

Efficacy of 2 - Chloro - 2' , 6' - dimethyl - N - (methoxymethyl) acetanilide (CDMMA) on ethanol bioproduction

Suresh Prasad*, Rafat Perween^{1**} and Rekha Kumari Bharti²

*Department of Chemistry Nalanda College Bihar Sharif, Nalanda -803101, Bihar

^{1,2}Department of Chemistry, M.G. College, Gaya - 823001, Bihar

Email : prabhanshurajmits@gmail.com , rafat4nov@gmail.com, rekhabharti15121976@gmail.com

**Main Authoress

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Abstract: The efficacy of CDMMA on bioenergetic transformation of molasses to ethanol by *Saccharomyces cerevisiae* NCIM - 1154 has been examined. It has been found that the chemical mutagen under observation, i.e CDMMA has been found encouraging and has upgraded the yield of ethanol significantly when allowed to ferment under optimised parameters, i.e., temperature, pH, incubation period and other levels are controlled to optimize microbial growth and ethanol production. Ethanol production by fermentation technology is widely used process to produce ethanol.

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

Introduction

The most revealing findings about mutation have come from the studies of mutagens as well as radiation energy. General reviews¹⁻¹² shows that there is a rather widespread agreement as to the best strategy for a programme of strain development of screening designed to improve best and potent mutant of microbes.

Mutation is related with a variety of chemicals and biochemicals in several bacteria, fungi and yeasts e.g., nitrogen and sulphur mustards and other related compounds¹³⁻¹⁶. Singh¹⁵ concluded that hydrazine sulphate enhances the yield of lactic acid, while p-nitrophenyl hydrazine, 2-4-dinitrophenyl hydrazine and lithium fluorids has been detrimental mutagens for *L.acidophilus* and production of lactic acid. Reeta Rani concluded that methoxy caffeine enhances the production

of ergot-alkaloids while sodium azide, hydrazine hydrochloride, and lithium fluoride has been detrimental and valueless for the fermentative production of ergot-alkaloids by the fungus *Claviceps purpurea*. 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 - 1154 species have not been studied¹⁷⁻⁴⁸.

The present study was undertaken to assess and analyse the alcoholic fermentation by *Saccharomyces cerevisiae* NCIM -1154 exposed to CDMMA.

Experimental

The influence of 2 - chloro - 2' , 6' - dimethyl - N - (methoxymethyl) acetanilide (CDMMA) 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%,
 (NH₄)₂HPO₄ : 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 2 - Chloro - 2', 6' - dimethyl - N - (methoxymethyl) acetanilide (CDMMA) 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 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 2 - Chloro - 2', 6' - dimethyl - N - (methoxymethyl) acetanilide (CDMMA) 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 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°C and analysed colorimetrically after 50, 55, and 60 hours for alcohol⁴⁹ formed and molasses sugars⁵⁰ left unfermented.

Results and Discussion :

The influence of 2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide

The data recorded in the table-1 shows that 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide has stimulatory effect on bioenergetic

Table - 1
Efficacy of 2 - Chloro - 2', 6' - dimethyl - N - (methoxymethyl) acetanilide (CDMMA)
on ethanol bioproduction

Concentration of Mutagen used $A \times 10^{-x} M$	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	8.03	2.425	–
$1.0 \times 10^{-5} M$	55	8.17	2.285	+1.743
$2.0 \times 10^{-5} M$	55	8.25	2.215	+2.739
$3.0 \times 10^{-5} M$	55	8.33	2.130	+3.735
$4.0 \times 10^{-5} M$	55	8.40	2.086	+4.607
$5.0 \times 10^{-5} M$	55	8.50	1.976	+5.853
$6.0 \times 10^{-5} M$	55	8.57	1.896	+6.724
$7.0 \times 10^{-5} M$	55	8.65	1.819	+7.721
$8.0 \times 10^{-5} M^{**}$	55	8.75**	1.756	+8.966
$9.0 \times 10^{-5} M$	55	8.59	1.899	+6.973
$10.0 \times 10^{-5} M$	55	8.38	2.125	+4.358

* 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 (\pm) 1.5–3%.

conversion of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM -1154 The data (table-1) reveals that the chemical mutage 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide stimulates the alcoholic fermentation process and enhances the yield of alcohol upto its (2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide) concentrations from 1.0×10^{-5} M to 10.0×10^{-5} M in two phases :

In the first phase, ie; from 1.0×10^{-5} M to 8.0×10^{-5} M the effect of 2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide on the productivity (yield) of alcohol was gradually in increasing order and attains its best role at 8.0×10^{-5} M where maximum yield of alcohol, ie; 8.75 ml/100 ml is fetched in 55 hours of optimum incubation period which is 8.966% higher in comparison to control fermentor flasks (8.03 ml/100 ml). In the second phase of mutagenic chemical effect the molar concentration, ie; from 9.0×10^{-5} M to 10.0×10^{-5} M the production of alcohol has been enhanced but the order of alcohol productivity is reversed in respect to increasing molar concentrations of 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide . However, the bioenergetic conversion of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM -1154 under the influence of each concentration of 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide used has been stimulating and the yield of alcohol has been found greater than that obtained in the control fermentor flasks. In both the phases the order of

productivity and % of alcohol formed is as under :
Concentration of 2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide from 1.0×10^{-5} M to 8.0×10^{-5} M.

Productivity of alcohol: 1.743%, 2.739%, 3.735%, 4.607%, 5.853% 6.724%, 7.21%, and 8.966%

Concentration of 2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide from 9.0×10^{-5} M to 10×10^{-5} M.

Productivity of alcohol : 6.973% and 4.358%.

Exposure of yeast strain to 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide may produce a variety of effects. Depending upon the concentration of 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide to which yeast strain *Saccharomyces cerevisiae* NCIM -1154 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 2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide at lower concentrations is stimulatory and at higher concentrations is deterioratory for the bioenergetic conversion of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM -1154

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