

The Influence of benzyl chloride on fermentative bioproduction of ethanol

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Abstract: The influence of benzyl chloride on bioproduction of ethanol by yeast *Saccharomyces cerevisiae* DK-18 has been assessed. It has been found that the mutagen, i.e., benzyl chloride 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 22.827% higher in comparison to control fermentor flasks, i.e., 6.79 ml/100mL in 56 hrs of incubation period, 4.7 pH and 33°C temperature with 22% (w/v) molasses solution.

(Keywords: Molasses, ethanol, mutagens, benzyl chloride, *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. Since the 1960s, however, mutagens have defined a transdisciplinary 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 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.⁵⁻¹⁸

Literature survey reveals that a little work has been done on fermentative production of ethanol by *Saccharomyces cerevisiae* DK-18 exposed to benzyl chloride, therefore, the author has employed benzyl chloride on fermentative production of ethanol by *Saccharomyces cerevisiae* DK-18.

Experimental

The influence of benzyl chloride 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.

Table - 1
The Influence of benzyl chloride on ethanolic alcohol fermentation

Concentration of mutagen used $A \times 10^{-x}$ M	Incubation Period in hours	Yield of ethanol* in ml/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in yield of ethanol in 56 hrs.
Control	46	4.14	5.029	—
(–) Mutagen	56	6.79	3.996	—
	62	5.56	3.869	—
1.0×10^{-5} M	46	4.23	4.939	—
(+) Mutagen	56	6.95	3.838	+ 2.356
	62	5.56	3.784	—
2.0×10^{-5} M	46	4.34	4.829	—
(+) Mutagen	56	7.15	3.637	+ 5.301
	62	6.10	3.574	—
3.0×10^{-5} M	46	4.48	4.689	—
(+) Mutagen	56	7.35	3.436	+ 8.247
	62	6.13	3.365	—
4.0×10^{-5} M	46	4.80	4.367	—
(+) Mutagen	56	7.90	2.889	+ 16.347
	62	6.68	2.798	—
5.0×10^{-5} M	46	4.92	4.249	—
(+) Mutagen	56	8.10	2.689	+ 19.293
	62	7.10	2.593	—

* Each value represents mean of three observations ** Optimum concentration of mutagen used.

*** Optimum yield of ethanol in 56 hours. (+)Values indicate % increase in the yield of ethanol after 56 hours. Experimental deviation ($\pm$) 1.5–3%.

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 benzyl chloride 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 benzyl chloride 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 mutagen in ml,

i.e; from 1.0 mL to 10.0 mL.

and x = molarity of the solution respectively.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 33°C and analysed colorimetrically after 46, 56, and 62 hours for ethanol¹⁹ formed and molasses sugars²⁰ left unfermented.

Concentration of mutagen used $A \times 10^{-x}$ M	Incubation Period in hours	Yield of ethanol* in ml/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in yield of ethanol in 56 hrs.
Control	46	4.14	5.029	—
(-) Mutagen	56	6.79	3.996	—
	62	5.56	3.869	—
6.0×10^{-5} M **	46	5.05	4.119	—
(+)Mutagen	56	8.34***	2.448	+ 22.827
	62	7.25	2.383	—
7.0×10^{-5} M	46	4.71	4.459	—
(+)Mutagen	56	7.76	3.028	+ 14.285
	62	6.67	3.017	—
8.0×10^{-5} M	46	4.42	4.749	—
(+)Mutagen	56	7.33	3.459	+ 7.952
	62	6.22	3.394	—
9.0×10^{-5} M	46	4.26	4.905	—
(+)Mutagen	56	7.01	3.779	+ 3.240
	62	6.10	3.693	—
10.0×10^{-5} M	46	4.18	4.986	—
(+)Mutagen	56	6.87	3.916	+ 1.178
	62	5.70	3.862	—

* Each value represents mean of three observations ** Optimum concentration of mutagen used.

*** Optimum yield of ethanol in 56 hours. (+)Values indicate % increase in the yield of ethanol after 56 hours. Experimental deviation ($\pm$) 1.5–3%.

Results and Discussion

The influence of benzyl chloride :

The data given in the table-1 shows that the mutagen, i.e., benzyl chloride has been found stimulatory for the fermentative bioproduction of ethanol from molasses by *Saccharomyces cerevisiae* DK-18. From the data given in the table it is obvious that benzyl chloride influences the alcoholic fermentation process in different phases. The main characteristics of the benzyl chloride is as follows:

- (i) Benzyl chloride is stimulatory at its all molar concentrations used during course of the alcoholic fermentation, i.e.; from 1.0×10^{-5} M to 10.0×10^{-5} M.

- (ii) The molar concentration 1.0×10^{-5} M, 2.0×10^{-5} M and 3.0×10^{-5} M of benzyl chloride influences the yield of alcohol in a approximately regular doubling order after each stage; ie: 2.356%, 5.301% and 8.247%.
- (iii) The higher molar concentrations, i.e.; 6.0×10^{-5} M to 10.0×10^{-5} M of benzyl chloride influences the yield of alcohol and therefore, the % yield of alcohol has been found to be as mentioned below: 22.827%, 14.285%, 7.952%, 3.240% and 1.178% respectively.

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