International Journal of Biotechnology and Microbiology
www.biotechnologyjournals.com
Online ISSN: 2664-7680, Print ISSN: 2664-7672
Received: 15-05-2022, Accepted: 30-05-2022, Published: 17-06-2022
Volume 4, Issue 1, 2022, Page No. 91-95

Production of citric acid by *Aspergillus niger* isolated from soils of Aurangabad District, Maharashtra

Chitra Bagmar
Sir Sayyed college of Arts Commerce and Science, Aurangabad, Maharashtra, India

Abstract
A total of twenty five isolates of *Aspergillus Niger* obtained from soils of Aurangabad region were tested for their efficiency of citric acid production. The isolate number 16 showed maximum citric acid production. This isolate was selected for further study. It was found that the surface fermentation method was more efficient for citric acid production than submerged fermentation on the basis of sucrose consumption. The percentage yield of citric acid on the basis of sugar consumption was higher by surface culture method. The results of titratable acidity also supports the above findings. Citric acid produced was purified and further identified by chromatographic technique.

Keywords: isolate, *Aspergillus niger*, citric acid, fermentation

Introduction

Citric acid is found as the natural constituent of citrus fruits, pineapple, pears, peaches, figs and other fruit juices. Citric acid from these products is known as "natural citric acid' in contrast to citric acid produced by microbial fermentations. (Prescott and Dunn, 1987) [9]

Commercial citric acid finds many uses in food and beverage industries, in pharmaceuticals etc.

Various microorganisms possess the ability to convert carbohydrates to high yields of organic acids. One of the industrially most important fungi producing important organic acids like citric acid, gluconic acid etc. is *Aspergillus niger*.

Citric acid can be produced by fermentation from *Aspergillus niger* Fermentation process involves the utilisation of this potential of microorganisms to obtain products of economical value (Casida, 1999).

Until the early part of the 19th century, citric acid was produced mostly from lemon juice. Today, most of the citric acid used comes from fungal fermentations. Although chemical synthesis of this organic acid is possible, as yet there is no competitive synthetic process developed that is superior to fungal fermentations. (Prescott and Dunn 1987) [9]

Over the years, a large no of organisms including fungi, yeast, and bacteria have been screened for citric acid production. They include *Aspergillus niger Aspergillus clavatus. A flavis. Penicillium luteum, citrinum, Ustalina vulgaris. Mucor piriformis.*

Aspergillus niger, has been employed for many years for the commercial production of citric acid. It is superior to other microorganisms for the commercial synthesis of citric acid because of its better production yield. It is easy to handle, can ferment various cheap raw materials and delivers high yields.

However, the worldwide demand for citric acid is increasing faster than its production and more economical processes are required.

The selection of a favorable fermentation medium probably is the most critical factor in obtaining high level accumulations of citric acid by *Aspergillus niger*, Also, various cultural conditions decide the successful fermentation of citric acid. A variety of carbon sources, such as sucrose, citrus molasses, cane juice, starch from various sources. cane and beet molasses have been used for citric acid production.It has been reported that of all the carbohydrates used for production of citric acid sucrose is to be the most suitable one.

The chemical nature of sugar source has a marked effect on citric acid production by *Aspergillus niger*.

Sucrose has relatively low molecular weight and it is readily transported into microbial cells for hydrolysis by intracellular enzymes (Hossain *et al* 1984) [8]

Material and Methods

Ten soil samples were collected from fertile and nonfertile areas of Aurangabad district with the help of a small shovel. These samples from upper 15-20 cm of the profiles were collected in sterile plastic bags (Approx.1 kgs) The lumps of the soil were broken as far as possible and the stones, stubble etc, were removed. Soil samples were then dried. The samples were preserved at 4°C and then were further set for microbial analysis.

Primary screening of *Aspergillus niger*

Aspergillus niger was isolated from soil using dilution plate technique. Serial dilution upto 10^{-6} were prepared and 0.1ml of it was spread on Potato Dextrose Agar, Rose Bengal Agar plates. The plates were incubated at room temperature for 48 hrs.

Identification of *Aspergillus niger*

Identification was carried out by staining the mold with lactophenol and cotton blue and by observing under the microscope.

Maintenance of cultures

The cultures of *Aspergillus niger* were maintained on Potato Dextrose Agar/Rose Bengal agar slants and were stored in a refrigerator at 4°C.

Screening of *Aspergillus niger* strains for citric acid production.

Twenty five isolates of Aspergillus niger were obtained from different soil samples. Screening of these isolates for citric acid production was carried out on Millis agar medium modified by Chaudhary (1974). All the isolates of *Aspergillus niger* were spot inoculated on the Millis agar plates in triplicate. The plates were incubated at room temperature for 48-36 hrs. Yellow zones were observed around the *Aspergillus niger* colonies in case of citric acid strains. The acid unitage values of each ere determined by measuring the diameter of the acid zone.

Citric acid production studies

Inoculum preparation

The strain of *Aspergillus niger* showing maximum acid units was selected for production studies. Five plates of Potato Dextrose Agar were inoculated with selected isolate and incubated for 120 hrs at room temperature to obtain a large number of spores. The spore suspension was prepared by scrapping the spores from the plates The suspension was prepared in sterile distilled water with 0.001% sodium dodecyl sulfate. The initial spore number per ml of the spore suspension was determined by using haemocytometer.

Surface culture method

For surface culture method, 1300 ml of sterile Prescott and Doegler's medium pH 2.3 was taken in a disinfected plastic tray upto 1.5-inch height. This medium was then inoculated with 70 ml of the *Aspergillus niger* spore suspension. It was kept undisturbed at room temperature for 7-8 days.

Submerged culture method.

For the submerged culture method, 250 ml of shu and Johnson's medium was taken in flask inoculated with 25 ml of the mold spore suspension. The pH of the medium was adjusted to 2.8 by using 1N HCl using a pH meter. The flask was kept on shaker at room temperature for eight days.

Temperature plays an important role in citric acid production. Temperature between 25 and 30°C is usually employed for culturing of *Aspergillus niger*. The optimum temperature for citric acid production is 30°C, but temperature of the medium increases above 30°C and thus the biosynthesis of citric acid is decreased.

Measurement of initial and final sugar content of the medium

The initial and final sugar contents of both the surface and submerged culture media were determined with the help of the land refractometer.

pH measurement during progress of fermentation-Extraction of fermented liquor

The Fermented broths of both surface and submerged culture methods were filtered through cotton. The mycelial mat in case of surface culture method was washed with small amount of distilled water 2-3 times and finally it was squeezed out (so that all the traces of Fermented liquor was obtained)

Determination of titratable acidity of Fermented liquor

Titration of citric acid (fermented liquor) The filtrate obtained is titrated against an alkali of known strength using phenolphthalein as indicator. The end point is the formation of pale pink colour. The volume of alkali used for neutralization is used to find the normality and the percentage of acid in the sample.

Relation 1 ml of 0.1 (N) NaOH = 7.0 mg of citric acid

Chromatographic detection of citric acid

Preparation of solute: 0.2% solution of standard citric acid was prepared in distilled water while the fermented liquor was used as test sample.

Preparation of Solvent: for detectiion of citric acid, following solvent was used n-Butanol: Formic acid: Water 10:2:15) the lower layer was discarded.

Loading: A strip of Whatman filter Paper No.1 was used. The origin was marked with pencil from one end of paper. The known standard citric acid sample and the test samples from surface and submerged culture

(fermented liquor) were spot inoculated with capillaries and after drying the paper it was subjected to development.

Development: The filter paper strip was kept in the chamber with the solvent in such a way that the sample end of the strip just dips into the solvent. The chamber was sealed with a glass plate. The solvent was allowed to run until the solvent front reached the supper edge of paper.

Etection: The paper was removed from the chamber, dried and solvent front was marked. The detecting agent used was 4% paradimethyl aminobenzaldehyde in acetic acid with few crystals of sodium acetate. This reagent was sprayed on the chromatogram and it was heated at 140°C for 30 seconds. Citric acid gave a deep red colour. The Rf values of standard citric acid spot and of samples were recorded and used for identification of citric acid spots of test samples.

Recovery and purification of citric acid

For the recovery of citric acid from the fermented liquor, a solution of calcium hydroxide was prepared. It was added into the fermented liquor to obtain pH eight. A precipitate of calcium citrate was formed. This precipitate was removed and the clear filter was collected.

Results and Discussion

A Total of 25 isolates of *Aspergillus niger* were obtained from the soil samples of Aurangabad. Acid unitage values of all the *Aspergillus* spp. isolated from soil is given in Table 1. It is seen from the table that acid unitage value varies from 0.0 to 5.2 cms. Isolate number 16 shows maximum acid units so that isolate was selected for further study. The efficiency of isolate number sixteen (*Aspergillus niger)* was further tested when sucrose was consumed in the surface fermentation method as compared to the submerged fermentation method.(Table 2) It can be concluded that the surface fermentation method is more efficient for citric acid production than the submerged method. The P^H values of citric acid fermentation medium was tested on various days of incubation (Table 3) It was noted that the P^H was reduced from 2.5 to 1.5 in surface culture method, whereas the P^H was reduced from 2.8 to 1.9 after eight days of incubation.

Table 1: Acid unitage values of Aspergillus spps isolated from soil.

Isolate no.	Acid unitage in cm
1	0.0
2	0.0
3	4.7
4	0.0
5	0.0
6	2.0
7	1.9
8	1.7
9	1.6
10	2.0
11	1.8
12	2.2
13	1.9
14	1.1
15	2.2
16	5.2
17	2.6
18	1.9
19	00
20	3.0
21	1.8
22	2.4
23	2.1
24	2.0
25	1.4

Table 2: Initial and final sugar percentage of citric acid fermentation medium inocuated with *Aspergillus niger*

Fermentation type	Sucrose percentage-Initial	Sucrose percentage-final
Surface	14	9.4
Submerged	14	10

Table 3: P^H values of citric acid fermentation medium after incubation. (days)

Day	Surface culture method	Submerged culture method
1st	2.3	2.8
2nd	2.2	2.7
3rd	2.0	2.6
4th	1.9	2.4
5th	1.9	2.3
6th	1.8	2.2
7th	1.7	2.1
8th	1.5	1.9

The titratable acidity of citric acid fermentation liquor was determined and is given in Table 4. It is seen from the table that acidity due to citric acid was 6.6 by surface culture method and 0.4 by submerged culture method. Thus these values again support our earlier findings that surface culture method is better than submerged culture method.

Table 4: Titratable acidity of citric acid fermented liquor.

	Amount of NaOH required to neutralize aliquots of Fermented liquor	Initial acidity of the medium	Acidity due to citric acid (2 ml)	Acidity due to citric aciid per ml of the medium
Surface fermentation	7.2	0.6	6.6	3.3
Submerged fermentation	0.8	0.4	0.4	0.2

Table 5: Yield of citric acid per 100 ml of Fermented broth of selected isolate

Type of fermentation	Percent yield of citric acid /100 ml on medium basis
Surface culture method	0.17 gm
Submerged culture method	0.0248 gm

Table 6: Percent yield of citric acid on the basis of sugar content per 100 ml of fermentation broth.

Type of fermentation	Percent yield on sugar basis
Surface culture method	3.70 gm
Submerged culture method	0.62 gm

References

1. Abraham TK, Chaudhari KL. Fermentation of citric acid by Aspergillus niger: 1. Role of impurities present in different grades of sucorse. 2. Use of molasses as a substitute for sucrose. Indian J. of Microbiol,1977:17(2):9395.
2. Clark DS, lentz CP. Submerged citric acid fermentation of sugarbeet molasses: effe.ct of pressure and recirculation of oxygen. Can J. Microbiol, 1971, 7.
3. Chaudhary AQ, Pirt SJ. The influence of metal complexing agents on citric acid production of Aspergillus niger. J. Gen. Microbiol, 1966, 43.
4. Doelger WP, Prescott SC. Citric acid fermentation. Ind. Eng. Chem, 1934, 26.
5. Dhankar HS. Production of citric acid by soil fungus niger) from pretreated sugar cane molasses. M.Sc. Thesis. Haryana Agric. Unive, Hissary, India, 1972.
6. Elholi MA, Al-Delaimy KS. Citric acid production from whey with sugars and additives by Aspergillus niger. Afr J Biotechnology,2003:2(10):356-359.
7. Gupta S. Isolation of a high citric acid yielding mutant strain of Aspergillus niger resistant to metal ions inhibition. In abstracts of International conference on microbial biotechnology trade and public held at Hyderabad, 2000, 68.
8. Hossain M, Brooks JD, Maddox IS. The effect of sugar source on citric acid production by Aspergillus niger. Appl Microbiol Biotechnol,1984:19:393-397. doi: 10.1007/BF00454376.
9. Kappor KKK, Chaudhary, Tauro P. "Citric Acid" in Prescott and Dunn's Industrial microbiology. (edited by Gerald Reed). 4th Ed. CBS Publishers and distributors, Delhi, 1987.
10. Luciana PS. Citric acid production by Aspergillus niger in solid state fermentation using Cassava residue. In abstracts of the International conference on microbial biotechnology trade and public policy held at Hyderabad, 2000, 78.
11. Pathade GR. Citric acid production by Aspergillus niger. A dissertation submitted to Shivaji University, Kolhapur, 1981.

12. Pau Loke Show ORCID Icon, Kehinde Opeyemi Oladele, Qi Yan Siew, Fitri Abdul Aziz Zakry, John Chi-Wei Lan Tau Chuan Ling Overview of citric acid production from Aspergillus niger, 2014, 271-283.
13. Prescott SC, Dunn CG. Industrial microbiology. 3rd Ed. McGraw-Hill book company, inc., New York, 1959.
14. Singh S, Kulkarni SD, Jha K. Use of banana and tomato wastes as microbial substrates for citric acid production. In abstracts of 41st Annual conference, Birla institute of science and research statue circle held at Jaipur, 2000.