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

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Abstract: The efficacy of chloralhydrate on bioproduction of citric acid by fungal strains such as *Aspergillus fischeri* NCIM – 508, *Aspergillus foetidus* NCIM–510, *Aspergillus giganticus* 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., chloralhydrate has stimulatory effect on bioproduction of citric acid by *Aspergillus oryzae* NCIM - 944 and enhances the yield of citric acid to an extent of 8.490% higher in comparison to control fermenter flasks, i.e., 7.868g/100 ml under the optimized conditions.

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

Introduction

In the rapidly developing field of environmental mutagenesis, the use of standardized protocols has permitted the evaluation of large numbers of chemicals for mutagenic and potential carcinogenic activity.

The influence of different chemical mutagens in different fermentations process has been investigated by a number of workers[1-20]. A large number of chemical mutagens are recorded as a good agent for different microbes and microbial processes[21-52]. Thus, from the above brief review it is evident that chemical mutagens are required for genetic manupulation and exploitation specially for citric acid fermentation and in view of this the author has studied the influence of chloral hydrate on production of citric acid by *Aspergillus oryzae* NCIM - 944

Experimental

The influence of chloralhydrate 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), NH_4NO_3: 0.65% , KH_2PO_4 : 0.65%, $MgSO_4.7H_2O$: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 chloralhydrate was prepared and from the above coumarin 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 coumarin. 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 4-hydroxy-7-methoxy-3-phenyl coumarin in 1st,2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

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

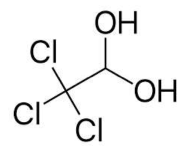
1.0×10^{-5}M to 10.0×10^{-5}M respectively.

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

The above fermenter flasks were then sterilized, cooled inoculated, incubated at 30^0C and analysed after 8, 10 and 12 days for citric acid[53] formed and molasses left unfermented[54].

Results and Discussion

The influence of chloralhydrate on production of citric acid by *Aspergillus oryzae* NCIM - 944.



Chloralhydrate

The data recorded in the table-1 shows that chloralhydrate also has stimulatory effect on

Table - 1

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

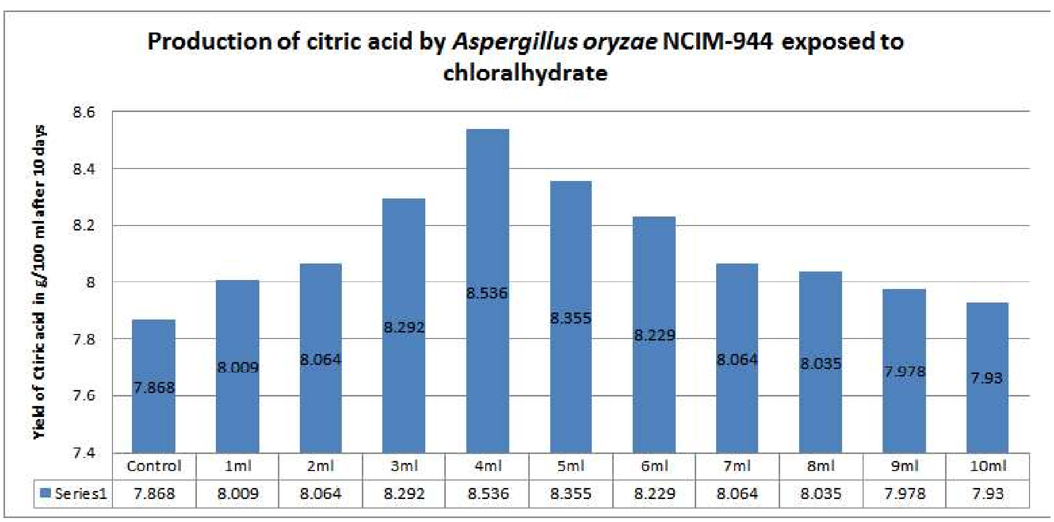
Concentration of mutagens used $A \times 10^{-x}$ M	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in yield of citric acid in comparison to control.
Control	8	5.269	5.036	-
(-) Mutagen	10	7.868	2.439	-
	12	6.786	2.336	-
1.0×10^{-5}M	8	5.353	4.949	-
(+) Mutagen	10	8.009	2.293	+ 1.792
	12	7.003	2.196	-
2.0×10^{-5}M	8	5.390	4.913	-
(+) Mutagen	10	8.064	2.239	+2.491
	12	7.063	2.136	-
3.0×10^{-5}M	8	5.542	4.759	-
(+) Mutagen	10	8.292	2.003	+ 5.388
	12	7.196	2.009	-
4.0×10^{-5}M**	8	5.706	4.593	-
(+) Mutagen	10	8.536***	1.763	+ 8.490
	12	7.439	1.669	-
5.0×10^{-5}M	8	5.585	4.719	-
(+) Mutagen	10	8.355	1.943	+ 6.189
	12	7.253	1.846	-

Contd.....

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

Concentration of mutagens used A X 10^{-x} 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	5.269	5.036	-
(-) Mutagen	10	7.868	2.439	-
	12	6.786	2.336	-
6.0x10^{-5}M	8	5.500	4.832	-
(+) Mutagen	10	8.229	2.079	+4.588
	12	7.123	2.073	-
7.0x10^{-5}M	8	5.390	4.913	-
(+) Mutagen	10	8.064	2.239	+2.491
	12	7.063	2.136	-
8.0x10^{-5}M	8	5.374	4.929	-
(+) Mutagen	10	8.035	2.263	+2.122
	12	7.036	2.169	-
9.0x10^{-5}M	8	5.332	4.969	-
(+) Mutagen	10	7.978	2.326	+1.398
	12	6.873	2.229	-
10.0x10^{-5}M	8	5.300	5.003	-
(+) Mutagen	10	7.930	2.379	+0.788
	12	6.833	2.273	-

* 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%



Concentration of Chloralhydrate 1 × 10^{-5}M to 10 × 10^{-5}M production of citric acid by *Aspergillus oryzae* NCIM - 944. The data (vide table-1) reveals that thechemical mutagen chloralhydrate stimulates the production of citric acid by *Aspergillus oryzae* NCIM - 944 and enhances the yield of citric acid upto its chloralhydrate 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 chloralhydrate on the productivity (yield) of citric acid was gradually in increasing order and attains its best function at 4.0×10^{-5} M where maximum yield of citric acid, i.e; 8.536g/100 ml is given in 10 days of optimum incubation period which is 8.490% higher in comparison to control fermentor flasks (7.868g/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 citric acid has been enhanced but the order of citric acid productivity is reversed in respect to increasing molar concentrations of chloralhydrate. However, the production of citric acid by *Aspergillus oryzae* NCIM-944 under the influence of each concentration of chloralhydrate used has been found stimulating and the yield of citric acid has been found greater than that obtained in the control fermentor flasks. In both the phases the order of productivity and % of citric acid formed after 10 days is as under :

Concentration of chloralhydrate from 1.0×10^{-5} M to 4.0×10^{-5} M

Productivity of citric acid: 1.792%, 2.491%, 5.388% and 8.490%

Concentration of chloralhydrate from 5.0×10^{-5} M to 10×10^{-5} M.

Productivity of citric acid : 6.189%, 4.588%, 2.491%, 2.122%, 1.398% and 0.788%

The exposure on the fungal strain *Aspergillus oryzae NCIM* - 944 to chloralhydrate may produce a variety of effects. Depending upon the concentration of chloralhydrate to which the fungal strain *Aspergillus oryzae NCIM* - 944 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 chloralhydrate at lower concentrations is stimulatory and at higher concentrations is deterioratory for production of citric acid by *Aspergillus oryzae* NCIM - 944

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