

Bioproduction of ethanol by *Saccharomyces cerevisiae* NCIM-3256 exposed to N-acetylbenzidine

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Abstract :-The effect of N-acetylbenzidine on alcoholic fermentation by SmF technique has been studied. It has been found that the mutagen under trial, i.e., N-acetylbenzidine has stimulatory effect on alcoholic fermentation and enhances the yield of ethanol to an extent of 5.563% higher in comparison to control fermentor flasks, i.e., 7.01 ml/100mL in 50 hours of optimum incubation period, 5.1 pH and 27°C temperature with 24% molasses solution.

(Key words : Ethanolic fermentation, N-acetyl benzidine and *Saccharomyces cerevisiae* NCIM-3256)

Introduction

The chemical or physical agents that cause mutations are called mutagens. Radiation exerts its mutagenic effect either directly or by creating free radicals that in turn have mutagenic effects. Radiation and free radicals can lead to the formation of bonds between nitrogenous bases in DNA. Individuals affected by this condition are abnormally sensitive to light and are more prone to skin cancer than normal individuals¹⁻⁵. The effect of chemical mutagen in fermentation biotechnology specially lactic acid, citric acid and alcoholic fermentation has been studied extensively.⁶⁻¹² A large number of chemical mutagens have been employed to generate the mutants of different microbes but still there are some chemical mutagens whose influence on alcoholic fermentation by *Saccharomyces cerevisiae* NCIM- 3256 species have not been studied¹³⁻⁴¹.

The present study was undertaken to assess and analyse the alcoholic fermentation by *Saccharomyces cerevisiae* NCIM- 3256 exposed

to N-acetylbenzidine mutagenic chemical.

Experimental

The effect of N-acetylbenzidine on bioproduction of ethanol by submerged fermentation technique by *Saccharomyces cerevisiae* NCIM-3256. The composition of production medium for the bioproduction of ethanol by submerged fermentation technique by *Saccharomyces cerevisiae* NCIM-3256 is prepared as follows :

Molasses solution : 24%, Malt extract : 1.25%, Yeast extract : 1.25%, Peptone : 1.25%, (NH₄)₂HPO₄ : 1.25%, 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 alcoholic fermentation by the yeast *Saccharomyces cerevisiae* NCIM-3256 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 N-acetylbenzidine 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

Table - 1
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Concentration of mutagen used $A \times 10^{-x}$ M	Incubation Period in hours	Yield of alcohol* in ml/100 ml	Blackstrap molasses sugars* left unfermented in g/100 ml	% Difference in yield of alcohol in comparison to control after 50 hrs
Control	40	4.85	2.68	-
(-) Mutagen	50	7.01	1.20	-
	60	6.85	1.18	-
1.0×10^{-5} M	40	4.98	2.65	-
(+) Mutagen	50	7.05	1.18	+0.570613409
	60	6.88	1.16	-
2.0×10^{-5} M	40	5.00	2.62	-
(+) Mutagen	50	7.08	1.16	+0.998573466
	60	6.88	1.14	-
3.0×10^{-5} M	40	5.00	2.60	-
(+) Mutagen	50	7.08	1.15	+1.711840228
	60	6.90	1.12	-
4.0×10^{-5} M	40	5.02	2.58	-
(+) Mutagen	50	7.13	1.14	+2.710413694
	60	6.92	1.10	-
5.0×10^{-5} M	40	5.10	2.55	-
(+) Mutagen	50	7.32	1.12	+4.422253922
	60	6.96	1.08	-
6.0×10^{-5} M**	40	5.14	2.50	-
(+) Mutagen	50	7.40***	1.10	+5.563480741
	60	7.05	1.05	-
7.0×10^{-5} M	40	5.20	2.45	-
(+) Mutagen	50	7.45	1.08	+6.276747503
	60	7.10	1.02	-
8.0×10^{-5} M	40	5.18	2.46	-
(+) Mutagen	50	7.43	1.09	+5.991440798
	60	7.09	1.04	-
9.0×10^{-5} M	40	5.16	2.48	-
(+) Mutagen	50	7.38	1.11	+5.278174037
	60	7.08	1.06	-
10×10^{-5} M	40	5.13	2.51	-
(+) Mutagen	50	7.35	1.13	+4.85021398
	60	7.06	1.09	-

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

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 2-aminoethanethiol in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :

$a \times 10^{-x}M$,	$6.0 \times 10^{-5}M$,
$1.0 \times 10^{-5}M$,	$7.0 \times 10^{-5}M$
$2.0 \times 10^{-5}M$,	$8.0 \times 10^{-5}M$
$3.0 \times 10^{-5}M$,	$9.0 \times 10^{-5}M$
$4.0 \times 10^{-5}M$,	$10.0 \times 10^{-5}M$
$5.0 \times 10^{-5}M$,	

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 27°C and analysed colorimetrically⁴² after 40, 50, and 60 hours for ethanol formed.

Results and Discussion

The influence of N-acetylbenzidine on bioproduction of alcohol by submerged fermentation technique by *Saccharomyces cerevisiae* NCIM-3256.

The data recorded in the table-I shows that the chemical mutagen, i.e; N-acetylbenzidine

has also been found stimulatory for the biosynthesis of alcohol by submerged fermentation technique by *Saccharomyces cerevisiae* NCIM-3256 at all its molar concentrations, ie; from $1.0 \times 10^{-5}M$ to $10.0 \times 10^{-5}M$ in two phases.

In the first phase the molar concentration of chemical mutagens i.e; N-acetylbenzidine from $1.0 \times 10^{-5}M$ to $7.0 \times 10^{-5}M$ is significant and the production of alcohol gradually increases till a maximum value is reached at $7.0 \times 10^{-5}M$, i.e; 7.45 ml/100ml in 50 hours of optimum incubation period which is 6.276747503% higher in comparison to control, i.e; 7.01 ml/100ml.

In the second phase of chemical mutagenic effect, i.e., at $8.0 \times 10^{-5}M$ and onwards the molar concentration of N-acetylbenzidine on biosynthesis of alcohol by submerged fermentation technique by *Saccharomyces cerevisiae* NCIM-3256 has been increased but in lesser quantum in the presence of N-acetylbenzidine. So, the gradual addition of N-acetylbenzidine after the optimum concentration of $7.0 \times 10^{-5}M$ gradually slows down the biosynthesis of alcohol by submerged fermentation technique by *Saccharomyces cerevisiae* NCIM-3256 and the part of % increase at $8.0 \times 10^{-5}M$, $9.0 \times 10^{-5}M$ and $10.0 \times 10^{-5}M$ molar concentration of N-acetylbenzidine is as follows: 5.991440798%, 5.278174037% and 4.85021398% respectively.

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