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Influence of NNMG on ethanol production by *Saccharomyces cerevisiae* NCIM - 1154

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Abstract: N' - Nitro - N - nitroso - N - methylguanidine (NNMG) is a potent mutagen, capable of inducing point mutations and other genetic alterations. NNMG has been shown to be carcinogenic in some studies. NNMG is used in research to study mutagenesis, carcinogenesis and DNA repair mechanisms.NNMG is a hazardous chemical that requires careful handling and storage due to its mutagenic and potentially carcinogenic properties. In the present study influence of some chemical mutagens, i.e., NNMG on ethanol production by *Saccharomyces cerevisiae* NCIM - 1154 has been assessed. It has been found that NNMG under trial has stimulating influence and has enhanced the production of ethanol.

(Keywords : NNMG, *Saccharomyces cerevisiae* NCIM - 1154, EtOH).

Introduction

Fermentation has come a long way from its humble beginning as a technology for household food preservative into a sophisticated technology for the manufacture of pharmaceuticals biochemicals, enzymes, foods, beverages and food ingredients at industrial scale. Mutagens in fermentation technology are being explored for their potential to improve microbial production processes. Researchers are exploring eco-friendly chemical mutagens that can induce targeted mutations without harming the environment. Researchers are working on optimising fermentation processes to maximise yields and process efficiency. The focus is on developing sustainable fermentation process that minimise environmental impact while improving product yields.

Mutagen is a physical or biochemical agent that change the genetic material (DNA) of an organism and thus increases the frequency of mutations above the natural incidence. Mutagenic changes results from changes in hydrogen bonding property of bases or from mistakes in base pairing during DNA replication cause mutation by chemical agents. In activating alterations include removal of bases, dimer formation, cross linking of two DB DNA strands and single or double strands breaks which prevent DNA replication across altered site and include chromosome breaks and chromosome mutation. 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.[1-6]

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.[7-15]

Literature survey reveals that a very little work has been done on ethanol production by yeast *Saccharomyces cerevisiae* NCIM -1154 exposed to chemical mutagens, therefore, the authors have employed NNMG on ethanol production by yeast *Saccharomyces cerevisiae* NCIM -1154.

Experimental :

The influence of N'-nitro-N-nitroso-N-methylguanidine on bioenergetic conversion of molasses pollutant to ethanol.

The composition of production medium for the bioenergetic conversion of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM -1154 is prepared as follows :

Molasses : 22%, Malt extract: 0.40%
Yeast extract : 0.40%, Peptone : 0.60%
$(NH_4)_2HPO_4$: 0.30%, pH : 4.2

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

The pH of the medium was adjusted to 4.2 by adding requisite amount of lactic acid.

Now, the same production medium for bioenergetic conversion of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM -1154 was prepared for 99 fermentor-flasks, i.e., each containing 100 ml of production medium. These 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 solutions of N'-nitro-N-nitroso-N-methylguanidine 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

Table - 1
nfluence of NNMG on ethanol production by *Saccharomyces cerevisiae* NCIM - 1154

Concentration of Mutagen used A × 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 55 hrs.
Control	55	8.15	2.350	–
1.0 x 10^{-5} M	55	8.27	2.232	+1.472
2.0 x 10^{-5} M	55	8.30	2.202	+1.840
3.0 x 10^{-5} M	55	8.45	2.056	+3.680
4.0 x 10^{-5} M	55	8.54	1.962	+4.785
5.0 x 10^{-5} M	55	8.62	1.884	+5.766
6.0 x 10^{-5} M**	55	8.70***	1.810	+6.748
7.0 x 10^{-5} M	55	8.56	1.943	+5.000
8.0 x 10^{-5} M	55	8.47	2.032	+3.926
9.0 x 10^{-5} M	55	8.37	2.128	+2.699
10.0 x 10^{-5} M	55	8.36	2.131	+2.576

* Each value represents mean of three trials. ** Optimum concentration of mutagen used.
*** Optimum yield of alcohol in 55 hours. (+)Values indicate % increase in the yield of alcohol after 55 hours. Experimental deviation (±) 1.5–3%.

contained no chemical mutagens. The total volume in each fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

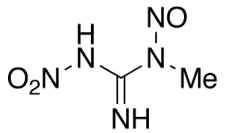
Thus, the concentration of N'-nitro-N-nitroso-N-methylguanidine in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :

$a \times 10^{-x}$M, 1.0×10^{-5} M to 10.0×10^{-5} M
Where,
a = amount of mutagens in ml, ie; from 1.0 ml to 10.0 ml.
x = molarity of the solution respectively.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 33^0 C and analysed colorimetrically after 50, **55**, and 60 hours for alcohol[16] formed and molasses[17] sugars left unfermented.

Results and Discussion

The influence of N'-nitro-N-nitroso-N-methylguanidine



N'-nitro-N-nitroso-N-methylguanidine

The data recorded in the table-1 shows that N'-Nitro-n-nitroso-N-methylguanidine has stimulatory effect on bioenergetic conversion of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM -1154

The maximum yield of alcohol, i.e., 8.70 ml/100 ml in the presence of N'-nitro-N-nitroso-N-methylguanidine was observed at 6.0×10^{-5} M molar concentration in 55 hours of optimum incubation period which is 6.748% higher in comparison to control fermentor flasks, i.e., 8.15 ml/100ml in the same times course and other same experimental parameters.

The higher molar concentrations of N'-nitro-N-nitroso-N-methylguanidine were not much favourable for the bioenergetic dissimilation of molasses to alcohol. So the gradual addition of the mutagen N'-nitro-N-nitroso-N-methyl guanidine after certain concentrations were not beneficial for the bioenergetic conversion of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM -1154

It has been observed that molar concentration of the mutagen, ie., N'-nitro-N-nitroso-N-methylguanidine from 1.0×10^{-5} M to 6.0×10^{-5} M enhances the yield of alcohol to a certain order being 1.472%, 1.840%, 3.680%, 4.785%, 5.766% and 6.748% higher in comparison to control flasks.

It has been observed further that after optimum concentration, i.e; 6.0×10^{-5} M, the addition of the same mutagen to the production medium causes fall in the yield of alcohol gradually and reached to 2.576%. However, at all the experimental concentrations of N'-nitro-N-nitroso-N-methylguanidine used, the yield of alcohol by submerged fermentation has been found higher in comparison to control fermentor flasks.

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