

Bioethanol production by *S. cerevisiae* AK-22 exposed to 2-ethylphenylhydrazine hydrochloride

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Abstract : The influence of chemical mutagen, i.e., 2-ethylphenylhydrazine hydrochloride on bioethanol production by *Saccharomyces cerevisiae* AK-22 has been assessed. It has been found that the chemical mutagen, i.e., 2-ethylphenylhydrazine hydrochloride at its molar concentration of 6.0×10^{-5} M has stimulatory effect on bioethanol production and enhances the yield of bioethanol to an extent of 20.113% higher in comparison to control fermenter flask, i.e. 5.27mL/100mL while molar concentration of 2-ethylphenylhydrazine hydrochloride under trial at 7.0×10^{-5} M and onwards inhibits and retards the bioethanol production. The molar concentration of 2-ethylphenylhydrazine hydrochloride has been employed in between 1.0×10^{-5} M to 10×10^{-5} M and has been found that at initial concentration, i.e., 1.0×10^{-5} M it is least effective and at higher concentrations it gives insignificant yield of ethyl alcohol. Experimental parameters has been optimized viz. : 32°C temperature, 4.6 pH, 50hrs incubation period with 16.5% (w/v) molasses solution alongwith other nutritional ingredients required by the yeast *Saccharomyces cerevisiae* AK-22 .

(Key words: Molasses, 2-ethylphenylhydrazine hydrochloride and yeast *S. cerevisiae* AK-22)

Introduction

Treatment with mutagenic agents can increase mutation frequencies by order of magnitude¹⁻². The influence of different chemical mutagens in different fermentations process has been investigated by a number of workers³⁻¹³. A large number of chemical mutagens are recorded as a good agent for different microbes and microbial processes¹⁴⁻²⁰.

Thus, from the above brief review it is

evident that chemical mutagens are required for genetic manipulation and exploitation specially for alcoholic fermentation and in view of this the author has studied the influence of 2-ethylphenylhydrazine hydrochloride on alcoholic fermentation by *S. cerevisiae* AK-22

Experimental

The influence of 2-ethylphenylhydrazine hydrochloride on bioethanol production by *S. cerevisiae* AK-22. The composition of production medium for the bioethanol production by *Saccharomyces cerevisiae* AK-22 is prepared as follows :

Molasses : 16.5 % (w/v), Malt-Extract: 0.300%
Yeast-Extract : 0.300%, Peptone : 0.300%, Distilled water : To make up 100 ml, pH : 4.6

Distilled water was added to make up the volume up to '100 ml'. The pH of the medium was adjusted to 4.6 by adding requisite amount of lactic acid.

Now, the same production medium for bioethanol production by *Saccharomyces cerevisiae* AK-22 was prepared for 99 fermentor-flasks, i.e., each containing 100 ml of the 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 solutions of 2-ethylphenylhydrazine hydrochloride was prepared and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0

Table - 1
Bioethanol production by *Saccharomyces cerevisiae* AK-22
exposed to 2-ethylphenylhydrazine hydrochloride

Concentration of mutagen used $A \times 10^{-x}$ M	Incubation Period in hours	Yield of bioethanol*in ml/100ml	Molasses* left unfermented in g/100 ml	% Diff. in the yield of bioethanol in 50 hrs
Control	48	3.28	3.852	—
	50	5.27	2.893	—
	52	4.10	2.739	—
1.0×10^{-5} M	48	3.29	3.842	—
	50	5.30	2.865	(+) 0.569
	52	4.13	2.756	—
2.0×10^{-5} M	48	3.36	3.773	—
	50	5.42	2.743	(+) 2.846
	52	4.26	2.634	—
3.0×10^{-5} M	48	3.41	3.723	—
	50	5.49	2.673	(+) 4.174
	52	4.29	2.539	—
4.0×10^{-5} M	48	3.50	3.632	—
	50	5.65	2.513	(+) 7.210
	52	4.45	2.411	—
5.0×10^{-5} M	48	3.73	3.410	—
	50	6.02	2.145	(+) 14.231
	52	4.95	2.054	—

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

*** Optimum yield of bioethanol in 50 hours. (+) Values indicate % increase in the yield of bioethanol after 50 hours. Experimental deviation ($\pm$) 1.5-3%.

Contd.....

ml of this solution was added to the fermentor-flasks of first 10 sets respectively. The control fermentor-flask contained no chemical mutagens. The total volume in each fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of 2-ethylphenylhydrazine hydrochloride in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :

$A \times 10^{-x}$ M, 1.0×10^{-5} M to 10.0×10^{-5} M

Where, A = amount of chemical mutagens in ml, i.e.; from 1.0 ml to 10.0 ml.

x = molarity of the solution.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 32^o C and analysed colorimetrically after 48, 50 and 52 hours for bioethanol formed²¹ and molasses²² pollutant left unfermented.

Results and Discussion

The influence of 2-ethylphenylhydrazine hydrochloride on bioethanol production by *Saccharomyces cerevisiae* AK-22

The data recorded in the table-1 shows that the chemical mutagen, i.e., 2-ethylphenylhydrazine hydrochloride has been found stimulatory for the bioethanol production

Concentration of mutagen used $A \times 10^{-x}$ M	Incubation Period in hours	Yield of bioethanol*in ml/100ml	Molasses* left unfermented in g/100 ml	% Diff. in the yield of bioethanol in 50 hrs
Control	48	3.28	3.852	—
	50	5.27	2.893	—
	52	4.10	2.739	—
6.0×10^{-5} M**	48	3.93	3.210	—
	50	6.33***	1.833	(+) 20.113
	52	5.19	1.730	—
7.0×10^{-5} M	48	3.67	3.463	—
	50	5.91	2.254	(+) 12.144
	52	4.80	2.145	—
8.0×10^{-5} M	48	3.47	3.663	—
	50	5.60	2.563	(+) 6.261
	52	4.56	2.436	—
9.0×10^{-5} M	48	3.37	3.762	—
	50	5.44	2.723	(+) 3.225
	52	4.38	2.632	—
10.0×10^{-5} M	48	3.31	3.825	—
	50	5.33	2.833	(+) 1.138
	52	4.17	2.730	—

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

*** Optimum yield of bioethanol in 50 hours. (+) Values indicate % increase in the yield of bioethanol after 50 hours. Experimental deviation ($\pm$) 1.5-3%.

by *Saccharomyces cerevisiae* AK-22 at all its concentrations, i.e; from 1.0×10^{-5} M to 10.0×10^{-5} M in two phases.

In the first phase the molar concentration of 2-ethylphenylhydrazine hydrochloride, i.e; from 1.0×10^{-5} M to 6.0×10^{-5} M is significant and the productivity of bioethanol gradually increases till a maximum is reached at 6.0×10^{-5} M, i.e.; 6.33ml/100ml in 50 hours of optimum incubation period which is 20.113% higher in comparison to control, i.e; 5.27ml/100ml.

In the second phase of mutagenic effect, i.e., at 7.0×10^{-5} M and onwards molar concentration of 2-ethylphenylhydrazine hydrochloride the bioethanol production has been increased but in lesser quantum in the presence of 2-

ethylphenylhydrazine hydrochloride. So, the gradual addition of 2-ethylphenylhydrazine hydrochloride after the optimum concentration of 6.0×10^{-5} M gradually slows down the bioethanol production by *Saccharomyces cerevisiae* AK-22 on the part of % increase at 7.0×10^{-5} M, 8.0×10^{-5} M, 9.0×10^{-5} M and 10.0×10^{-5} M molar concentration of 2-ethylphenylhydrazine hydrochloride is as follows: 12.144%, 6.261%, 3.225% and 1.138% respectively.

From the present investigation it is obvious that the chemical mutagens used, i.e., 2-ethylphenylhydrazine hydrochloride is useful for bioethanol bioproduction by *Saccharomyces cerevisiae* AK-22 and therefore, it can be employed for the improved/enhanced yield of alcohol.

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