J. Chemtracks 23 (1&2)13-16, 2021

Abstracted / Indexed in
A division of the American Chemical Society
Chemical Abstract U.S.A.

ISSN 0973-239X
CODEN:JCOHF7
IMPACT FACTOR : 4.950

Fermentative bioproduction of ethanol from molasses exposed to dibenzamine

Dhananjay Kumar[1], Renu Bala[2] and V. Mandal[3]

[1]Department of Chemistry, Magadh University, Bodh-Gaya (India) -824234
[2]Department of Chemistry, College of Commerce, Arts and Science, Patna
[3]Department of Chemistry, A.N. College, Patna
E-mail : dhananjaychemistry1@gmail.com

Manuscript received online 24 February 2021, accepted on 14 April 2021

Abstract: The effect of dibenzamine on bioproduction of ethanol by yeast *Saccharomyces cerevisiae* DK-18 has been assessed. It has been found that the mutagen, i.e., dibenzamine under observation has stimulatory effect on bioproduction of ethanol by yeast *Saccharomyces cerevisiae* DK-18 and enhances the yield of ethanol to an extent of 10.289% higher in comparison to control flat bottom fermentor flasks, i.e., 6.98 ml/100mL in 56 hrs of incubation period, 4.7 pH and 33^{0}C temperature with 22% (w/v) molasses solution.

(**Keywords:** Molasses, ethanol, mutagens, dibenzamine, *Saccharomyces cerevisiae* DK-18)

Introduction

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.[10-14]

Literature survey reveals that a very little work has been done on ethanol production by yeast *Saccharomyces cerevisiae* DK-18 exposed to chemical mutagens, therefore, the authors have employed dibenzamine on ethanol production by yeast *Saccharomyces cerevisiae* DK-18.

Experimental

The influence of dibenzamine on fermentative bioproduction of ethanol from molasses by *Saccharomyces cerevisiae* DK-18

The constitution of production medium for the fermentative bioproduction of ethanol from molasses by *Saccharomyces cerevisiae* DK-18 is prepared as follows :

MS Molasses Solution	:	22 % (w/v)
ME Malt-Extract	:	0.40%
YE Yeast-Extract	:	0.40%
PTN Peptone	:	0.40%
DW Distilled water	:	10 ml
To make up 100 ml		
pH	:	4.7

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

The pH of the medium was adjusted to 4.7 by adding requisite amount of lactic acid.

Now, the same production medium for fermentative bioproduction of ethanol from

molasses by *Saccharomyces cerevisiae* DK-18 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 dibenzamine 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 dibenzamine 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, 6.0×10^{-5} M,
2.0×10^{-5} M, 7.0×10^{-5} M,
3.0×10^{-5} M, 8.0×10^{-5} M,
4.0×10^{-5} M, 9.0×10^{-5} M,
5.0×10^{-5} M, 10.0×10^{-5} M

Where, A = amount of mutagen in ml, ie; from1.0 ml to 10.0 ml.
and x = molarity of the solution respectively.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 33^0 C and analysed colorimetrically after 46, 56, and 62 hours for ethanol[15] formed and molasses sugars[16] left unfermented.

Results and Discussion

The influence of dibenzamine:

The data recorded in the table-1 shows that dibenzamine has stimulatory effect on fermentative bioproduction of ethanol from molasses by *Saccharomyces cerevisiae* DK-18.

The data (table-1) reveals that the chemical mutagen dibenzamine stimulates the alcoholic fermentation process and enhances the yield of alcohol upto its (dibenzamine) 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 4.0×10^{-5} M the effect of dibenzamine on the productivity (yield) of alcohol was gradually in increasing order and attains its best role at 4.0×10^{-5} M where maximum yield of alcohol, ie; 7.68 ml/100 ml is fetched in 56 hours of optimum incubation period which is 10.289% higher in comparison to control fermentor flasks (6.98 ml/100 ml).

In the second phase of mutagenic chemical effect the molar concentration, ie; from 5.0×10^{-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 dibenzamine. However, the fermentative bioproduction of ethanol from molasses by *Saccharomyces cerevisiae* DK-18 under the influence of each concentration of dibenzamine 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 as under :

Concentration of dibenzamine from 1.0×10^{-5} M to 4.0×10^{-5} M .
Productivity of C_2H_5OH: 1.146%, 3.008%, 5.014% and10.289%, Concentration of dibenzamine from 5.0×10^{-5} M to 10×10^{-5} M .
Productivity of alcohol : 8.022%, 6.017%, 4.011%, 2.005%, 0.716% and 0.286%

Exposure of yeast strain to dibenzamine may produce a variety of effects. Depending upon the concentration of dibenzamine to which yeast

Table - 1
Fermentative bioproduction of ethanol from molasses exposed to dibenzamine

Concentration of mutagen used A X 10^{-x} M	Incubation Period in hours	Yield of ethanol*in ml/100ml	Molasses* left unfermented in g/100 ml	%Diff. in the yield of ethanol in 56 hrs
Control	46	4.23	4.915	–
(–) Mutagen	56	6.98	3.768	–
	62	5.90	3.685	–
1.0 x 10^{-5} M	46	4.27	4.875	–
(+)Mutagen	56	7.06	3.688	+1.146
	62	6.92	3.584	–
2.0 x 10^{-5} M	46	4.35	4.795	–
(+) Mutagen	56	7.19	3.559	+3.008
	62	6.95	3.452	–
3.0 x 10^{-5} M	46	4.44	4.706	–
(+) Mutagen	56	7.33	3.419	+5.014
	62	6.96	3.305	–
4.0 x 10^{-5} M**	46	4.65	4.495	–
(+) Mutagen	56	7.68***	3.068	+10.289
	62	6.98	3.039	–
5.0 x 10^{-5} M	46	4.56	4.585	–
(+) Mutagen	56	7.54	3.208	+8.022
	62	6.95	3.105	–
6.0 x 10^{-5} M	46	4.49	4.656	–
(+)Mutagen	56	7.40	3.348	+6.017
	62	6.89	3.283	–
7.0 x 10^{-5} M	46	4.39	4.755	–
(+)Mutagen	56	7.26	3.488	+4.011
	62	6.82	3.389	–
8.0 x 10^{-5} M	46	4.31	4.835	–
(+)Mutagen	56	7.12	3.628	+2.005
	62	6.79	3.589	–
9.0 x 10^{-5} M	46	4.25	4.895	–
(+)Mutagen	56	7.03	3.719	+0.716
	62	6.70	3.620	–
10.0 x 10^{-5} M	46	4.24	4.905	–
(+)Mutagen	56	7.00	3.748	+0.286
	62	6.62	3.684	–

* Each value represents mean of three observations ** Optimum concentration of mutagen used. *** Optimum yield of alcohol in 56 hours. (+)Values indicate % increase in the yield of alcohol after 56 hours. Experimental deviation (±) 1.5–3%.

strain *Saccharomyces cerevisiae* DK-18 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 dibenzamine at lower concentrations is stimulatory and at higher concentrations is deterioratory for the fermentative bioproduction of ethanol from molasses by *Saccharomyces cerevisiae* DK-18

References

1. Benigni Romualdo and Bossa Cecilia *Mutagenesis* **26** (3), 455 (2011)
2. L. Huang, A. R. Snyder, W.F. Morgan, *Oncogene* **22**, 5848 (2003)
3. M. Valko, H. Morris, M.T. Cronin, *Current medicinal chemistry* **12** (10), 1161 (2005)
4. A. Gibbs, J. Schwartzman, V. Deng, *J. Alumkal. Proc Natl Acad Sci U S A.* **106** (39): .1 6638, (2009)
5. N. P. Gullett, A.R. Ruhul Amin, S. Bayraktar, J. M. Pezzuto, D. M. Shin, F.R. Khuri, B. B. Aggarwal, Y. J. Surh, O. Kucuk Seminars in *Oncology* **37** (3), 258 (2010).
6. B. N. Ames, *Science* **204**, 587 (1979)
7. Charlotte Auerbach, J. M. Robson, & J. G. Carr *Science* **105**, 243 (1947)
8. B. Ames, N.; Lee, F. D.; Durston, W. E. . *United States of America* **70** (3): 782 (1973)
9. J. McCann, L.S. Gold, L. Horn, R. McGill, T.E. Graedel, J. Kaldor *J. Mutation Research* **205** (1-4): 183 (1988).
10. J. McCann, E. Choi, E. Yamasaki, B.N. Ames, *Proceedings of the National Academy of Sciences* (USA) **72** (12),5135 (1975)
11. V. C. Dunkel, E. Zeiger, D. Brusick, E. McCoy, D. McGregor, K. Mortelmans, H.S. Rosenkranz, V. F. Simmon. *Environ Mutagen* **7**, 1 (1985)
12. R. Doll, R. Peto *Journal of the National Cancer Institute* **66** (6): 1191 (1981)
13. Raghwendra Kumar, H. K. K. Kishore, L.D. P. Yadav, S. K. Sahay, Akhauri Prashant Kumar and S. P. Singh *J. Chemtracks* **15**(2), 365-368 (2013)
14. R. Kumar, B. Kumari and S. P. Singh *J. Chemtracks* **22**(1&2), 181-184 (2020)
15. L. P. Mccloskey and L. L. Replogle *Am.J. Enol. Vitie* **25**, 194 (1974).
16. M. Dubois, K. A.Gilles, J. K. Hamilton and F. Smith, *Anal. Chem.* **28**, 350 (1956)