

Studies on biotic production of bioethanol exposed to isocarboxazide

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Abstract : In the current communication some of the more important steps has been taken for the higher production of bioethanol exposed to some chemical mutagens specially isocarboxazide 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. It has been noted that *Saccharomyces cerevisiae* NT-138 has been found most successful bioethanol producer. It has been observed that the chemical mutagen, i.e. isocarboxazide has stimulatory and significant effect on bioethanol production and enhances the yield of bioethanol to an extent of 18.074% higher in comparison to control, i.e., 6.75ml/100 ml 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, isocarboxazide and *Saccharomyces cerevisiae* NT-138)

Introduction

A genetic alteration that is present for the first time in one family member as a result of a variant (or mutation) in a germ cell (egg or sperm) of one of the parents, or a variant that arises in the fertilized egg itself during early embryogenesis. Also called de novo mutation, de novo variant, and new variant.

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

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. 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 ethanol production by yeast *Saccharomyces cerevisiae* NT-138 exposed to chemical mutagens, therefore, the authoress have employed isocarboxazide on ethanol production by yeast *Saccharomyces cerevisiae* NT-138

Experimental

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

MS Molasses Solution : 22.5 % (w/v)
ME Malt-Extract : 1.25%
YE Yeast-Extract : 1.25%
PTN Peptone : 1.25%

DW 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 ninety nine 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 ten sets each comprising nine fermentor-flasks. The remaining nine fermentor-flasks out of ninety nine fermentor-flasks were kept as control and these were also rearranged in three subsets each consisting of three fermentor flasks.

Now, M/1000 solutions of isocarboxazide was made 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 ten 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 isocarboxazide in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below:

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

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 $33.5^{\circ}C$ and analysed colorimetrically after 25, 30, 45, 55, 60, 65 and 70 hours for ethanol¹⁷ formed and molasses sugars¹⁸ left unfermented.

Results and Discussion

The influence of isocarboxazide:

The data recorded in the table-1 shows that isocarboxazide has stimulatory effect on fermentative bioproduction of bioethanol from molasses by *Saccharomyces cerevisiae* NT-138. The data (table-1) reveals that the chemical mutagen isocarboxazide stimulates the alcoholic fermentation process and enhances the yield of bioethanol upto its (isocarboxazide) concentrations from $1.0 \times 10^{-5} M$ to $10.0 \times 10^{-5} M$ in two phases :

In the first phase, i.e.; from $0.5 \times 10^{-5} M$ to $7.5 \times 10^{-5} M$ the effect of isocarboxazide on the productivity (yield) of bioethanol was gradually in increasing order and attains its best role at $7.5 \times 10^{-5} M$ where maximum yield of bioethanol, i.e; 7.97 ml/100 ml is fetched in 55 hours of optimum incubation period which is 18.074% higher in comparison to control fermentor flasks (6.75 ml/100 ml).

In the second phase of mutagenic chemical effect the molar concentration, ie; from $8.0 \times 10^{-5} M$ to $10.0 \times 10^{-5} M$ the production of bioethanol has been enhanced but the order of bioethanol productivity is reversed in respect to increasing molar concentrations of isocarboxazide.

However, the fermentative bioproduction of bioethanol from molasses by *Saccharomyces cerevisiae* NT-138 under the influence of each concentration of isocarboxazide used has been stimulating and the yield of C_2H_5OH has been found greater than that obtained in the control fermentor flasks. In both the phases the order of productivity and % of C_2H_5OH formed is

Table 1
Biotic production of bioethanol exposed to Isocarboxazide

Concentration of Mutagen used $A \times 10^{-X} M$	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	2.85	6.613	-
	30	3.40	6.062	-
	45	4.00	5.463	-
	55	6.75	4.242	-
	60	6.18	4.165	-
	65	5.55	3.561	-
	70	4.45	3.211	-
$0.5 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.01	5.453	-
	55	6.79	4.205	+ 0.592
	60	6.20	4.109	-
	65	****	-	-
	70	****	-	-
$1.0 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.03	5.432	-
	55	6.81	4.182	+ 0.888
	60	6.22	4.080	-
	65	****	-	-
	70	****	-	-
$1.5 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.05	5.413	-
	55	6.84	4.154	+1.333
	60	6.25	4.045	-
	65	****	-	-
	70	****	-	-

Contd...

Table - 2
Biotic production of bioethanol exposed to Isocarboxazide

Concentration of Mutagen used $A \times 10^{-x} M$	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	2.85	6.613	-
	30	3.40	6.062	-
	45	4.00	5.463	-
	55	6.75	4.242	-
	60	6.18	4.165	-
	65	5.55	3.561	-
	70	4.45	3.211	-
$2.0 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.08	5.383	-
	55	6.92	4.074	+ 2.518
	60	6.28	4.047	-
	65	****	-	-
	70	****	-	-
$2.5 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.10	5.363	-
	55	6.99	4.002	+ 3.555
	60	6.30	3.986	-
	65	****	-	-
	70	****	-	-
$3.0 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.12	5.343	-
	55	7.04	3.953	+4.296
	60	6.35	3.834	-
	65	****	-	-
	70	****	-	-

Contd...

Table -1
Biotic production of bioethanol exposed to Isocarboxazide

Concentration of Mutagen used $A \times 10^{-x} M$	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	2.85	6.613	-
	30	3.40	6.062	-
	45	4.00	5.463	-
	55	6.75	4.242	-
	60	6.18	4.165	-
	65	5.55	3.561	-
	70	4.45	3.211	-
$3.5 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.15	5.314	-
	55	7.06	3.934	+ 4.592
	60	6.38	3.843	-
	65	****	-	-
	70	****	-	-
$4.0 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.18	5.283	-
	55	7.12	3.874	+5.481
	60	6.41	3.747	-
	65	****	-	-
	70	****	-	-
$4.5 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.20	5.263	-
	55	7.15	3.844	+5.925
	60	6.43	3.743	-
	65	****	-	-
	70	****	-	-

Contd...

Table - 1
Biotic production of bioethanol exposed to Isocarboxazide

Concentration of Mutagen used $A \times 10^{-x} M$	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	2.85	6.613	-
	30	3.40	6.062	-
	45	4.00	5.463	-
	55	6.75	4.242	-
	60	6.18	4.165	-
	65	5.55	3.561	-
	70	4.45	3.211	-
$5.0 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.22	5.243	-
	55	7.18	3.815	+ 6.370
	60	6.45	3.750	-
	65	****	-	-
	70	****	-	-
$5.5 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.25	5.214	-
	55	7.24	3.755	+ 7.259
	60	6.48	3.653	-
	65	****	-	-
	70	****	-	-
$6.0 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.29	5.169	-
	55	7.37	3.625	+9.185
	60	6.50	3.751	-
	65	****	-	-
	70	****	-	-

Contd...

Table - 1
Biotic production of bioethanol exposed to Isocarboxazide

Concentration of Mutagen used $A \times 10^{-X} M$	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	2.85	6.613	-
	30	3.40	6.062	-
	45	4.00	5.463	-
	55	6.75	4.242	-
	60	6.18	4.165	-
	65	5.55	3.561	-
	70	4.45	3.211	-
$6.5 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.32	5.139	-
	55	7.51	3.485	+ 11.259
	60	6.55	3.359	-
	65	****	-	-
	70	****	-	-
$7.0 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.36	5.099	-
	55	7.78	3.215	+ 15.259
	60	6.60	3.189	-
	65	****	-	-
	70	****	-	-
$7.5 \times 10^{-5} M^{**}$	25	****	-	-
	30	****	-	-
	45	4.40	4.059	-
	55	7.97***	3.024	+18.074
	60	6.65	3.011	-
	65	****	-	-
	70	****	-	-

Contd...

Table - 1
Biotic production of bioethanol exposed to Isocarboxazide

Concentration of Mutagen used $A \times 10^{-x} M$	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	2.85	6.613	-
	30	3.40	6.062	-
	45	4.00	5.463	-
	55	6.75	4.242	-
	60	6.18	4.165	-
	65	5.55	3.561	-
	70	4.45	3.211	-
$8.0 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.38	5.079	-
	55	7.64	3.354	+ 13.185
	60	6.61	3.245	-
	65	****	-	-
	70	****	-	-
$8.5 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.35	5.109	-
	55	7.45	3.545	+ 10.370
	60	6.58	3.454	-
	65	****	-	-
	70	****	-	-
$9.0 \times 10^{-5} M$	25	****	-	-
	30	****	-	-
	45	4.20	5.261	-
	55	7.17	3.826	+6.222
	60	6.41	3.762	-
	65	****	-	-
	70	****	-	-

Contd...

Table - 1
Biotic production of bioethanol exposed to Isocarboxazide

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	2.85	6.613	-
	30	3.40	6.062	-
	45	4.00	5.463	-
	55	6.75	4.242	-
	60	6.18	4.165	-
	65	5.55	3.561	-
	70	4.45	3.211	-
9.5 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	4.10	5.359	-
	55	6.96	4.035	+ 3.111
	60	6.33	4.014	-
	65	****	-	-
	70	****	-	-
10.00×10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	4.03	5.430	-
	55	6.81	4.188	+ 0.888
	60	6.23	4.089	-
	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 (±) 1.5–3%. **** Insignificant value

as under : Concentration of isocarboxazide from 0.5×10^{-5} M to 7.5×10^{-5} M. Productivity of C_2H_5OH : 0.592%, 0.888%, 1.333%, 2.518%, 3.555%, 4.296%, 4.592%, 5.481%, 5.925%, 6.370%, 7.259%, 9.185%, 11.259%, 15.259% and 18.074%. Concentration of isocarboxazide from 8.0×10^{-5} M to 10×10^{-5} M.

Productivity of bioethanol : 13.185%, 10.370%, 6.22%, 0.3111%, and 0.888%

Exposure of yeast strain to isocarboxazide may produce a variety of effects. Depending upon the concentration of isocarboxazide to which yeast strain *Saccharomyces cerevisiae* NT-

138. were exposed may influence 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.

Thus, it is concluded that isocarboxazide at lower concentrations is stimulatory and at higher concentrations is deterioratory for the fermentative bioproduction of bioethanol from molasses by *Saccharomyces cerevisiae* NT-138.

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