

Studies on biotic production of bioethanol exposed to phenelzine

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Abstract : In the present communication the efficacy of some chemical mutagen, i.e., phenelzine for production of bioethanol by some microbial strains such as *Thermoanaerobacter matheranii* NT-166, *T. pseudoethanolicus* NT-120, *T. pentosaceus* NT-132, *Zymomonas mobilis* NT-145 and *Saccharomyces cerevisiae* NT-138 has been assessed. It has been noted that *Saccharomyces cerevisiae* NT-138 has been found most successful bioethanol producer. It has been found that the chemical mutagen, i.e; phenelzine has stimulatory and significant effect on bioethanol production and enhances the yield of bioethanol to an extent of 16.183% higher in comparison to control, i.e., 6.55ml/100 ml at 6.0×10^{-5} M molar concentration of phenelzine when 22.5% (w/v) molasses solution is allowed to ferment for 55 hrs of optimum incubation period, 4.8 pH, and 33.5°C temperature along with some other nutritional ingredients required by the fungal strain under trial.

(Keywords : Bioethanol, fermentation, mutagen, phenelzine and *Saccharomyces cerevisiae* NT-138)

Introduction

A mutagen is a substance or agent that causes DNA impairment that results in the alteration of the DNA sequence. This alteration of the DNA sequence is known as mutation. Any agent causing mutation is called mutagen. Mutagens can be physical mutagens, chemical mutagens, or biological mutagens. The ability of a substance to induce the alterations in the base pairs of DNA or mutation is known as mutagenicity. Mutagens can be classified into 3 types based on their origin. They are as follows: Physical mutagens: These include ionizing radiation, such as X-rays, gamma rays and alpha

particles. Ultraviolet radiations can also behave as potential mutagens. Chemical mutagens: Elements such as arsenic, nickel and chromium are considered to be mutagens.¹⁻⁹

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.¹⁰⁻¹⁶ Literature survey reveals that a very little work has been done on bioethanol production by yeast *Saccharomyces cerevisiae* NT-138 exposed to chemical mutagens, therefore, the authoress have employed phenelzine on bioethanol production by yeast *Saccharomyces cerevisiae* NT-138

Experimental

The influence of phenelzine on biotic production of bioethanol from molasses by *Saccharomyces cerevisiae* NT-138. The constitution of production medium for the biotic production of bioethanol from molasses by *Saccharomyces cerevisiae* NT-138 is prepared as follows :

Molasses Solution : 22.5 % (w/v)
Malt-Extract : 1.25%
Yeast-Extract : 1.25%
Peptone : 1.25%
Distilled water : 10 ml
To make up 100 ml
pH : 4.8

Distilled water was added to make up the volume up to '100 ml'. The pH of the medium was

adjusted to 4.8 by adding requisite amount of lactic acid.

Now, the same production medium for fermentative bioproduction of ethanol from molasses by *Saccharomyces cerevisiae* NT-138 was prepared for four hundred forty one (441) fermentor-flasks, i.e., each containing hundred ml of production medium.

These fermentor-flasks, i.e., each containing hundred mL of production medium. These fermentor-flasks were then arranged in twenty sets each comprising twenty one fermentor-flasks. The remaining twenty one fermentor-flasks out of four hundred forty one fermentor-flasks were kept as control and these were also rearranged in seven subsets each consisting of three fermentor flasks.

Now, M/1000 solutions of phenelzine was made and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0 mL of this solution was added to the fermentor-flasks of first twenty 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 phenelzine in 1st, 2nd, 3rd, 4th, ... 20th subsets were approximately as given below:

$$A \times 10^{-x}M, \\ 0.5 \times 10^{-5} M \text{ to } 10.0 \times 10^{-5} M$$

Where, A = amount of mutagen in ml, ie; from 0.5 ml to 10.0 ml.
and x = molarity of the solution respectively.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 33.5°C and analysed colorimetrically after 25, 30, 45, 55, 60,

65 and 70 hours for bioethanol¹⁷ formed and molasses sugars¹⁸ left unfermented.

Results and Discussion

The influence of phenelzine on biotic production of bioethanol by *Saccharomyces cerevisiae* NT-138.

The data given in the table-1 shows that the mutagen, i.e ; phenelzine has been found stimulatory for biotic production of bioethanol by *Saccharomyces cerevisiae* NT-138. From the data given in the table-1, it is obvious that phenelzine influences the ethanolic fermentation process in different phases. The main characteristics of the ethanol formation exposed to phenelzine is as follows:

(i) Phenelzine is stimulatory at its all molar concentrations used during course of the bioethanol formation by the yeast *Saccharomyces cerevisiae* NT-138, i.e; from $0.5 \times 10^{-5} M$ to $10.0 \times 10^{-5} M$.

(ii) The molar concentration of chemical mutagen, i.e.; phenelzine from $0.5 \times 10^{-5} M$ to $6.0 \times 10^{-5} M$ influences the yield of bioethanol in approximately regular increasing order after each stage; i.e, 1.068%, 1.526%, 1.984%, 3.053%, 3.511%, 6.259%, 6.717%, 9.312%, 9.770%, 14.198%, 14.503% and 16.183%.

(iii) The other molar concentrations of phenelzine i.e; $6.5 \times 10^{-5} M$ to $10.0 \times 10^{-5} M$ influences the yield of bioethanol in almost fast decreasing manner and could give the yield of bioethanol with major difference, i.e., 15.114%, 8.091%, 7.022%, 5.190%, 4.427%, 1.984%, 0.763% and 0.458% respectively. However, the maximum yield of bioethanol has been found at $6.0 \times 10^{-5} M$ concentration of phenelzine, i.e., 7.61ml/100 ml which is 16.183% higher in comparison to control fermentor flasks, i.e. 6.55/ 100 mL.

Table -1 Biotic production of bioethanol exposed to phenelzine

Concentration of Mutagen used A X 10 ^{-x} M	Incubation Period in hours	Yield of bioethanol* in ml/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in yield of bioethanol after 55 hours in comparison to control.
Control	25	2.73	6.728	-
	30	3.26	6.195	-
	45	3.89	5.568	-
	55	6.55	4.440	-
	60	6.00	4.319	-
	65	5.47	4.002	-
	70	4.36	3.856	-
0.5 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	3.91	5.547	-
	55	6.62	4.373	+ 1.068
	60	6.02	4.237	-
	65	****	-	-
	70	****	-	-
1.0 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	3.93	5.528	-
	55	6.65	4.343	+ 1.526
	60	6.04	4.235	-
	65	****	-	-
	70	****	-	-
1.5 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	3.95	5.509	-
	55	6.68	4.312	+2.984
	60	6.06	4.225	-
	65	****	-	-
	70	****	-	-
2.0 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	3.98	5.488	-
	55	6.75	4.244	+ 3.053
	60	6.09	4.143	-
	65	****	-	-
	70	****	-	-
2.5 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	4.00	5.458	-
	55	6.78	4.212	+ 3.511
	60	6.10	4.117	-
	65	****	-	-
	70	****	-	-
3.0 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	4.05	5.408	-
	55	6.96	4.031	+6.259
	60	6.15	4.012	-
	65	****	-	-
	70	****	-	-

* Each value represents mean of three trials. ** Optimum concentration of the chemical mutagen used. *** Optimum yield of bioethanol in 55 hours. (+)Values indicate % increase in the yield of ethanol in comparison to control. Experimental deviation (±) 1.5–3%. **** Insignificant value

$3.5 \times 10^{-5} \text{M}$	25	****	-	-
	30	****	-	-
	45	4.06	5.398	-
	55	6.99	4.000	+ 6.717
	60	6.16	3.095	-
	65	****	-	-
$4.0 \times 10^{-5} \text{M}$	70	****	-	-
	25	****	-	-
	30	****	-	-
	45	4.09	5.368	-
	55	7.16	3.832	+ 9.312
	60	6.18	3.723	-
$4.5 \times 10^{-5} \text{M}$	65	****	-	-
	70	****	-	-
	25	****	-	-
	30	****	-	-
	45	4.10	5.356	-
	55	7.19	3.004	+9.770
$5.0 \times 10^{-5} \text{M}$	60	6.20	3.701	-
	65	****	-	-
	70	****	-	-
	25	****	-	-
	30	****	-	-
	45	4.18	5.278	-
$5.5 \times 10^{-5} \text{M}$	55	7.48	6.810	+ 14.198
	60	6.25	6.705	-
	65	****	-	-
	70	****	-	-
	25	****	-	-
	30	****	-	-
$6.0 \times 10^{-5} \text{M}^{**}$	45	4.19	5.268	-
	55	7.50	3.493	+ 14.503
	60	6.26	3.339	-
	65	****	-	-
	70	****	-	-
	25	****	-	-
$6.5 \times 10^{-5} \text{M}$	30	****	-	-
	45	4.25	5.209	-
	55	7.61**	3.378	+16.183
	60	6.30	3.286	-
	65	****	-	-
	70	****	-	-
$7.0 \times 10^{-5} \text{M}$	25	****	-	-
	30	****	-	-
	45	4.10	5.356	-
	55	7.08	3.909	-
	60	6.20	3.815	-
	65	****	-	-
$7.5 \times 10^{-5} \text{M}$	70	****	-	-
	25	****	-	-
	30	****	-	-
	45	4.10	5.356	-
	55	7.08	3.909	-
	60	6.20	3.815	-
$8.0 \times 10^{-5} \text{M}$	65	****	-	-
	70	****	-	-
	25	****	-	-
	30	****	-	-
	45	4.10	5.356	-
	55	7.08	3.909	-
$8.5 \times 10^{-5} \text{M}$	60	6.20	3.815	-
	65	****	-	-
	70	****	-	-
	25	****	-	-
	30	****	-	-
	45	4.10	5.356	-
$9.0 \times 10^{-5} \text{M}$	55	7.08	3.909	-
	60	6.20	3.815	-
	65	****	-	-
	70	****	-	-
	25	****	-	-
	30	****	-	-

* Each value represents mean of three trials. ** Optimum concentration of the chemical mutagen used. *** Optimum yield of bioethanol in 55 hours. (+)Values indicate % increase in the yield of ethanol in comparison to control. Experimental deviation ($\pm$) 1.5–3%. **** Insignificant value

7.5×10 ⁻⁵ M	25	****	-	
	30	****	-	
	45	4.08	5.376	
	55	7.01	3.978	
	60	6.19	3.886	
	65	****	-	
	70	****	-	
8.0 ×10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	4.03	5.424	-
	55	6.89	4.099	+ 5.190
	60	6.16	4.075	-
	65	****	-	-
	70	****	-	-
8.5×10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	4.05	5.406	-
	55	6.84	4.148	+ 4.427
	60	6.12	4.082	-
	65	****	-	-
	70	****	-	-
9.0×10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	3.92	5.536	-
	55	6.68	4.308	+1.984
	60	6.04	4.281	-
	65	****	-	-
	70	****	-	-
9.5 ×10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	3.89	5.568	-
	55	6.60	4.388	+ 0.763
	60	6.02	4.284	-
	65	****	-	-
	70	****	-	-
10.0×10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	3.88	5.577	-
	55	6.58	4.408	+ 0.458
	60	6.01	4.311	-
	65	****	-	-
	70	****	-	-
*****	25	****	-	-
	30	****	-	-
	45	****	-	-
	55	****	-	-
	60	****	-	-
	65	****	-	-
	70	****	-	-

* Each value represents mean of three trials. ** Optimum concentration of the chemical mutagen used. *** Optimum yield of bioethanol in 55 hours. (+)Values indicate % increase in the yield of ethanol in comparison to control. Experimental deviation ($\pm$) 1.5–3%. **** Insignificant value

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