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PRODUCTION OF CITRIC ACID BY FERMENTED BROTH METHOD

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ABSTRACT

Citric acid demand increased in the different industries (fermented food items, beverages and pharmaceutical industries). Ancient source of citric acid was citrus fruits which contain 6 to 9% of citric acid. It was obtained by the processing of citrus fruits and precipitation of calcium salt. Traditional method citrus fruits could not sufficient for fulfill the growing demand of citric acid production. Hence alternative source of citric acid is a microbial origin. Many microorganisms are involved in the production of citric acid.

In the present study *Aspergillusniger* is considered more suitable than the other microorganisms for production of citric acid by fermented broth method. Many factors are affecting invitro production of citric acid. It was confirmed from tabular reading that sucrose was a suitable at 13 % to 15 % carbon source(Currie, 1917). Many factors were affecting fungal origin production of citric acid like strain of microorganism, temperature, carbon source, PH etc. were considered. Pure strain of *Aspergillus niger* wasobtained from soil samples from different localities and from lemon fruits, 2% methanol added into culture medium gives higher yield, 13% sucrose was better carbon source than the glucose, 4.5 P^Hacidic was suitable for healthy production of citric acid in vitro condition. Healthy growth was found at 28-30 ^{0} CtemperatureAftab Nadeem et al **(2010).**

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

Introduction:

Citric acid is widely used an acidifying agent and antioxidant in a fermented food items, beverages and pharmaceutical industries (Kapoor et. al, 1982). Microbial fungal origin Citric acid was obtained by submerged fermentation of *Aspergillus niger.* The yield of citric acid depends upon composition of culture medium and on the microbial strain(Aftab Nadeem, et al,2010). Citric acid production using *A. niger* is influenced by the process variables such as

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initial sucrose concentration, pH, nutrient concentration, additive, incubation period, temperature etc. However demand for Citric acid production is increasing faster than its production and hence requires more economical process. 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.AnandKishor, et al 2008).

In the present study screened different factors for the microbialorigin of citric acid like methanol, strain of microorganism, carbon source, PH etc. were considered. Isolation and maintained pure cultures of *Aspergillus niger* from soil sample collected from different localities, suitable 2% methanol added into culture medium gives higher yield, 13% sucrose was gives higher yield than the glucose, 4.5 P^H was found more suitable for production ofcitric acid in vitro and in 28-30^0 temperature healthy growth was found (ChaturvediMadhusudan 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 which produces high amount of citric acid. Primary screening determines which microorganism is able to produce a citric acid followed by secondary screening to determine capacity of that organism producing quantitatively (Abdullah – Al –Mahin et al 2004).

2. Isolation of Microorganism: The natural source of *Aspergillus niger* is the soil. Collection of soil samples from different locations with respect to P^H of the soil. In this way ten soil samples were collected and diluted 1/100, 1/1000 and 1/10000 of each sample. The soil samples were diluted,analyzed and they were purified by sub culturing on Czepecks Dox agar slants. Culture Plates were incubated at 30^0C and isolated cultures were observed and they were purified by sub culturing on Czepecks Dox agar slants. Isolation of *A. niger*from lemon fruits and pure culture isolated and maintained. Similar to soil screening lemon strain tested for citric acid production.

Similarly fresh and healthy lemon fruits were soaked in the water placed in closed container for a 5- 7 days. After incubation pure cultures of *Aspergillus niger* maintained on culture medium.

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3. Screening for organic acid production: Spore from slant cultures were inoculated on sterile Czepeck's Dox agar medium incorporated with Bromo-cresol green dye. Inoculated plates were incubated at 28^0C for one to two days and checked colour change blue to yellow indicates organic acid production.

Sr. No.	Area	PH	Culture	Yield mg/ml
1.	Lemon fruits		**LF1**	**4.7**
			LF2	3.7
			LF3	4.3
			LF4	2.8
			LF5	3.9
2.	Forest soil	6.2	FS1	2.5
		6.1	FS2	3.2
		5.9	**FS3**	**4.1**
		5.4	FS4	2.9
		6.1	FS5	3.5
3.	College Campus garden Soil	4.8	CC1	2.6
		5.2	CC2	2.7
		4.9	CC3	1.6
		5.1	CC4	1.8
		4.8	**CC5**	**2.9**

To study the effect of media component on citric acid fermentation, all the components 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 analyzed for the production of citric acid by selected strains of *Aspergillus niger*.

Table No.1: Screening for citric acid yieldand selection of strains

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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**		
%	**LF1**	**FS3**	**CC5**	**LF1**	**FS3**	**CC5**	**LF1**	**FS3**	**CC5**	**LF1**	**FS3**	**CC5**
10	1.6	2.1	1.9	1.4	1.1	1.3	1.1	0.8	0.8	1.2	1.6	1.7
11	1.9	1.8	1.9	1.4	1.6	1.3	0.5	0.8	0.9	1.1	1.5	1.3
12	2.5	2.6	2.8	1.8	1.6	1.9	0.9	0.7	1.0	1.2	1.5	1.4
13	**4.7**	**3.9**	**3.6**	**2.9**	**2.4**	**1.8**	**0.6**	**0.9**	**1.6**	**1.6**	**1.8**	**1.2**
14	3.1	2.9	2.6	2.4	2.1	2.3	1.1	1.5	1.7	1.9	2.0	1.6
15	2.9	2.4	2.1	1.9	1.8	1.8	0.9	0.8	0.7	1.7	1.9	1.4
16	1.8	1.4	1.3	1.0	1.2	1.3	0.5	0.7	0.6	0.9	0.9	0.8

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

Sr.No.	pH	**LF1**	**FS3**	**CC5**
1.	2.5	2.6	2.8	2.9
2.	3.0	2.8	2.8	3.0
3.	3.5	3.0	3.1	3.3
4.	4.0	2.9	3.5	**3.9**
5.	**4.5**	**3.9**	**3.7**	3.8
6.	5.0	1.8	2.1	1.9
7.	5.5	1.3	1.2	1.5

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:

Table No.1 Soil from various areas were collected and different cultures isolated from it. These were checked for citric acid production.Similarly different cultures of *A. niger*isolated from lemon and college campus garden soils. The soil pH was checked of the collected soils. Samples were tested for citric acid production and results were noted (SING, S.P, 1998).The highest yield was reported from LF1, FS3 and CC5. It was found that the highest yield was found when

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the pH of soil was 4.5, above this pH the yield was less. These three higher yielding strains were used for further experiments(Ashish Kumar and V.K. Jain, 2008)

Table2, when all the three cultures were tested for variation in sugar concentration (Sucrose and glucose). It was found that glucose is not a suitable carbon source 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.

Table3,pH was play important role in the production of citric acid invitro. pH acidic (2.5 to 5.5) were tested for citric acid production. It was found that the pH of 4.5 was found to be suitable for all the three cultures for the yield of citric acid (Gupta J.K., Heding L.G. &Jorgensen O.B. (1976). This showed the contrasts with the finding of Prescott and Dunn (1987) which claims that the initial pH for sucrose.

Table4,Ascending chromatography was done by using the solvent system n-Butanol, formic acid and water in the proportion of 10:2:5 respectively. It was prepared by separating funnel and out of the two layers upper organic layer was used. The fermented broth concentrated by evaporating it in a Petri dish. Chromatogram removed, dried and sprayed with 0.4% Bromo-Cresol Green prepared in ethanol (pH is equal to 6.7) and Rf values were calculated and recorded. Calculated Rf value of samples were compared with the different organic acids and from this it was confirmed that fermentation broth contains citric acid

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