

The influence of N-nitrosodiethylamine on novel biotechnological method for production of ethanol by *Saccharomyces cerevisiae* RK-16

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Abstract : The efficacy of N-nitrosodiethylamine on production of ethanol by *Saccharomyces cerevisiae* RK-16 has been assessed. It has been found that the mutagen under trial, i.e; N-nitrosodiethylamine at 6.0×10^{-5} M molar concentration enhances the production of ethanol by *Saccharomyces cerevisiae* RK-16 to an extent of 20.363% higher in comparison to control fermenter flask, i.e, 5.50 ml/100 in 65 hours of optimum incubation period, 18.5% (w/v) molasses solution 4.5 pH and 30°C temperature alongwith other nutritional ingredients required by the yeast.

(Keywords: Alcoholic fermentation, molasses, mutagen, N-nitrosodiethylamine, *Saccharomyces cerevisiae* RK-16)

Introduction

A mutagen is defined as any physical or chemical substance that can change the genetic material of an organism, thereby causing a mutation. Chemical mutagens are compounds that increase the frequency of some types of mutation.¹⁻⁷ Mutagenic agents are used to induce favorable mutations at high frequency that include ionizing radiation and chemical mutagens.⁸⁻¹² Many chemicals have clastogenic (chromosome damaging) effects on plants via reactive oxygen-derived radicals.¹³ Many researchers compared the mutagenic efficiencies of different mutagens on different crops and their results seem to be entirely specific for particular species and even varieties. While many researchers found chemical mutagens are to be more effective than physical

ones^{14,15} and many others researchers found the reverse case¹⁶.

A number of workers¹⁷⁻¹⁸ have reported the role of chemical mutagens in enhancing genetic variability in higher plants because it is the fundamental characteristics to successful breeding programs in vegetatively and sexually propagated plants¹⁹. A large number of chemical mutagens are recorded as a good agent for different microbes and microbial processes²⁰⁻³⁶. 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 N-nitrosodiethylamine on production of ethanol by *Saccharomyces cerevisiae* RK-16

Experimental

The efficacy of chemical mutagens, i.e, N-nitrosodiethylamine on novel biotechnological method for production of ethanol by *Saccharomyces cerevisiae* RK-16

The constitution of bioproduction medium for the novel biotechnological method for production of ethanol by *Saccharomyces cerevisiae* RK-16 is prepared as follows :

Molasses : 18.5%, Malt extract : 1.80%
Yeast extract : 1.80%, Peptone : 1.80%
(NH₄)₂HPO₄ : 1.80%, pH : 4.5

Table - 1
The influence of N-Nitrosodiethylamine on novel biotechnological method
for production of ethanol by *Saccharomyces cerevisiae* RK-16

Concentration of mutagen used A X 10 ^{-x} M	Incubation Period in hours	Yield of alcohol* in ml/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in yield of alcohol in comparison to control.
Control	65	5.50	1.659	—
1.0 × 10 ⁻⁵ M	65	5.62	1.541	+2.181
2.0 × 10 ⁻⁵ M	65	5.68	1.481	+3.272
3.0 × 10 ⁻⁵ M	65	5.80	1.359	+5.454
4.0 × 10 ⁻⁵ M	65	6.23	0.935	+13.272
5.0 × 10 ⁻⁵ M	65	6.53	0.639	+18.727
6.0 × 10 ⁻⁵ M**	65	6.62***	0.559	+20.363
7.0 × 10 ⁻⁵ M	65	6.18	0.986	+12.363
8.0 × 10 ⁻⁵ M	65	5.74	1.420	+4.363
9.0 × 10 ⁻⁵ M	65	5.56	1.606	+1.090
10.0 × 10 ⁻⁵ M	65	5.53	1.631	+0.545

* Each value represents mean of three trials. ** Optimum concentration of the chemical mutagen used. *** Optimum yield of alcohol in 65 hours. (+)Values indicate % increase in the yield of alcohol in comparison to control. Experimental deviation (±) 1.5–3%.

Distilled water was well added to make up the volume up to '100 ml'.

The pH of the medium was adjusted to 4.5 by adding desired quantity of lactic acid.

Now, the same production medium for novel biotechnological method for production of ethanol by a fungal yeast strain of *Saccharomyces cerevisiae* RK-16 was prepared for ninety nine flat bottom fermentor-flasks, i.e., each flat bottom fermentor flask containing 100 ml of production medium. These flat bottom fermentor-flasks, i.e., each containing 100 ml of bioproduction medium. These flat bottom fermentor-flasks were then well arranged in ten sets each comprising nine flat bottom fermentor-flasks. The remaining nine flat bottom fermentor-flasks out of ninety nine flat bottom fermentor-flasks were kept as control and these were also rearranged in three subsets each consisting of three flat bottom fermentor flasks.

Now, M/1000 solutions of chemical

mutagen, i.e., N-nitrosodiethylamine 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 mutagenic solution was added to the flat bottom fermentor-flasks of first ten sets respectively. The control flat bottom fermentor-flask contained no chemical mutagens. The total volume in each flat bottom fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

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

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

Where, a = amount of chemical mutagens in ml, ie; from 1.0 ml to 10.0 ml.

x = molarity of the solution.

The flat bottom fermentor-flasks were then carefully steam sterilized, cooled, inoculated and incubated at temperature 30°C and then

carefully analysed colorimetrically after 45, 65, and 75 hours for alcohol formed³⁷ and molasses³⁸ solution sugars left unused.

Results and Discussion

The influence of chemical mutagen, i.e., N-nitrosodiethylamine on novel biotechnological method for production of ethanol by *Saccharomyces cerevisiae* RK-16

The data shown in the table-1 describes that the chemical mutagen N-nitrosodiethylamine has also been found stimulatory for the novel biotechnological method for bioproduction of ethanol by fungal yeast strain of *Saccharomyces cerevisiae* RK-16 at all its concentrations, i.e.; from 1.0×10^{-5} M to 6.0×10^{-5} M in two phases.

In the first phase the molar concentration of chemical mutagen, i.e.; N-nitrosodiethylamine from 1.0×10^{-5} M to 6.0×10^{-5} M is significant and the productivity of ethanol gradually enhances till a maximum value is reached at 6.0×10^{-5} M, i.e.; 6.62 ml/100ml in 65 hours of optimum incubation period which is 20.363% higher in comparison to control, i.e.; 5.50 ml/100ml.

In the second phase of chemical mutagenic effect, i.e., at 6.0×10^{-5} M and onwards the molar concentration of chemical mutagen, i.e., N-Nitrosodiethylamine the bioproduction of

ethanol has been increased but in lesser quantum in the presence of chemical mutagen, i.e., N-nitrosodiethylamine. So, the gradual addition of chemical mutagen, i.e., N-nitrosodiethylamine after the optimum concentration of 6.0×10^{-5} M gradually slows down the biotransformation of molasses solution to ethanol and the yield of ethanol on the part of % increase at 7.0×10^{-5} M, to 10.0×10^{-5} M molar concentration of chemical mutagen, i.e., N-nitrosodiethylamine is as follows: 12.363%, 4.363%, 1.090%, and 0.545% respectively.

From the present investigation it is very clear that the chemical mutagens used, i.e., N-nitrosodiethylamine is very useful for novel biotechnological method for production of ethanol by fungal yeast strain of *Saccharomyces cerevisiae* RK-16 and therefore, can be employed for the improved yield of ethanol.

It is interesting to note that the mutagenic chemicals used in the present research investigation in the molar concentrations, i.e., from 1.0×10^{-5} M to 10.0×10^{-5} M has given better yield of ethanol in each case in comparison to respective controls. So far as effectiveness of mutagenic chemicals used in the present research investigation is concerned N-nitrosodiethylamine is most effective for production of ethanol by *Saccharomyces cerevisiae* RK-16.

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