

Bioproduction of citric acid by LSF technique by *Aspergillus niger* NCIM-1856 exposed to 2,3-dibromopropyl benzonate

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Abstract : The efficacy of 2,3-dibromopropyl benzonate on bioproduction of citric acid by LSF (Liquid State Fermentative) technique by fungal strains such as *Aspergillus awamori* NCIM-1869, *Aspergillus foetidus* NCIM-1659, *Aspergillus nidulans* NCIM-1651, *Aspergillus oryzae* NCIM-1756 and *Aspergillus niger* NCIM-1856 has been assessed. It has been observed that the fungal strain *Aspergillus niger* NCIM-1856 has been found most significant and effective for the citric acid fermentation process. It has been found that the mutagen, i.e., 2,3-dibromopropyl benzonate has stimulatory effect on production of citric acid by *Aspergillus niger* NCIM-1856 and enhances the yield of citric acid to an extent of 8.531% higher in comparison to control fermentor flasks, i.e., 6.998g/100 ml under the optimized conditions, viz. 29°C temperature, 2.1 pH, 8 days of incubation period with 16% sucrose solution.

(Keywords : Citric acid fermentation, 2,3-dibromopropyl benzonate and *Aspergillus niger* NCIM-1856)

Introduction

Mutagenesis is the process whereby sudden heritable changes occur in the genetic information of an organism not caused by genetic segregation or genetic recombination, but induced by chemical, physical or biological agents.¹⁻⁸ Biological, chemical, or physical agent, process, or substance that causes permanent genetic change (mutation) in a cell (other than which occurs during normal cell growth) due to genetic alterations or loss of genes or chromosomes. The influence of different chemical mutagens in different fermentations process has been investigated by a number of workers⁹⁻¹². A large

number of chemical mutagens are recorded as a good agent for different microbes and microbial processes¹³⁻³⁷. 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 2,3-dibromopropyl benzonate on bioproduction of citric acid by LSF technique by *Aspergillus niger* NCIM-1856.

Experimental

The influence of 2,3-dibromopropyl benzonate on bioproduction of citric acid by LSF technique by *Aspergillus niger* NCIM-1856. The composition for production of citric acid by LSF technique by *Aspergillus niger* NCIM-1856 has been prepared as follows :

Sucrose: 16% w/v, NH_4NO_3 : 0.45%
 KH_2PO_4 : 0.45%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.50 %
pH: 2.1

The pH of the production medium was adjusted to 2.1 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 fermentor flask, i. e., "100ml" production medium for citric acid fermentation by *Aspergillus niger* NCIM-1856. Now, the same production medium for citric acid fermentation by *Aspergillus niger* NCIM-1856 was prepared for 99-fermentor flask, i. e; each contained

Table - 1
Bioproduction of citric acid by LSF technique by *Aspergillus niger* NCIM-1856
exposed to 2,3-dibromopropyl benzonate

Concentration of mutagen used $A \times 10^{-x} M$	Incubation Period in days	Yield of citric acid* in g/100 ml	Sucrose* left unfermented in g/100 ml	% of citric acid increased after 8 days
Control (-) Mutagen	6	5.348	4.895	—
	8	6.998	3.194	—
	10	6.379	3.172	—
$1.0 \times 10^{-5} M$ (+) Mutagen	6	5.422	4.889	—
	8	7.096	3.188	(+) 1.400
	10	6.468	3.167	—
$2.0 \times 10^{-5} M$ (+) Mutagen	6	5.496	4.882	—
	8	7.194	3.183	(+) 2.800
	10	6.556	3.164	—
$3.0 \times 10^{-5} M$ (+) Mutagen	6	5.529	4.879	—
	8	7.236	3.181	(+) 3.400
	10	6.595	3.160	—
$4.0 \times 10^{-5} M$ (+) Mutagen	6	5.572	4.873	—
	8	7.292	3.176	(+) 4.201
	10	6.646	3.155	—
$5.0 \times 10^{-5} M$ (+) Mutagen	6	5.610	4.869	—
	8	7.342	3.171	(+) 4.915
	10	6.690	3.152	—
$6.0 \times 10^{-5} M$ (+) Mutagen	6	5.635	4.860	—
	8	7.376	3.169	(+) 5.401
	10	6.723	3.148	—
$7.0 \times 10^{-5} M$ (+) Mutagen	6	5.710	4.856	—
	8	7.475	3.166	(+) 6.816
	10	6.812	3.144	—
$8.0 \times 10^{-5} M^{**}$ (+) Mutagen	6	5.802	4.851	—
	8	7.595 ^{***}	3.163	(+) 8.531
	10	6.920	3.139	—
$9.0 \times 10^{-5} M$ (+) Mutagen	6	5.678	4.864	—
	8	7.432	3.171	(+) 6.201
	10	6.774	3.144	—
$10.0 \times 10^{-5} M$ (+) Mutagen	6	5.528	4.873	—
	8	7.236	3.175	(+) 3.400
	10	6.595	3.149	—

* 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 8 days. Experimental deviation ($\pm$) 1.5-3%

'100ml' of production medium.

The above 99-fermentor flasks were then arranged to 11-sets each comprising of 9-fermentor flasks. Each set was then rearranged in 3-subsets, each consisting of 3-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.

After preparing the above sets of fermentor flasks M/1000 solution of 2,3-dibromopropyl benzonate was prepared and from the above mutagenic 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 fermentor flasks contained no mutagen.

Now, the total volume in each fermentor flasks was made upto 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of 2,3-dibromopropyl benzonate in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th 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 2,3-dibromopropyl benzonate in ml, i.e., 1.0 ml .. to 10 ml. x = Molarity of the 2,3-dibromopropyl benzonate solution.

The above fermentor flasks were then

sterilized, cooled inoculated, incubated at 29°C and analysed colorimetrically³⁸ after 6,8 and 10 days for citric acid formed and sucrose³⁹ left unfermented during the course of present investigation.

Results and Discussion

The results of the influence of 2,3-dibromopropyl benzonate on LSF technique for citric acid production by *Aspergillus niger* NCIM-1856 has been recorded in the table-1. It is obvious from the results that the presence of above mutagen in the production medium has enhanced the yield of citric acid at its all molar concentration but to a lesser amount.

It has been noticed that the presence of above mutagenic compounds, i.e., 2,3-dibromopropyl benzonate was found to be beneficial and encouraging for the improved yield of citric acid up to its concentration from 1.0×10^{-5} M to 8.0×10^{-5} M but not so significant and above this concentration the mutagen under trial has retarded the yield of citric acid.

The maximum citric acid production was observed at 8.0×10^{-5} M concentration of 2,3-dibromopropyl benzonate on LSF technique for citric acid production by *Aspergillus niger* NCIM-1856, i.e., 7.595 g/100ml which is 8.531% higher in comparison to control fermentor flasks, i.e., 6.998g/100 ml in 8 days of optimum incubation period.

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