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*NOTE*

# Studies on Fermentative Transformation of Molasses to Citric Acid by *A. niger* Exposed to Some Chemical Mutagens

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Mutagenic action of 1,1-dimethyl hydrazine, 1,2-dimethyl hydrazine dihydrochloride, hydrazine sulphate an hydrazine acetate were studied on fermentative transformation of molasses to citric acid by *A. niger*. It was found that hydrazine sulphate and hydrazine acetate enhance the production of citric acid to a great extent while 1,1-dimethyl hydrazine and 1,2-dimethyl hydrazine dihydrochloride retard the yield.

Producdtion of citric acid by fermentation has been placed in the category of difficult processes. In India, several attempts have been made to develop an indigenous citric acid process, but all of them proved to be unsuccessful. Work on the development of a process for the production of citric acid by fermentation was started in this Laboratory a few years ago. A series of hydrazine derivatives and other chemical mutagens[1–9] have been used in this laboratory for different microbial processes. Besides some other chemical mutagens[10–17] have also been studied by many workers on different microbial strains. The present communication deals with the assessment and analysis of the fermentative production of citric acid by *A. niger* V-18 exposed to some chemical mutagens of hydrazine groups.

*Medium*: The composition of the production medium for 100 mL flask each was prepared as follows: Molasses (22% w/v); $NH_4NO_3$ (0.224%); $MgSO_4 \cdot 7H_2O$ (0.025%); $KH_2PO_4$ (0.10%), pH 5.4; distilled water, to make up 100 mL; inoculum: 0.05 mL conidial suspension of *A. niger* V-18; incubation periods; 8, 10 and 12 days; optimum incubation period: 10 days; sterilization; the production medium was sterilized at 15 lbs for 20–25 min. in an autoclave.

*Organism*: *Aspergillus niger* V-18 was used as citric acid producing strain. It was stored in a refrigerator and reinoculated before immediate use. The strain was inoculated into 1 L baffled shake flasks containing 100 mL medium. These were grown at 30°C overnight and then inoculated into the fermentor. After inoculation the pH was kept constant with KCl-HCl buffer solution.

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*Assay method*: Evaluation of the citric acid formed and cane molasses left unfermented was made colorimetrically.[18, 19]

The results obtained in the study of the influence of different chemical mutagens on fermentative transformation of molasses to citric acid by *A. niger* V-18 for maximum production in 10 days of optimum incubation period are tabulated in Table -1

TABLE-1
CITRIC ACID FERMENTATION EXPOSED TO SOME CHEMICAL MUTAGENS

| Chemical mutagens | Optimum concentration of the mutagens used | Max. yield of citric acid* in control flasks in g/100 mL | Max. yield of citric acid* in the presence of different mutagens in g/100 mL | % Difference in the yield of citric acid increse/decrease in 10 days of optimum incu ation period |
|---|---|---|---|---|
| 1,1-dimethyl hydrazine | $1.0 \times 10^{-5}$ M | 6.3895 | 6.2066 | (–) 2.8620 |
| 1,2-dimethyl hydrazine dihydrochloride | $10 \times 10^{-5}$ M | 6.3893 | 6.0861 | (–) 4.7449 |
| Hydrazine sulphate | $6.0 \times 10^{-5}$ M | 6.3894 | 7.2070 | (+) 12.7966 |
| Hydrazine acetate | $5.0 \times 10^{-5}$ M | 6.3920 | 7.2108 | (+) 12.8107 |

*Each value represents mean of three trials. +ve/–ve values indicate % increase//decrease in the yield of citric acid in 10 days of optimum incubation period. Experimental deviation ± 1.5 to 2.5 %

The results show that 1,1-dimethyl hydrazine has toxic effect on fermentative transformation of molasses to citric acid by *A. niger* V-18 at all concentrations used. The maximum yield of citric acid 6.2066 g/100 mL was observed at $1.0 \times 10^{-5}$ M and even this was lower than that obtained in the control flasks. The influence of 1,2-dimethyl hydrazine dihydrochloride has been found very poor for the production of citric acid. Its specific mutagenic action has been recorded at approximately lower concentration. It has inhibitory effect and the yield of citric acid has been found to be very low *i.e.* 6.0861 g/100 mL which is 4.7449% lower in comparison to control flasks.

The influence of hydrazine sulphate and hydrazine acetate on the fermentative production of citric acid was found to be very significant. The maximum yield in the presence of hydrazine sulphate was found to be 7.2070 g/100 mL which is 12.7966% higher in comparison to the control flasks while the yield of citric acid in the presence of hydrazine acetate was more encouraging and was found to be 7.2108 g/100 mL being 12.8107% higher in comparison to the control flasks in the same experimental conditions and optimum incubation period.

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