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Effect of Alcohols on the Production of Citric Acid by *Aspergillus niger* using Solid State Fermentation

Hamad Ashraf, Asad-ur-Rehman and Ikram-ul-Haq
Biotechnology Research Centre, Department of Botany, GC University, Lahore, Pakistan

Abstract: The present study is concerned with the effect of alcohols such as ethanol and methanol on the biosynthesis of citric acid by *Aspergillus niger* strain GCB-3. Methyl alcohol in the concentration of 25 ml kg^{-1} of the substrate gave maximum production i.e. 45.85 g kg^{-1} while when ethanol was added the maximum production achieved was 40.65 g kg^{-1}. The sugar consumption was 111g kg^{-1} and 110 g kg^{-1} respectively. The kinetic parameters such as product yield coefficient ($Y_{p/x}$), and volumetric rate for product formation (Q_p) and substrate consumption (Q_s) were also found to be significant at 25 ml kg^{-1} methanol concentration.

Key words: Citric acid, ethyl alcohol, methyl alcohol, molasses, wheat bran

Introduction

The production of citric acid by fermentation has been a worth praising achievement in the field of industrial microbiology. There are three principal methods available today for fungal biosynthesis of this acid i.e. submerged, surface culture and solid state technique. The solid state fermentation was first described by Cahn (1935). In this case fermentation medium is impregnated in porous solid material such as sugarcane bagasse (Prescott and Dunn's, 1987). Among the variety of stimulants tested for improving citric acid production, methanol and to a smaller extent ethanol are beneficial in increasing citric acid yields (Ali *et al.*, 2002 and Saha *et al.*, 1999). In the present investigation the effect of various concentrations of methanol and ethanol was studied for the enhancement of citrate production using wheat bran and molasses.

Materials and Methods

Organism and Culture Maintenance: *Aspergillus niger* strain GCB-3 was taken from the available stock culture of Biotechnology Research Centre, Department of Botany, GC University, Lahore. This culture was maintained on potato dextrose agar slants at 4°C in a cool lab.

Inoculum Preparation: The conidial inoculum was used in the present study. Conidia from 5-7 days old culture were used for inoculation. The conidial suspension was prepared in sterilized 0.005% Monoxal O.T. (Dioctyl ester of sodium sulfosuccinic acid). Ten ml of solution of Monoxal O.T. was added to each slant having profuse conidial growth on its surface. The test tube was shaken vigorously for breaking the clumps of conidia.

Sterilization: All the media unless or otherwise stated were autoclaved at 15 lb in^{-2} pressure (121°C) for 15 minutes.

Fermentation Technique: The solid state technique was employed. The wheat bran and molasses were used as basal substrate. Ten g of wheat bran and 5 g of molasses were taken in 250 ml cotton wool plugged conical flasks. The mixture was stirred with a glass rod in order to homogenize it. The pH of the solution was adjusted to 6. One ml of the conidial suspension was transferred to each flask after sterilization. The flasks were then incubated at rotary incubator shaker for 168 h.

Analytical Methods: The sugar was estimated according to the method of Tasun *et al.* (1970). The citric acid from the fermented mash was extracted by adding 100 ml distilled water to each flask. The flask were rotated at rotary incubator shaker for 1 h. The contents of the flask were filtered through Whatmann filter paper no. 44. The citric acid was calculated by Pyridine acetic anhydride method as reported by Marrier and Boulet, (1958).

Results and Discussion

Various alcohols have been found to affect citric acid fermentation profoundly. They exert stimulatory effect on citrate biosynthesis (Roukas and Kotzekidou, 1987). The use of methanol in citric acid fermentation was first reported by Moyer (1953). Fig. 1 exhibits the effect of methanol on citric acid accumulation by *Aspergillus niger*

GCB 3 in solid state fermentation. The citric acid was accumulated in maximum amounts (45.85 g kg^{-1}) when methanol was added to the substrate at the concentration of 25 ml kg^{-1}. The sugar consumption was 111 g kg^{-1}. The addition of ethanol, however, was not found to be much significant.

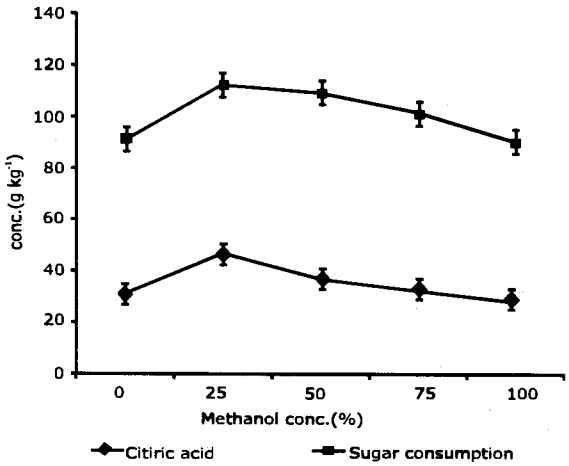

Fig. 1: Effect of methanol concentration on citric acid production by *Aspergillus niger* GCB 3 using agricultural by products

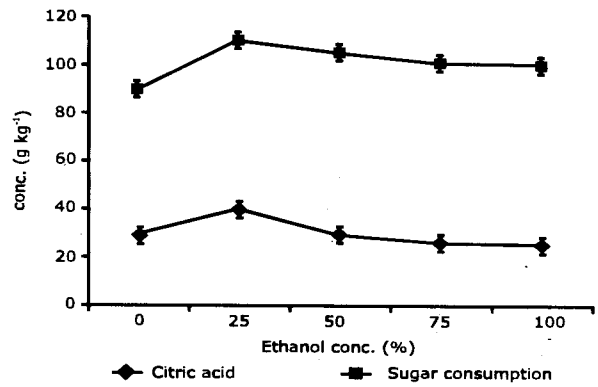

Fig. 2: Effect of ethanol concentration on citric acid production by *Aspergillus niger* GCB 3 using agricultural by products

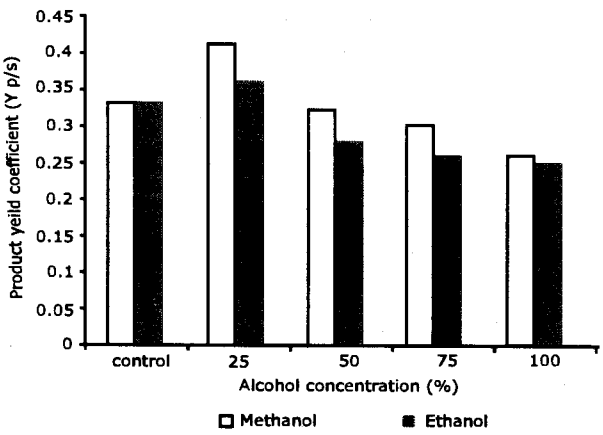

Fig. 3: Comparison of product yield coefficient for citric acid fermentation at various alcohol concentrations

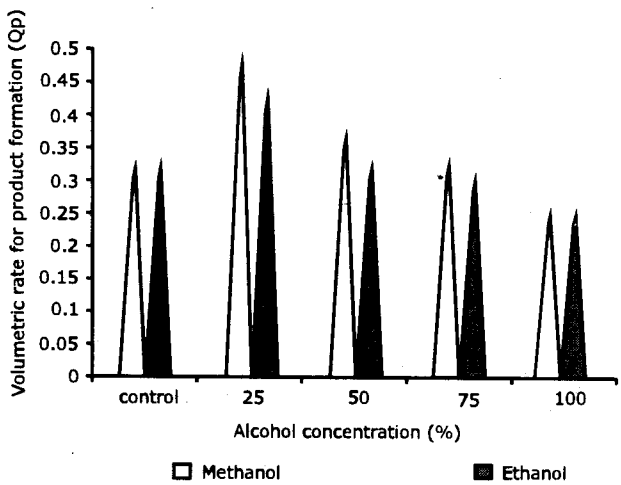

Fig. 4: Comparison of volumetric rate for product formation in citric acid fermentation at various alcohol concentrations

The maximum amount of citric acid produced in case of ethanol was 40.65 g kg^{-1} at the expense of 110 g kg^{-1} sugar (Fig. 2). Methanol concentration (25 ml kg^{-1}) was sufficient in conditioning mycelia for citric acid production without impairing their metabolism. This finding is in accordance with the work reported by Chaudhary *et al.* (1978). The production of citric acid was decreased by increasing the concentration of alcohols. It may be due to the toxic inhibitory effect of high concentration of alcohols. This is in accordance with the work reported earlier (Manonmani and Srikantiah, 1988). Also in the presence of methanol (25 ml kg^{-1}), the utilization of sugar by the fungus was more better i.e. 41.30 %* than in case of ethanol (36.95 %)*.

The kinetics of the research work was studied after Pirt (1975).The study of various kinetic parameters such as product yield coefficient ($Y_{p/x}$ = 0.41), product formation rate (Q_p = 0.47) and volumetric rate for substrate consumption (Q_s = 1.15) also revealed that methanol in 25ml kg^{-1} concentration was sufficient for maximum

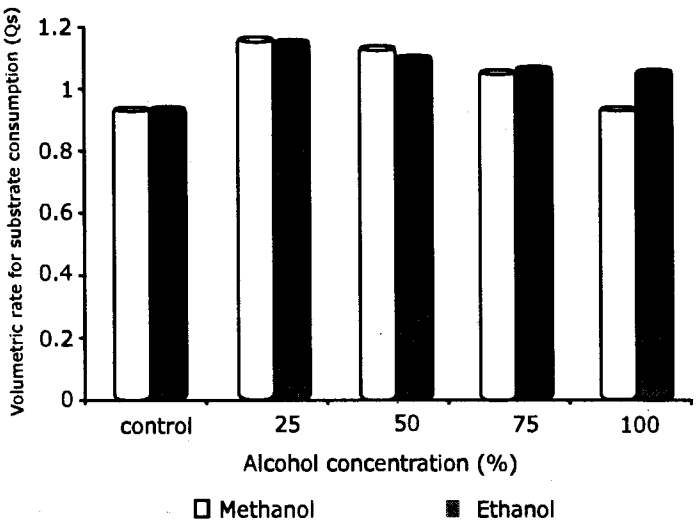

Fig. 5: Comparison of volumetric rate for substrate consumption in citric acid fermentation at various alcohol concentrations

accumulation of citric acid in the fermented mash (Fig.3 , 4 and 5). The volumetric productivity of citric acid and its yield by sugar consumption was higher in the presence of methanol (25 ml kg^{-1}) and varies significantly than other concentrations. Thus the addition of methanol at the level of 25 ml kg^{-1} was selected for the maximum secretion of citric acid from the mycelia.

* = percentage of citric acid produced on the basis of sugar consumed.

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