735	—
(-) Mutagen	50	6.21	2.45943	—
	55	6.03	2.21689	—
1.0 × 10 ⁻⁵ M	45	5.70	3.18789	+0.52910
(+) Mutagen	50	6.32	2.34943	+1.77133
	55	6.12	2.12689	+1.49253
2.0 × 10 ⁻⁵ M	45	5.75	3.13741	+1.41093
(+) Mutagen	50	6.38	2.28951	+2.73752
	55	6.18	2.06680	+2.48756
3.0 × 10 ⁻⁵ M	45	5.81	3.07759	+2.46913
(+) Mutagen	50	6.45	2.21986	+3.86473
	55	6.20	2.04683	+2.81923
4.0 × 10 ⁻⁵ M	45	5.87	3.01728	+3.52733
(+) Mutagen	50	6.50	2.16926	+4.66988
	55	6.25	1.98756	+3.64842
5.0 × 10 ⁻⁵ M**	45	5.98	2.90781	+5.46737
(+) Mutagen	50	6.63***	2.03948	+6.76328
	55	6.40	1.84689	+6.13598

fermentation with yeast *Saccharomyces cerevisiae* NCIM-2232 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 ethanesulfonylchloride 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 to 10th sets respectively. The control fermentor-flask contained no mutagen. The total volume in each fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

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

A × 10^{-x}M,
 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

Where, A = amount of mutagen in ml, ie;
 from 1.0 ml to 10.0 ml.
 and x = molarity of the solution respectively.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 30^o C and analysed colorimetrically after 45, 50, and 55

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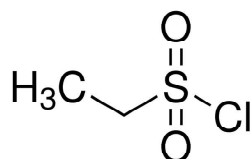
Concentration of mutagen used A X 10 ^{-x} M	Incubation Period in hours	Yield of ethanol* in ml/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in yield of ethanol in comparison to control after 50 hours
Control	45	5.67	3.21735	—
(-) Mutagen	50	6.21	2.45943	—
	55	6.03	2.21689	—
6.0 × 10 ⁻⁵ M	45	5.93	2.95759	+4.58553
(+) Mutagen	50	6.57	2.09986	+5.78710
	55	6.36	1.88687	+5.47263
7.0 × 10 ⁻⁵ M	45	5.81	3.07764	+2.46913
(+) Mutagen	50	6.46	2.20956	+4.02576
	55	6.23	2.01689	+3.31674
8.0 × 10 ⁻⁵ M	45	5.71	3.17778	+0.70546
(+) Mutagen	50	6.39	2.27957	+2.89855
	55	6.14	2.10683	+1.82421
9.0 × 10 ⁻⁵ M	45	****	—	—
(+) Mutagen	50	****	—	—
	55	****	—	—
10.0 × 10 ⁻⁵ M	45	****	—	—
(+) Mutagen	50	****	—	—
	55	****	—	—

* Each value represents mean of three trials. ** Optimum concentration of the chemical mutagen used.
 *** Optimum yield of ethanol in 50 hours. (+) Values indicate % increase in the yield of ethanol in comparison to control. Experimental deviation (±) 1.5–3%.

hours for ethanol¹⁵ formed and molasses sugars¹⁶ left unfermented.

Results and Discussion

The influence of ethanesulfonylchloride on ethanol bioproduction by yeast *Saccharomyces cerevisiae* NCIM-2232.



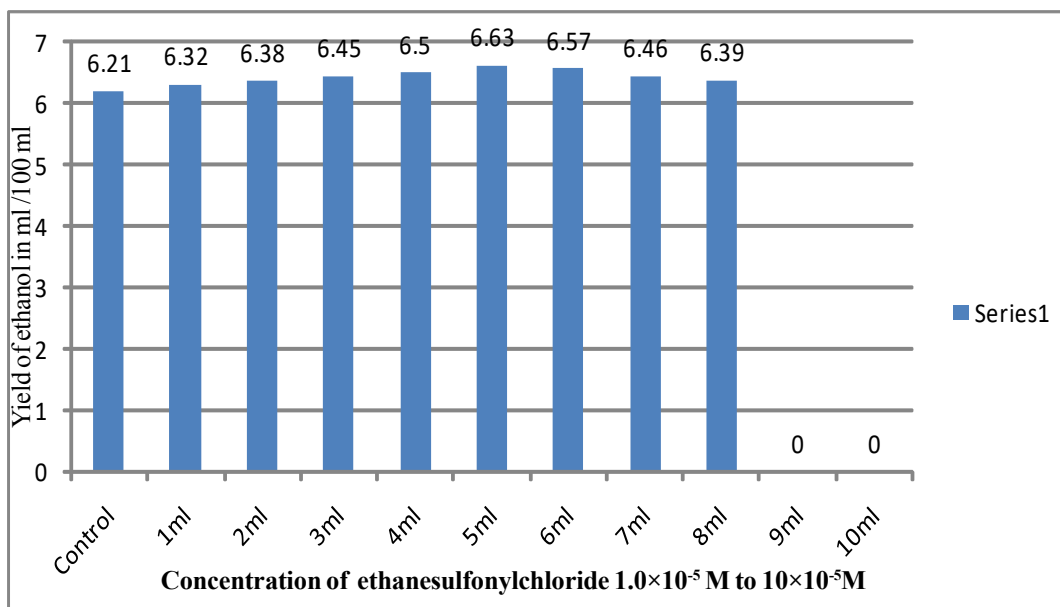
Ethanesulfonylchloride

The data recorded in the table-1 shows that ethanesulfonyl chloride also has stimulatory effect on ethanol bioproduction by yeast *Saccharomyces cerevisiae* NCIM-2232.

The data (table-1) reveals that the chemical mutagen ethanesulfonyl chloride stimulates the ethanol bioproduction by yeast *Saccharomyces cerevisiae* NCIM-2232 and enhances the yield of ethanol upto its (ethanesulfonylchloride) concentrations from 1.0 x 10⁻⁵ M to 8.0x10⁻⁵ M in two phases :

In the first phase, ie; from 1.0 x 10⁻⁵ M to 5.0 x 10⁻⁵ M the effect of ethanesulfonylchloride

Table - 1
Ethanol production by yeast *Saccharomyces cerevisiae* NCIM-2232
exposed to ethanesulfonylchloride



on the productivity (yield) of ethanol was gradually in increasing order and attains its best function at 5.0×10^{-5} M where maximum yield of ethanol, ie; 6.63ml/100 ml is obtained in 50 hours of optimum incubation period which is 6.76328% higher in comparison to control fermentor flasks (6.21 ml/100 ml).

In the second phase of mutagenic chemical effect the molar concentration, ie; from 6.0×10^{-5} M to 8.0×10^{-5} M the production of ethanol has been enhanced but the order of ethanol productivity is reversed in respect to increasing molar concentrations of ethanesulfonylchloride. However, the ethanol formation with the yeast *Saccharomyces cerevisiae* NCIM-2232 under the influence of each concentration of ethanesulfonylchloride used has been found stimulating and the yield of ethanol has been found higher than that obtained in the control fermentor flasks. In both the phases the order of productivity and % of ethanol formed after 50 hrs is as under :

Concentration of ethanesulfonylchloride from 1.0×10^{-5} M to 5.0×10^{-5} M

Productivity of ethanol: 1.77133%, 2.73752%, 3.86473%, 4.66988% and 6.76328%.

Concentration of ethanesulfonylchloride from 6.0×10^{-5} M to 8×10^{-5} M.

Productivity of ethanol : 5.79710%, 4.02576 and 2.89855%,

Concentration of ethanesulfonylchloride at 9.0×10^{-5} M and 10.0×10^{-5} M

Productivity of ethanol : Insignificant value.

The exposure on the yeast strain *Saccharomyces cerevisiae* NCIM-2232 to ethanesulfonyl chloride may produce a variety of effects. Depending upon the concentration of ethanesulfonyl chloride to which the yeast strain *Saccharomyces cerevisiae* NCIM-2232 were exposed may effect disruption of cells, precipitation of cell protein, inactivation of enzymes and leakage of amino acids from the cells. Although the special mode of action is not very clear, there is a consensus that the lethal effect is

associated with physical damage of the membrane structure of the cell surface, which initiates further deterioration.

- chloride at lower concentrations is stimulatory and at higher concentrations is deterioratory for the ethanol formation with the yeast *Saccharomyces cerevisiae* NCIM-2232 .

Thus, it is concluded that ethanesulfonyl

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