Production of citric acid (CA) by *Aspergillus niger* fermentation method

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Abstract - Citric acid is important tricarboxylic acid which is applied in the different fermented food items, beverages and pharmaceutical industries. CA is colourless and weak organic acid. Ancient source was citrus fruits which contain 6 to 9% of citric acid. The processing on citrus fruits and precipitation with calcium salt CA is extracted. Traditional method citrus fruits were not sufficient for fulfill required growing demand of citric acid production. Hence alternative source of citric acid is a microbial fungal origin. Many fungi were involved in the production of citric acid (Behera et al, 2021).

In the present study it was found that *Aspergillus niger* considered more suitable microorganism as compare to the other microorganisms. In the present study production of citric acid was carried out by isolating higher yielding strain by fermented broth method (Ajala A.S. et al, 2020). Microorganism *Aspergillus niger* was more suitable. In vitro many factors were affecting microbial production of citric acid. It was confirmed from tabular reading that sucrose was a suitable as compared to glucose as a carbon source (Currie, 1917) for in vitro citric acid production. Along with the sucrose many other factors were affecting fungal microbial origin production of citric acid like strain of microorganism, methanol, temperature, carbon source, pH, nutrients etc. were considered (Peksel & Kubicek, 2003). Pure strains of *Aspergillus niger* were obtained from soil samples collected from different localities and lemon fruits a natural resource of CA. It was clear from experimental results 2% methanol added into culture medium gives higher yield, 13% sucrose and 4.5 P^H was suitable for higher production of citric acid in vitro condition. Healthy growth of *Aspergillus niger* was found at 28-30 ^{0} C temperature in vitro, Aftab Nadeem et al (2010).

Key Words: Soil, lemon fruit, Fermentation, Carbon source, Citric acid, Aspergillus *niger etc.*

INTRODUCTION

Citric acid is widely used flavorings agents in different food stuff. CA is having medicinal antioxidant properties. Hence CA required for fermented food items, beverages, pharmaceutical industries and other industries (Kapoor et. al, 1982). Artificially microbial fungal (*A. niger*) produced Citric acid was produced in vitro by submerged fermentation method. The yield of citric acid was affects different parameters in the nutrient composition of culture medium and on the vigor of microbial strain (Aftab Nadeem, et al, 2010). Citric acid production using fungus *A. niger* was affected by the initial sucrose concentration, pH, other nutrient concentration, additive, incubation period, temperature etc. However, growing demand of Citric acid production is increasing day by day. Citrus fruits were not sufficient hence alternative method needed. The fitness of solution would be measured by determining the total weight of the proposed solution. Healthy growth of fungus means more quantity of citric acid (K. Anand Kishor, et al 2008).

In the present study screened different factors for production of citric acid like methanol, higher yielding strain of *A. niger*, carbon source, pH etc. were considered. Isolation and screening of *Aspergillus niger* from soil sample collected from different localities, suitable 2% methanol added into culture medium gives higher yield, 13% sucrose concentration was suitable for higher yield of CA than the glucose, 4.5 P^H was found more suitable and in 28-30^0 temperature healthy growth was found for production of citric acid (Chaturvedi Madhusudan et-- al 2010).

MATERIALS AND METHODS:

Aspergillus niger is a fungus recommended for the production of various metabolites. Citric acid is one of the important organic acids synthesized and released on synthetic medium by this fungus. Natural source of *Aspergillus niger* is a soil, hence soil from different localities from Nashik District was collected and tested for higher yielding strain of *Aspergillus niger*

for the production of citric acid. It also isolated from lemon fruits.

1. Screening of *Aspergillus niger* for citric acid production: Selection and isolation of micro-organism *A. niger* strain produces high amount of citric acid in vitro as a alternative source. Primary screening determines which microorganism is able to produce a citric acid followed by secondary screening to determine capacity of that organism producing quantitative yield (Abdullah – Al –Mahin et al 2004).

2. Isolation of *Aspergillus niger*: Isolation of selected strain from different localities soil and lemon fruits. Soil samples were collected from different locations with respect to P^H of the soil. Ten soil samples were collected and brought into the laboratories. Soil dilutions of 1/100, 1/1000 and 1/10000 of each sample were prepared separately. The soil samples were tested and purified by sub culturing on suitable culture medium (Czepecks Dox agar slants). Culture Plates were incubated at 30 ^{0}C in the culture room and isolated cultures were kept for observations. *Aspergillus niger* strains were purified by sub culturing on Czepecks Dox agar slants. Isolation of *A. niger* isolated from lemon fruits and brought into pure culture by sub culturing on culture medium and maintained. Similar to soil screening high yielding strains were analyzed and brought into pure cultures saved for further studies. Also from lemon fruits *A. niger* strains were also tested for citric acid production in vitro.

Similarly, fresh and healthy lemon fruits were selected. Cleaned lemon fruits were soaked in the water and placed in the closed container for a week (5-7 days). After incubation pure cultures of *Aspergillus niger* isolated from lemon fruits and maintained on suitable culture medium.

3. Screening for organic acid production: Spore from slant cultures were inoculated on sterile Czepeck's Dox agar culture medium plates incorporated with Bromo-cresol green dye. Inoculated *A. niger* culture plates were incubated at 28^{0}C temperature in the culture room for one to two days and analyzed color change blue to yellow indicates organic acid production.

To study the effect of different parameters in culture media on citric acid production were carried out. All the components in the culture medium except one to be studied are kept constant with respect to the control medium and one component concentration is changed in particular range. Fermented broths were tested for the yields of citric acid by selected pure strains of *Aspergillus niger* (Lende A. V. et al, 2021)

Table No.1: Screening for citric acid yield and selection of higher yielding strains

Sr. No.	Area	PH	Culture	Yield mg/ml
1.	Medium sized Lemon fruits (MLF)	2.6	MLF1	4.6
		2.9	MLF2	3.4
		3.5	MLF3	4.0
		3.1	MLF4	2.7
		3.4	MLF5	3.8
2.	Deola Forest soil(DFS)	6.2	DFS1	2.4
		6.1	DFS2	3.1
		5.9	DFS3	4.1
		5.4	DFS4	2.8
		6.1	DFS5	3.3
3.	College Campus garden Soil (CCG)	4.8	CCG1	2.5
		5.2	CCG2	2.7
		4.9	CCG3	1.5
		5.1	CCG4	1.7
		4.8	CCG5	2.9

Graph No.1: Screening for citric acid yield and selection of higher yielding strains

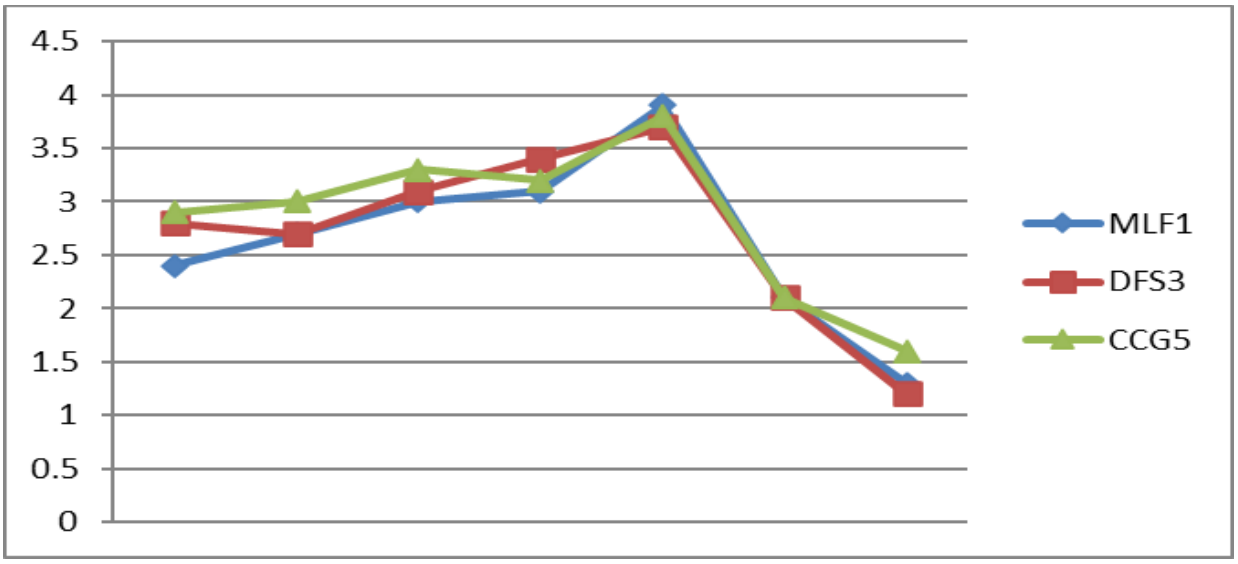


Table No.2: Effect of different carbon source % on the production of CA by *Aspergillus niger*

Carbon source	Yield mg/ml Sucrose			Yield mg/ml Glucose			Yield mg/ml Galactose			Yield mg/ml Fructose		
%	MLF 1	DFS 3	CCG 5	MLF 1	DFS 3	CCG 5	MLF 1	DFS 3	CCG 5	MLF 1	DFS 3	CCG 5
10	1.7	1.8	1.9	1.3	1.2	1.1	1.4	0.9	0.8	1.1	1.4	1.2
11	1.8	1.7	2.0	1.5	1.5	1.4	1.2	0.8	0.7	1.3	1.5	1.6
12	2.2	2.6	2.5	1.9	1.5	1.8	1.5	0.6	1.0	1.2	1.7	1.4
13	4.6	3.8	3.5	2.9	2.4	2.0	1.7	1.2	1.6	1.6	1.8	1.2
14	3.1	2.8	2.7	2.1	2.4	1.9	1.2	1.5	1.7	1.9	2.0	1.6
15	2.8	2.5	2.4	1.8	1.6	1.6	0.8	0.9	0.8	1.7	1.2	1.4
16	1.9	1.6	1.7	1.2	1.3	1.2	0.6	0.8	0.6	0.9	0.9	0.8

Graph No.2: Effect of different carbon source % on the production of CA by *Aspergillus niger*

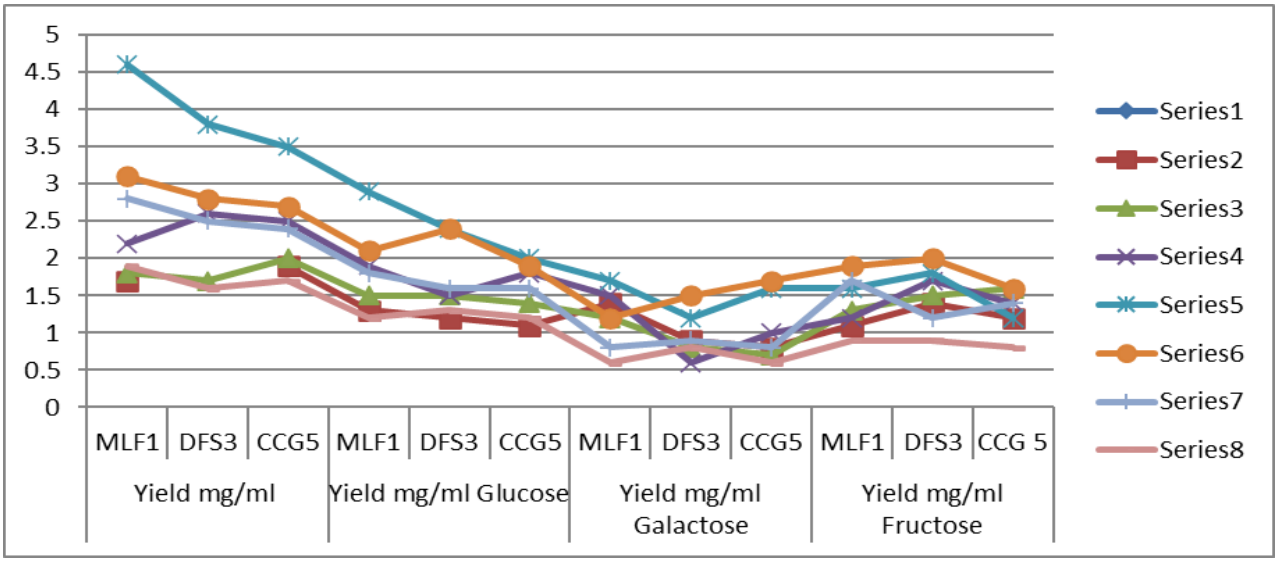


Table No. 3: Effect of pH on the production of CA by *Aspergillus niger*

Sr. No.	pH	Citric acid Yield mg/ml		
		MLF1	DFS3	CCG5
1.	2.5	2.4	2.8	2.9
2.	3.0	2.7	2.7	3.0
3.	3.5	3.0	3.1	3.3
4.	4.0	3.1	3.4	3.2
5.	4.5	3.9	3.7	3.8
6.	5.0	2.1	2.1	2.1
7.	5.5	1.3	1.2	1.6

Graph No. 3: Effect of pH on the production of CA by *Aspergillus niger*

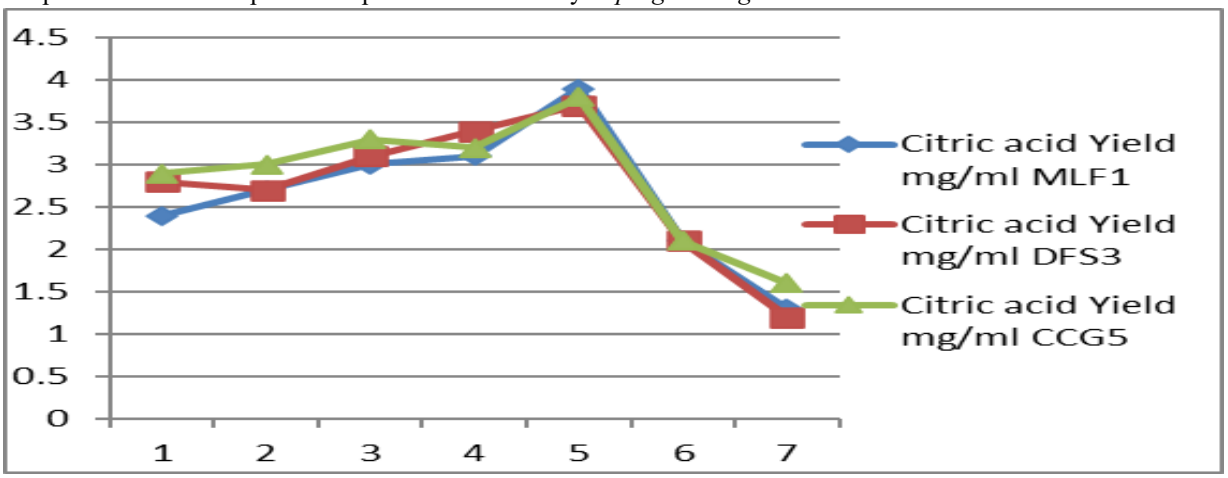


Table No.4: Ascending chromatography of organic acid and test solution

Sr. No.	Sugar	Distance travelled by solvent	Distance travelled by solute	Rf	% Rf
1.	Citric acid	10.0	4.0	0.4	40
2.	Oxalic acid	10.0	5.0	0.5	50
3.	Succinic acid	10.0	6.0	0.6	60
4.	Test	10.0	4.0	0.4	40

RESULT AND DISCUSSION

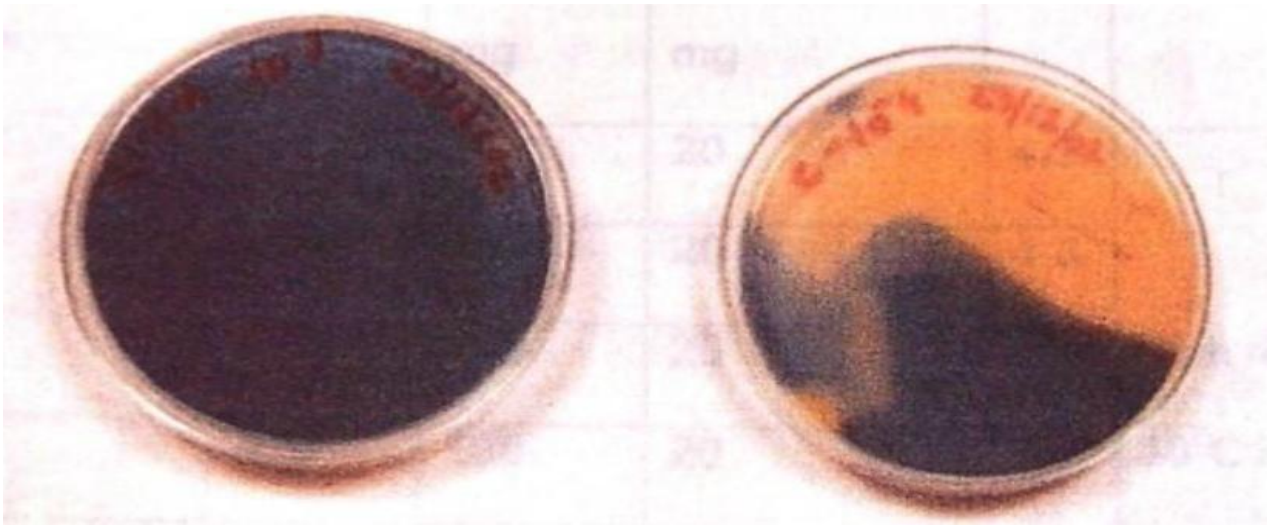
It was clear from the Table No.1 that Soil samples were collected from various areas of Deola forest and college garden. Serial dilutions were of soil samples were prepared and pure cultures isolated from it. These pure cultures were checked for citric acid production. Similarly, different cultures isolated from lemon fruits and college campus garden soils and brought into pure cultures of *A. niger*. The soil pH was determined of the collected soils samples. Samples were tested separately for citric acid production and results were noted (Sing, S.P, 1998). The highest yield was reported from MLF1, DFS3 and CCG5 strains. It was also found that the highest yield of CA was found when the pH was 4.5, above this pH the yield was less. These three higher yielding strains were conserved and used for further experiments (Ashish Kumar and V.K. Jain, 2008)

The result presented in the Table no. 2 found that the most suitable carbon source among the selected carbon source in the culture medium. It was clear from result presented in the Table No. 2 that sucrose is the best carbon source for the production of citric acid as compared to glucose, galactose and fructose. when all the cultures were tested for CA production, it was found that 13% sucrose was found more suitable than the other sugar concentration. It was found that glucose, galactose and fructose were not a suitable carbon sources for citric acid production. Culture medium with sucrose showed higher yields. It was reported that the at sugar concentration 13% all the three cultures gave the higher yield. It was also found that sugar concentration (15% to 18%) the yield reported was less as compared to 13%. Kovats (1960)

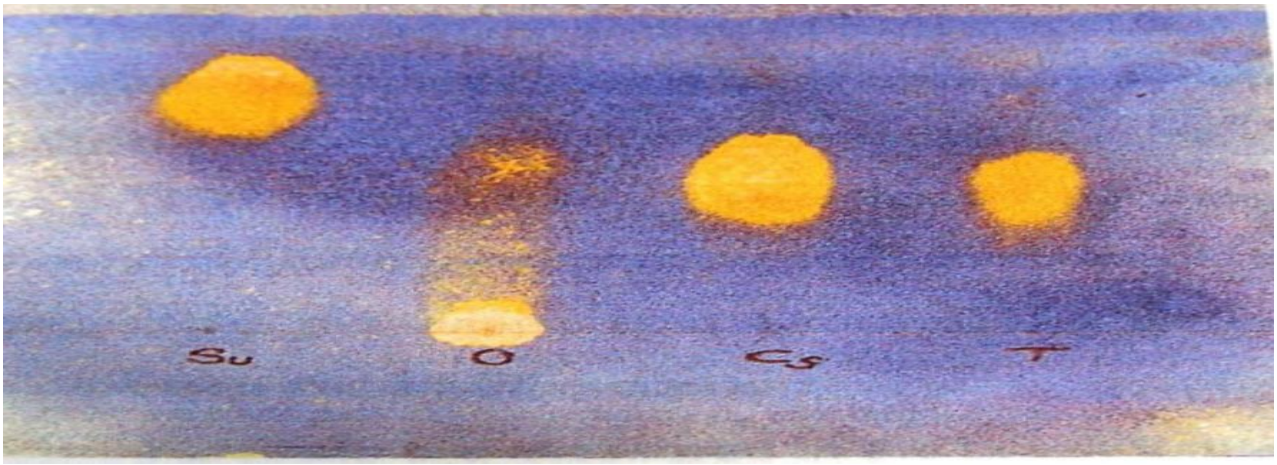
reported that higher sugar concentration (15 to 18%) greater amount of residual sugars remains in the medium and process become uneconomical.

It is clear from result presented in the Table No. 3 that pH of culture medium was play vital role in the production of citric acid invitro. Acidic pH of culture medium was required for the healthy production of citric acid. Different concentrations of pH tested for CA production of selected higher yielding strains. Different pH cultures were prepared (2.5 to 5.5) and screened for citric acid production. It was found that the pH of 4.5 was found to be most suitable for all the three selected strains for the yield of citric acid (Gupta J.K., Heding L.G. & Jorgensen O.B. (1976). Thus the further experiment pH was 4.5 is taken. This showed the contrasts with the finding of Prescott and Dunn (1987) which claims that the initial pH for sucrose.

It was found from result presented in the Table No. 4, that ascending chromatography was done by using the solvent system n-Butanol, formic acid and water in the proportion of 10:2:5 respectively. Organic acid is separated by separating funnel and out of the two layers upper organic layer was used. The fermented broth filtered through Whatman filter paper. Filtered broth allows evaporating and concentrated broth loaded on chromatogram. After incubation period Chromatogram was removed, dried and sprayed with 0.4% Bromo-Cresol Green prepared in ethanol (pH is equal to 6.7) Rf vales were determined. Rf values were calculated and recorded. Calculated Rf value of samples were compared with the standard Rf values of different organic acids and from this it was confirmed that fermentation broth contains citric acid.



Photograph 1: Primary Screening of Organic Acid Production in Culture Medium by using Bromocresol Green Dye



Photograph 2: Chromatogram of different organic acids
(Su-Succinic acid, O-Oxalic acid, Cs-Standard citric acid, T-Fermented broth)

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