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EFFECT OF MEDIA COMPONENTS AND PHYSICAL PARAMETERS ON CITRIC ACID FERMENTATION

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ABSTRACT:

Worldwide demand of Citric acid is about 6.0 X 105 tons per year (Karaff and Kubicek, 2003). Approximately 75% commercial use of citric acid is for food and 12% for pharmaceutical industries (Haq et al, 2001). Many microorganisms have been evaluated for citric acid production including bacteria such as Bacillus licheniformis, B. subtilis, Corynebacterium sp (Kapoor et al,1983), Fungi such as Aspergillus niger, A. awamori, A. foetidus, Penicillium restrictum etc. (Mattey and Allen, 1990), Yeast such as Candida lipolytica, C. intermedia and Saccharomyces cerevisiae(Crolla and Kennedy,2001).

In the present study different factors affecting microbial production of citric acid like methanol, strain of microorganism, carbon source, PH etc. were considered. Isolation of Aspergillus niger from soil sample collected from different localities, 2% methanol added into culture medium gives higher yield, 13% sucrose was better carbon source than the glucose, 4.5 P^H was suitable for production of citric acid in vitro and in 28-30^0 temperature healthy growth was found.

Key Words*: Fermentation broth, citric acid, P^H, methanol, Carbon source, Aspergillus niger etc.*

INTRODUCTION:

Citric acid is mainly produced by submerged fermentation of *Aspergillus niger.* The yield depends upon composition of medium and on the micro organism strain (Aftab Nadeem, Quratulain Sayed, Shahjahan Baig, Muhammed Irfan and Muhammed Nadeem, 2010). Citric acid production using *A. niger* is influenced by the process variables such as initial sucrose concentration, pH, nutrient concentration, additive, incubation period, temperature etc. However demand for Citric acid production is increasing faster than the its production and hence requires more economical process. The fitness of solution would be measured by determining the total weight of the proposed solution. The higher the weight, greater the fitness provided that solution (K. Anand Kishor, M. Praveen Kumar, V. Ravi Krishna and G. Venkat Reddy, 2008).

Citric acid, a tricarboxylic acid widely used an acidifying agent and antioxidant in a food, beverages and pharmaceutical industries (Kapoor et. al, 1982; Wang and Liu,1996).

In the present study locally isolated *Aspergillus niger* employed for the production of Citric acid.

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 and use further.

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 citric acid quantitatively.

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 diluted samples were observed and they were purified by sub culturing on Czepecks Dox agar slants. Culture Plates were incubated at 28 ^{0}C and isolated cultures were observed and they were purified by sub culturing on Czepecks Dox agar slants.

3. Screening for organic acid production: Spore from slant cultures were inoculated on sterile Czepeck's Dox agar medium plates incorporated with Bromo-cresol green dye. Plates were incubated at 28^{0}C for 24 to 28 hrs. and checked colour change blue to yellow indicates organic acid production.

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. Broths were tested for citric acid production by selected strains of *Aspergillus niger*.

Table No.1: Isolation of soil from different area and test for citric acid production

Sr.No.	Area	Culture	Soil pH	Yield mg/ml
1.	Nashik Caves area	**NS1**	4.4	**3.8**
		NS2	5.2	3.6
		NS3	4.8	**4.1**
		NS4	5.6	2.9
2.	Shaptshrungi Garh	**SG1**	5.3	2.8
		SG2	5.5	3.1
		SG3	4.8	**3.9**
		SG4	5.7	2.8
3.	Deola forest	**DF1**	5.6	2.6
		DF2	6.2	2.4
		DF3	5.4	**2.9**
		DF4	5.1	2.8
4.	Deola college Garden	**DCG1**	6.5	2.2
		DCG2	6.3	**2.4**
		DCG3	7.6	1.8
		DCG4	7.8	1.6

Table No.2: Effect of Sucrose % on Yield of citric acid.

Sucrose %	**Yield**(mg/ml)		
	NS1	**NS3**	**SG3**
12	2.2	1.5	1.2
13	**3.5**	**3.2**	**3.4**
14	2.9	2.5	2.7
15	2.4	2.6	2.2
16	2.3	2.7	2.7
17	2.1	2.8	2.5
18	1.6	1.4	0.8

Table No.3: Effect of different $NaNo_3$ concentration on Yield of citric acid.

Sr.No.	**$NaNO_3$ mg/l**	**NS1**	**NS3**	**SG3**
1.	200	2.2	2.0	1.7
2.	250	2.4	2.1	1.5
3.	300	2.6	2.2	1.8
4.	350	3.1	2.8	1.3
5.	**400**	**3.7**	**3.9**	**2.9**
6.	450	2.8	2.6	2.0
7.	500	2.1	2.6	1.7
8.	550	1.8	2.1	1.6
9.	600	1.6	1.9	1.4

Table No.4: Effect of different KH_2PO_4 concentration on Yield of citric acid.

Sr.No.	KH_2PO_4 mg/l	NS1	NS3	SG3
1.	25	2.9	2.8	2.2
2.	50	2.8	3.2	2.4
3.	75	3.7	3.8	2.6
4.	**100**	**4.7**	**4.5**	**3.5**
5.	125	3.6	4.2	3.1
6.	150	3.1	3.2	2.2

Table No.5: Effect of different $MgSO_4$ concentration on Yield of citric acid

Sr.No.	$MgSO_4$ mg/l	NS1	NS3	SG3
1.	10	0.7	0.9	1.1
2.	15	1.9	2.2	1.3
3.	**20**	**3.8**	**3.6**	**3.5**
4.	25	3.1	2.9	3.4
5.	30	1.8	2.1	2.3

Table No. 6: Effect of different pH on citric acid production.

Sr.No.	pH	NS1	NS3	SG3
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.7: Paper chromatography of organic acid

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 was collected and different cultures isolated from it. These were checked for citric acid production. The highest yield was reported from NS1, NS3 and SG3. It was found that the highest yield was found when the pH of soil was 4.5, above this pH the yield was less.

Table No.2, When all the three cultures were tested for variation in sugar concentration (Sucrose), 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.

Table No.3 A wide range (200 to 600 mg) of $NaNO_3$ for all the three cultures was tested and found that at the concentration of 400 mg the yield of citric acid was higher, this result matched with Dhankar's (1974) finding. It was showed that at lower and higher concentration of NaNO3 the yield reported was poor and higher yield of citric acid at 400 mg of NaNO3 was found to be optimum.

Table No. 4 A wide range of $KH2Po_4$ was checked for citric acid production, it was found that 100 mg of $KH2Po_4$ was suitable for citric acid production for all the three cultures. The higher concentration of $KH2Po_4$ leads to less yield and promotes growth. This result confirms findings of Khan's and Szuc's that higher KH_2Po_4 decreases production of citric acid.

Table No. 5 Variation of $MgSo_4$ ranging from 10 to 30 mg, it was found that higher yield was given by a concentration of 20 mg of $MgSo_4$ which helped for both growth and citric acid production. These results compared with findings of Prescott and Dunn as Mg is essential for a variety of enzyme reactions in the cell.

Table No. 6 A pH of 4.5 was found to be suitable for all the three cultures for the yield of citric acid. The culture SG3 showed higher yield at pH 4.0. This showed the contrasts with the finding of Prescott and Dunn which claims that the initial pH for sucrose.

Table No. 7 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. Rf value of samples compared with the organic acid and from this it was confirmed that fermentation broth contains citric acid

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