

The influence of N-methyl hydroxylamine on citric acid fermentation by *A.niger* HK²-5

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Abstract : The influence of N-methyl hydroxylamine on citric acid fermentation by *A. niger* HK²-5 has been studied. It has been found the mutagen N-methyl hydroxylamine has stimulatory effect on citric acid fermentation and enhances the yield of citric acid to an extent of 12.95376% higher in comparison to control fermentation flask, i.e., 9.063 g/100ml under the optimized condition.

(Keywords: Citric acid, fermentation, N-methyl hydroxylamine, *A. niger* HK²-5)

Introduction

A large variety of compounds^{1,2} like peroxides, caffeine, gaseous butadiene, ethylene and thiourea causes mutation in different microbes. Peroxides and epoxides as mutagenic chemicals were also reported by a group of workers^{3,4}, as a very specific mutagens. Nishi *et al*⁵ and others⁶⁻¹⁰ have worked on different mutagenic chemicals and some mutagens on different microorganisms on fermentation process such as N-methyl-N-nitroso urea, EMS, or x-rays to induce the microbial process and achieve the improved yields. Mahana¹¹ and Khan¹² have also reported the mutagenic properties of ethyl methan sulphonate (EMS). 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 N-methyl hydroxylamine on production of citric acid.

Experimental

Preparation of production medium

Molasses : 30% w/v, NH₄NO₃ : 0.25%, MgSO₄·7H₂O : 0.025%, KH₂PO₄ : 0.10%, pH : 2.2, Distilled water : To make up 100 ml, Temperature : 31°C, pH was adjusted 2.2 by adding requisite amount of KCl-HCl buffer solution.

Assay Methods : Citric acid¹³ formed and molasses¹⁴ left and used was estimated colorimetrically.

M / 1000 solution of N-methyl hydroxylamine of mutagen has been used.

Results and Discussion

The influence of N-methyl hydroxylamine (CH₃-NH-OH) has been reported (vide table-1). The data shows that N-methyl hydroxylamine was found to be increasing the production of citric acid upto its molar concentration from 1.0×10^{-5} M to 6.0×10^{-5} M and above this molar concentrations the production of citric acid was found to be decreasing. However, production of citric acid at all the experimental concentrations of N-methyl hydroxylamine used were higher than that of control fermentor flasks. Hydroxylamine behaves as a typical mutagen. N-methyl hydroxylamine is substituted with methyl group in parent hydroxylamine. Methyl group has marked influence and enhances hypnotic action of the compound whereas the entrance of -OH group into a compound generally enhances its biological properties. Thus, it may be concluded that due to the presence of -CH₃ and -OH groups the compound becomes physiologically active and appreciably held responsible for higher fermentative transformation of molasses to citric acid by *A.niger* HK²-5

Table - 1
The influence of N-methyl hydroxylamine on citric acid fermentation by *A. niger* HK²-5

Concentration of mutagen used	Incubation Period in days	Yield of citric acid in g/100 ml	Molasses left unfermented in g/100 ml	% Difference in yield of citric acid increase (+) in 15 days
Control	15	9.063	3.436	—
1.0×10^{-5} M	15	9.080	3.429	+0.18757
2.0×10^{-5} M	15	9.098	3.422	+0.38618
3.0×10^{-5} M	15	9.299	3.346	+2.60399
4.0×10^{-5} M	15	9.676	3.203	+6.76376
5.0×10^{-5} M	15	10.092	3.045	+11.35385
6.0×10^{-5} M**	15	10.237***	2.992	+12.95376
7.0×10^{-5} M	15	10.228	2.994	+12.85446
8.0×10^{-5} M	15	10.198	3.005	+12.52344
9.0×10^{-5} M	15	9.886	3.123	+9.08087
10.0×10^{-5} M	15	9.762	3.170	+7.71267

*Mean of three trials ** Optimum concentration of chemical mutagen, i.e., N-methyl hydroxylamine

*** Optimum yield of citric acid (+) Values indicate % increase in the yield of citric acid

In 15 days of optimum incubation period the maximum production of citric acid was found to be 10.237 g/100 ml at 6.0×10^{-5} M concentration of N-methyl hydroxylamine which is 12.95376%

higher in comparison to control fermentor flasks in the same incubation period and experimental conditions.

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