

Production of citric acid by *Aspergillus oryzae* NCIM-944 exposed to O-ethylthiocarbamate

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Manuscript received online 04 April 2020, accepted on 05 June 2020

Abstract: The efficacy of O-ethylthiocarbamate on bioproduction of citric acid by fungal strains such as *Aspergillus fischeri* NCIM-508, *Aspergillus foetidus* NCIM-510, *Aspergillus giganteus* NCIM-568, *Aspergillus luchuensis* NCIM-991 and *Aspergillus oryzae* NCIM-944 has been assessed. It has been observed that the fungal strain *Aspergillus oryzae* NCIM-944 has been found most significant and effective for the citric acid fermentation process. It has been found that the compound, i.e., O-ethylthiocarbamate has stimulatory effect on bioproduction of citric acid by *Aspergillus oryzae* NCIM-944 and enhances the yield of citric acid to an extent of 9.599% higher in comparison to control fermenter flasks, i.e., 7.866g/100 ml under the optimized conditions.

(Keywords: Citric acid fermentation, O-ethylthiocarbamate and *Aspergillus oryzae* NCIM-944)

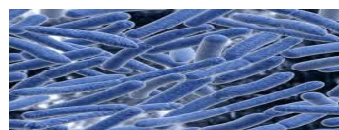
Introduction

The influence of different chemical mutagens in different fermentations process has been investigated by a number of workers¹⁻²⁰. A large number of chemical mutagens are recorded as a good agent for different microbes and microbial processes²¹⁻⁵³. Thus, from the above brief review it is evident that chemical mutagens are required for genetic manipulation and exploitation specially for citric acid fermentation and in view of this the author has studied the influence of O-ethylthiocarbamate on production of citric acid by *Aspergillus oryzae* NCIM-944

Experimental

The influence of O-ethylthiocarbamate on

production of citric acid by *Aspergillus oryzae* NCIM-944.



Aspergillus oryzae

The composition of the production medium for production of citric acid by *Aspergillus oryzae* NCIM-944 has been prepared as follows: Molasses:24.5%(w/v), NH₄NO₃: 0.65%, KH₂PO₄: 0.65%, MgSO₄·7H₂O:0.65%, pH: 2.2 The pH of the production medium was adjusted to 2.2 by adding requisite amount of KCl-HCl buffer solution, and this pH was also ascertained by a pH meter. The above composition medium represents volume of a fermenter flask, i.e., "100ml" production of citric acid by *Aspergillus oryzae* NCIM-944. Now, the same production medium for production of citric acid by *Aspergillus oryzae* NCIM-944 was prepared for 99-fermenter flask, i.e; each contained '100ml' of production medium.

The above 99-fermenter flasks were then arranged to 11-sets each comprising of 9-fermenter flasks. Each set was then rearranged in 3-subsets, each consisting of 3-fermenter flasks. The remaining 9-fermenter flasks out of 99-fermenter flasks were kept as control and these were also rearranged in 3-subsets each consisting of 3-fermenter flasks.

After preparing the above sets of fermenter flasks M/1000 solution of O-

ethylthiocarbamate was prepared and from the above mutagen solution 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 ml was added to the fermentation flasks of above 1st to 10th sets respectively. The control fermenter flasks contained no mutagen. Now, the total volume in each fermenter flasks was made upto 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of O-ethyl thiocarbamate in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

$A \times 10^{-x} \text{ M}$

$1.0 \times 10^{-5} \text{ M}$ to $10.0 \times 10^{-5} \text{ M}$ respectively.

i.e., Where A = amount of mutagen, in ml, i.e. 1.0 ml to 10 ml, x = Molarity of the mutagen solution

The above fermenter flasks were then sterilized, cooled inoculated, incubated at 30°C and analysed after 8, 10 and 12 days for citric acid⁵⁴ formed and molasses left unfermented⁵⁵.

Results and Discussion

The influence of O-ethyl thiocarbamate on production of citric acid by *Aspergillus oryzae* NCIM - 944.

The data recorded in the table-1 shows that the chemical mutagen, i.e; O-ethyl thiocarbamate has also been found stimulatory for the production of citric acid by *Aspergillus oryzae* NCIM - 944 at all its molar concentrations, i.e; from $1.0 \times 10^{-5} \text{ M}$ to $10.0 \times 10^{-5} \text{ M}$ in two phases. In the first phase the molar concentration of chemical mutagens i.e; o-ethyl thiocarbamate from $1.0 \times 10^{-5} \text{ M}$ to $5.0 \times 10^{-5} \text{ M}$ is stimulatory and the production of citric acid gradually increases till a maximum value is reached at $5.0 \times 10^{-5} \text{ M}$, i.e; 8.643g/100ml in 10 days of optimum incubation period which is 9.599% higher in comparison to control, i.e; 7.886g/100ml.

Table - 1
Production of citric acid by *Aspergillus oryzae* NCIM-944 exposed to O-ethylthiocarbamate

Concentration of mutagens used $A \times 10^{-x} \text{ M}$	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in yield of citric acid in comparison to control.
Control	8	5.365	4.939	-
(-) Mutagen	10	7.886	2.416	-
	12	6.767	2.319	-
$1.0 \times 10^{-5} \text{ M}$	8	5.429	4.871	-
(+) Mutagen	10	7.996	2.309	+ 1.394
	12	6.893	2.203	-
$2.0 \times 10^{-5} \text{ M}$	8	5.515	4.789	-
(+) Mutagen	10	8.122	2.176	+ 2.992
	12	7.026	2.079	-
$3.0 \times 10^{-5} \text{ M}$	8	5.622	4.679	-
(+) Mutagen	10	8.280	2.023	+ 4.996
	12	7.186	2.026	-
$4.0 \times 10^{-5} \text{ M}$	8	5.724	4.576	-
(+) Mutagen	10	8.430	1.879	+ 6.898
	12	7.336	1.773	-
$5.0 \times 10^{-5} \text{ M}^{**}$	8	5.869	4.439	-
(+) Mutagen	10	8.643***	1.656	+ 9.599
	12	7.643	1.553	-

Contd.....

Concentration of mutagens used A X 10 ⁻⁵ M	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in yield of citric acid in comparison to control.
Control	8	5.365	4.939	-
(-) Mutagen	10	7.886	2.416	-
	12	6.767	2.319	-
6.0x10 ⁻⁵ M	8	5.751	4.543	-
(+) Mutagen	10	8.469	1.832	+7.392
	12	7.363	1.736	-
7.0x10 ⁻⁵ M	8	5.660	4.643	-
(+) Mutagen	10	8.335	1.969	+5.693
	12	7.236	1.863	-
8.0x10 ⁻⁵ M	8	5.590	4.713	-
(+) Mutagen	10	8.232	2.068	+4.387
	12	7.130	2.063	-
9.0x10 ⁻⁵ M	8	5.493	4.809	-
(+) Mutagen	10	8.091	2.203	+2.559
	12	7.096	2.106	-
10.0x10 ⁻⁵ M	8	5.429	4.873	-
(+) Mutagen	10	7.996	2.306	+1.394
	12	6.893	2.209	-

* Each value represents mean of three trials ** Optimum concentration of mutagen used

*** Optimum yield of citric acid (+) values indicate % increase in the yield of citric acid after 10 days. Experimental deviation (±) 1.5-3%

In the second phase of chemical mutagenic effect, i.e., at 6.0 x 10⁻⁵ M and onwards the molar concentration of O-ethyl thiocarbamate on production of citric acid by *Aspergillus oryzae* NCIM - 944 has been found increased but in lesser quantum in the presence of O-ethyl thiocarbamate. So, the gradual addition of O-ethyl thiocarbamate

after the optimum concentration of 5.0 x 10⁻⁵ M gradually slows down the production of citric acid by *Aspergillus oryzae* NCIM - 944 and the part of % increase at 6.0 x 10⁻⁵ M to 10.0 x 10⁻⁵ M molar concentration of O-ethyl thiocarbamate is in decreasing order.

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