Microbial production of citric acid by *Aspergillus niger* different carbon sources

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ABSTRACT

A static fermentation was developed for citric acid production from are flower of *Madhuk*a *longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba* (200 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH_2PO_4 (100 mg), $NaNO_3$ (400 mg), $MgSO_4.7H_2O$ (20 mg), and adjust 4.5 initial pH with HCL and 0.1 NaOH. The flask was cotton plugged and autoclaved at 121^0 C for 15 minutes. After cooling at room temperature each medium inoculated with 1.0 ml (6.0×10^6) of *A. niger* (selected strains i.e. A, B, and C) conidial suspension and incubated at 28^0 C in static incubator for 8 days. After fermentation, the medium was diluted with distilled water (1:4 W/V). The medium was filtered and the filtrate was used for the subsequent analysis. In this study were found that the *A. niger* –B gives the highest yield of citric acid that is 8.3 grams in given condition, medium and incubation time.

Key Words: A static fermentation, *Aspergillus niger*, citric acid,from are flower of *Madhuk*a *longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba*

INTRODUCTION

One of the most important fungi used in industrial microbiology, *Aspergillus niger* has been employed for many years for the commercial production of citric acid (Schuster et al., 2002). Citric acid is produced commercially from the fermentation of bulk hydrated materials and by-product of sugar production by *A.* niger (Lesniak et al., 2002). However, the worldwide demand for citric acid is increasing faster than its production and more economical processes are required (Alvarez-Vasquez et al., 2000). It was well known fact that growth and production of *A. niger* are strongly affected by the medium composition, fermentation parameters and stimulators. Thus, citric acid productivity by *A. niger* can be improved by optimizing the fermentation conditions.

Citric acid is an intermediate in the TCA cycle and its accumulation is strongly influenced by the balance of nutrients. The type and concentration of the carbon source,

especially glucose and sucrose, has a significant effect on citric acid production. In general, the final concentration of citric acid increases as the initial concentration of the carbon source is increased (Papassiopi et al., 1999). Also, citric acid production by *A. niger* also depends on presence of other nutrients such as nitrogen, phosphorous, potassium and other salts (Jianlong and Ping, 1998; Wen and Chen, 2001).The limitation or starvation of nitrogen, phosphorus or other trace elements during the fermentation resulted in the limited growth of *A. niger* and to the enhancement of citric acid production (Mirminachi et al., 2002). In addition to the basal nutrients, to improve citric acid production, stimulators such as organic solvents, Phytate and lipids can be applied (Jianlong and Ping, 1998).

MATERIALS AND METHODS

For the proposed research work the investigator has framed work into different parts.

1) **Isolation of organism (*Aspergillus niger*):**
   a) **Isolation of Rhizosphere Soil From 54 places and 23 species of trees of Nandurbar District:**

The natural source of *Aspergillus niger* is soil. For the isolation of high citric acid yielding strains of *Aspergillus niger*, for this propose soil samples from 54

b) Serial dilution method:

In this way, 54 different samples were collected. Each sample was diluted in serial dilution so as to get 1/100, 1/1000 and 1/10000 dilution of each sample. These dilutions (0.1 ml) of each sample were spread separately on sterile Potato-Dextrose agar (PDA) medium containing streptomycin and Penicillin to avoid growth of bacteria.

c) Classification, Identification and Maintenance of the fungal cultures:

The young *Aspergillus niger* colonies were picked up and transferred to potato dextrose agar (PDA) slants. The cultural and morphological characteristics of *A. niger*isolate were observed.

The PDA slants were then inoculated by transferring a small amount of *A. niger*conidia from the petri plates and incubating at 30^0 C (4-6 days) for maximum sporulation. Culture was kept in refrigerator at 4^0C for further study.

d) Screening isolated cultures for citric acid production:

To study the *Aspergillus niger* strain producing organic acid from carbon substrates can be detected by incorporation of pH indicators bromocresol dye in Potato Dextrose agar medium and growing fungal strain on it. A color change of medium from blue to yellow in the vicinity of colony indicates organic acid production.

e) Preparation of conidial inoculation:

Conidial inoculation was used in the present study. Conidia from 4-6 days old slant culture were used for the inoculation. Ten milliliters of sterilized distilled water was added to slant having profuse conidial growth on its surface. An inoculum needle was used to break the conidial clamps. The tubes were shaken vigorously to obtain a homogenous mixture of

the conidial suspension. The homogenous mixture of the conidial suspension was added in distilled water and makes a final 100 ml. The soil suspension was further diluted to 10^4 - 10^6 times. One milliliter of this diluted suspension was transferred to submerged fermentation medium for estimation of citric acid.

f) Designing of media for fermentation of citric acid production:

Growth and production of microorganism are strongly affected by the medium composition such as concentrations of carbon, nitrogen, phosphorous, and potassium. Thus, citric acid productivity by *A. niger* can be improved by optimizing the medium composition. In this respect there was select for Carbon source which are flower of *Madhuka longifolia*, Flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba.*

g) Fermentation technique:

The selected carbon sources which are flower of *Madhuka longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba.* Were cut in small spices and crushed in mixture machine with 20 ml distilled water and the mixture containing KH_2PO_4, $NaNO_3$, $MgSO_4.7H_2O$, and adjustment of pH of medium was added into individual 250 ml cotton plugged conical flasks. The flasks were autoclaved at 15.0 lbs/in^2 pressure for 15 minutes. After cooling at room temperature the flasks were inoculated with 1.0 ml of conidial suspension and incubated at 30^0 C in incubator. The ingredients of the flasks were then filtered and filtrate was used for the estimation of citric acid.

h) Estimation of citric acid production:

After fermentation medium was diluted with distilled water (1:4 W/V). The medium was then filtered and filtrate was used for the subsequent analysis.

Citric acid was determined titrimetrically by using 0.1 NaOH and phenolphthalein as indicator and calculated as % according to the formula:

$$\% \text{ citric acid} = \frac{\text{Normality X volume of 0.1 M NaOH X Equivalent weight of citric acid X dilution factor}}{\text{Weight of sample (g) X 10}}$$

(Kareem, S. O.et al. 2010)

RESULT AND DISCUSSION

The investigation made under this work can be summarized as follows:

a) Isolation and Screening of *Aspergillus niger* strain:

Fifty four *Aspergillus niger* strains were isolated from Nandurbar district among the 54 different strains isolated from different soil types only three strain were found to be competent for citric acid production. These strains selected by following method:

First screening: Done in 54 rhizosphere soil sample. It gives higher potential power of strain of *Aspergillus niger* for citric acid production, in this manner 5 strain are selected from 54 rhizosphere soil sample strain of *A. niger* which are collected from soil sample of village Bhagdari, Khuntamovli, Leghapani, Morakhi and Valmba.

2nd screening: The prepared medium of PDA of 10 ml are filled in petri plate in laminar air flow and allowed to cool at room temperature. Approximately small quantity of the conidial spot five *A. niger* was given aseptically to each of these petri plates. The petri plates incubated at 30^0 C for 12 hours. Yellow zones due to citric acid formation were formed. On the basis of larger citric acid zone compared with control, the best strains of *Aspergillus niger* were picked and transferred to the PAD slants this strains are isolated from soil sample of Leghapani, Bhagdari and Valmba village from trees of *Bauhinia variegate* L., *Madhuka longifolia* (Koem.) and *Tectona grandis* L. respectively.

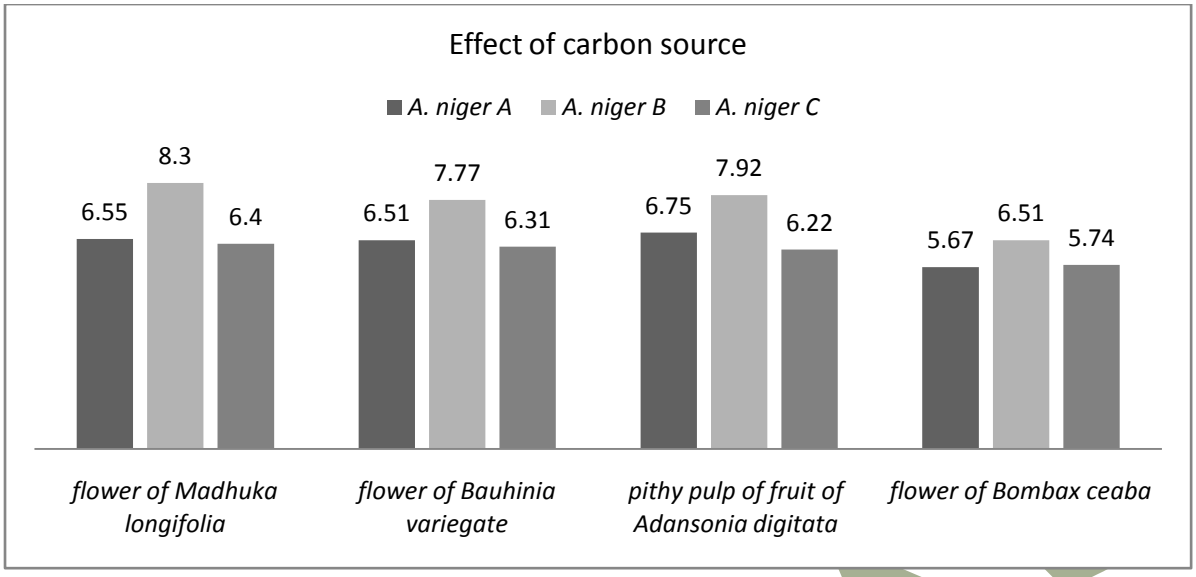
The cultures were incubated at 30^0 C for 4-6 days until maximum sporulation. Three *A. niger*isolates were selected from five strains of *A. niger* for further submerged fermentation.

Abbreviation for these three strains:

Sr. No.	Place	Name of trees	Abbreviation
1	Leghapani	*Bauhinia variegate* L	A
2	Bhagdari	*Madhuka longifolia* (Koem.)	B
3	Valmba	*Tectona grandis* L	C

Effect of carbon sources:

Medium preparation: The selected carbon sources which are flower of *Madhuka longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba* (200 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH_2PO_4 (100 mg), $NaNO_3$ (400 mg), $MgSO_4.7H_2O$ (20 mg), and adjust 4.5 initial pH with HCL and 0.1 NaOH. The flask was cotton plugged and autoclaved at 121^0 C for 15 minutes. After cooling at room temperature each medium inoculated with 1.0 ml (6.0×10^6) of *A. niger* (selected strains i.e. A, B, and C) conidial suspension and incubated at 28^0 C in static incubator for 8 days. After fermentation, the medium was diluted with distilled water (1:4 W/V). The medium was filtered and the filtrate was used for the subsequent analysis.



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