J. Chemtracks 23 (1&2)21-24, 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

Biotechnique for ethanol bioproduction by *Saccharomyces cerevisiae* DK-18 exposed to 2-Naphthylhydroxylamine

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 17 March 2021, accepted on 26 June 2021

Abstract: The efficacy of 2-Naphthylhydroxylamine on bioproduction of ethanol by yeast *Saccharomyces cerevisiae* DK-18 has been assessed. It has been found that the mutagen, i.e., 2-Naphthylhydroxylamine 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 17.201% higher in comparison to control fermentor flasks, i.e., 6.86 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, 2-Naphthylhydroxylamine, *Saccharomyces cerevisiae* DK-18)

Introduction

Chemical mutagens are standard tools for mutagenesis in a variety of organisms, and they are a primary means of creating mutations in phenotype-based screens in most genetic systems. Although varied in the experimental design, all whole animal screens involve the generation of lines harboring mutated chromosomes followed by the examination of the resulting phenotypes in the heterozygous or homozygous state. In contrast, gene-based screens rely on the identification of lines that carry.

Mutagens are simultaneously both required and avoided substances. They are "substances of transformation," but also "genetic poisons." Their transformative qualities destined mutagens to become unavoidable instruments within genetics and molecular biology. Since the 1960s, however, mutagens have defined a trans-disciplinary problem of risk policy. Substances such as radioactive particles from fall-out and the nuclear industries, pharmaceuticals, chemical supplements in the foodstuffs industry or pesticides (like DDT) were silent, efficient and ubiquitous.[1-4] The precarious status between efficiency and (dangerous) autonomy formed the key characteristics of mutagens that nurtured the ambivalent career of mutants. Daily life became populated by horrifying, but also superhuman creatures.[5-15]

Literature survey reveals that a little work has been done on SmF biotechnique for ethanol production by *Saccharomyces cerevisiae* DK-18 exposed to 2-Naphthylhydroxylamine, therefore, the authors have employed 2-Naphthylhydroxylamine on biotechnique for ethanol production by *Saccharomyces cerevisiae* DK-18.

Experimental

The influence of 2-Naphthylhydroxylamine 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 :

Molasses Solution : 22 % (w/v), Malt-Extract : 0.40%, Yeast-Extract : 0.40%, Peptone : 0.40% Distilled water : 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 2-Naphthylhydroxylamine 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 2-Naphthylhydroxylamine 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[16] formed and molasses sugars[17] left unfermented.

Results and Discussion

The influence of 2-naphthylhydroxylamine :

The data recorded in the table-1 shows that the mutagen, i.e., 2-naphthylhydroxylamine has been found stimulatory for the fermentative bioproduction of ethanol from molasses by *Saccharomyces cerevisiae* DK-18 at all its concentrations, ie; from 1.0×10^{-5} M to 10.0×10^{-5} M in two phases.

In the first phase the molar concentration of mutagen, i.e; 2-naphthylhydroxylamine from 1.0×10^{-5} M to 7.0×10^{-5} M is significant and the productivity of alcohol gradually increases till a maximum is reached at 7.0×10^{-5} M, ie; 8.04 ml/100ml in 56 hours of optimum incubation period which is 17.201% higher in comparison to control, i.e; 6.86 ml/100ml.

In the second phase of mutagenic effect, i.e., at 8.0×10^{-5} M and onwards the molar concentration of mutagen, i.e., 2-naphthyl-hydroxylamine the production of alcohol has been increased but in lesser quantum in the presence of 2-naphthylhydroxylamine So, the gradual addition of 2-naphthylhydroxylamine after the optimum concentration of 7.0×10^{-5} M gradually slows down the fermentative bioproduction of ethanol from molasses by *Saccharomyces cerevisiae* DK-18 and the yield of alcohol on the part of % increase at 8.0×10^{-5} M, 9.0×10^{-5} M, and 10.0×10^{-5} M molar concentration of 2-naphthylhydroxylamine is as follows:

12.244%, 6.122%, and 2.186% respectively.

Table - 1
Biotechnique for ethanol bioproduction by *Saccharomyces cerevisiae* DK-18 exposed to 2-Naphthylhydroxylamine

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	4.19	4.933	–
(–) Mutagen	56	6.86	3.461	–
	62	5.66	3.359	–
1.0 x 10-5 M	46	4.26	4.893	–
(+)Mutagen	56	6.94	3.385	+1.166
	62	5.70	3.281	–
2.0 x 10-5 M	46	4.27	4.853	–
(+) Mutagen	56	7.01	3.312	+2.186
	62	6.10	3.205	–
3.0 x 10-5 M	46	4.35	4.773	–
(+) Mutagen	56	7.15	3.169	+4.227
	62	6.12	3.093	–
4.0 x 10-5 M	46	4.48	4.649	–
(+) Mutagen	56	7.35	2.975	+7.142
	62	6.18	2.859	–
5.0 x 10-5 M	46	4.65	4.473	–
(+) Mutagen	56	7.63	2.691	+11.224
	62	6.40	2.588	–
6.0 x 10^{-5} M	46	4.81	4.315	–
(+)Mutagen	56	7.90	2.424	+15.160
	62	6.66	2.342	–
7.0 x 10^{-5} M**	46	4.90	4.223	–
(+)Mutagen	56	8.04***	2.285	+17.201
	62	7.01	2.159	–
8.0 x 10^{-5} M	46	4.69	4.436	–
(+)Mutagen	56	7.70	2.626	+12.244
	62	6.59	2.569	–
9.0 x 10^{-5} M	46	4.45	4.674	–
(+)Mutagen	56	7.28	3.049	+6.122
	62	6.23	3.035	–
10.0 x 10^{-5} M	46	4.28	4.849	–
(+)Mutagen	56	7.01	3.312	+2.186
	62	6.00	3.210	–

* 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%.

From the present investigation it is obvious that the chemical mutagens used, i.e., 2-naphthylhydroxylamine is useful for fermentative bioproduction of ethanol from molasses by *Saccharomyces cerevisiae* DK-18 and therefore, can be employed for the improved yield of alcohol.

It is interesting to note that the mutagenic chemicals exploited in the present investigation in the molar concentrations, i.e., from 1.0×10^{-5} M to 10.0×10^{-5} M has given better yield of alcohol in each case in comparison to respective controls.

References

1. B.P. Forster, J.D.Franckowiak, U. Lundqvist, *Annals of Botany*. **100**: 725 (2007)
2. P. Krysan, J.C. Young, P. Jester, *J. Integrative Biology*. **6**: 163 (2002)
3. X. Pan, H. Liu, J. Clarke *Nucleic Acids Res*. **31**:1245 (2003).
4. R.L. Phillips, S.M. Kaeppler, and P. Olhoft, *Proc. Natl. Acad. Sci.* USA. **91**:5222 (1994.)
5. R. Azpiroz-Leehan and K.A. Feldmann, *Genetics* **13**: 152-156 (1997.)
6. N. Bechtold, J. Ellis and G. Pelletier *In Planta Acad. Sci.: Life Sci.* **316**: 1194 (1993)
7. S.J. Clough, and A.F. Bent, *The Plant Journal* **16:** 735 (1998).
8. K.A. Feldmann, *The Plant Journal*.**1**: 71 (1991)
9. K.A. Feldmann and M.D. Marks *Genetics*. **208**: 1 (1987)
10. R.N. Radhamony, A.M.Prasad and R.E. Srinivasan, *Journal Biotechnology*. **8,** 1 (2005)
11. A. K. Adamu, H. Aliyu, *Science World Journal* 2 (4), 9 (2007)
12. Van, Den-Bulk RW., Loffer, H.J.M., Lindhout, W.H., Koornneef, M., *Theoretical Applied Genetics* **80**, 817 (1990).
13. B. Bertagne-Sagnard, G. Fouilloux, Y. Chupeau, *Journal of Experimental Botany* **47**, 189 (1996).
14. R. Kumar, B. Kumari and S. P. Singh *J. Chemtracks* **22**(1&2), 181-184 (2020)
15. Ashwini Kumar *J. Chemtracks* **22**(1&2), 405-408 (2020)
16. L. P. Mccloskey and L. L. Replogle *Am.J. Enol. Vitie* **25**, 194 (1974).
17. M. Dubois, K. A.Gilles, J. K. Hamilton and F. Smith, *Anal. Chem.* **28**, 350 (1956)

An International Peer Reviewed Research Journal of Chemistry
Abstracted / Indexed in : Chemical Abstract U.S.A.
JC- 1432/2K21 (ISSN:0973-239X), INDIA
Journal Chemtracks Vol. 23 (1&2),21-24, January to December 2021