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Fungal strains for mycological production of citric acid

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ABSTRACT

Citric acid has become an important raw material for general industrial use with many varied and expanding applications such as iron, steel, treatment and conditioning of industrial water supplies, preparation of alkyl resins, paints and in the printing of calico and textile industries. Mycological Production of citric acid includes preparation and sterilization of different media, culture medium, and seedling of culture tube, incubation of culture tubes, determination of citric acid formed and molasses left unfermented during the course of present investigation. The Present work deals mainly selection of potent strains of fungus.

1. Introduction

The production of citric acid by fungal strains is influenced by the carbohydrate sources such as molasses, concentration of molasses, incorporation of trace metals and some other compounds, monitoring of parameters to optimize the levels such as hydrogen ion concentration, temperature and incubation period. A probe is necessary for the above mentioned problems which will give the desired improved yield of citric acid.

Successful production of citric acid using potent strain of fungus is dependent on several factors of which the fungus, the culture conditions [1] and the raw materials used are most important. The success of a fermentation process depends to a very extent on the use of right type of fungal strain that can produce the desired end product at a minimum cost and in large quantities. The type of fungal strain used will depend on the product. *Aspergillus oryzae* [2] is a filamentous fungus used to saccharify rice, other grains and potatoes in making of alcoholic *beverages. Aspergillusflavus* is a fungal pathogen which causes post-harvest disease in cereal grains and legumes. *Aspergilluswenti* is used to process soybean. Aspergillus *foetidus* is used to produce koji for shochu- a distilled Japanese alcoholic beverage and it is also utilized for the production of many useful enzymes that serve differing purposes. *Aspergillus niger* [3–5] is a fungus and one of the most common species of the genus Aspergillus. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions, peanuts and is a common contaminant of food.

Fungal strains of the *Aspergillus niger* group of molds have usually given most successful results both in the laboratory and on an industrial basis. Many of these molds produce high yields, imposes fairly uniform biochemical characteristics and they are easily cultivated and produce a negligible quantity of undesirable end products. In the present investigation the following potent fungal strains of some molds have been employed by the author for the mycological production of citric acid using Molasses as feedstock.

Table 1: Fungal strains employed for mycological production of citric acid

1(K)	*Aspergillus oryzae*	NCIM-647
2 (L)	*Aspergillus flavus*	NCIM-650
3 (M)	*Aspergillus wentii*	NCIM-661
4 (N)	*Aspergillus foetidus*	NCIM-511
5 (O)	*Aspergillus niger*	NCIM-683

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NCIM stands for national Collection of industrial Microbes. The above citric acid producing strains of molds were employed for mycological production of citric acid and the observation are tabulated as in the Table 1 under heading result and discussion.

2. Experimental Method

The experiment was carried out with the above species of Aspergillus molds. Culture medium was prepared[6] as follows:

Table 2:

Sucrose:	0.100g
Malt-Extract:	0.075g
Yeast extract:	0.075g
Peptone :	0.075g
Agar-Agar:	1.00g
Distilled Water:	0.100 ml
pH:	1.8

The pH of the culture medium was adjusted to 1.8 by adding requisite amount of KCl-HCl buffer solution. Malt extract is a sweet substances used as a dietary supplement[7] for bacterial culture medium. Peptone mainly acts as a source of nitrogen and also carbon up to some extent. Agar-Agar is a gelatinous substances[8] serves as the primary structural support for the algae's cell wall.

Microorganism grow in a vessel known as culture tubes. About a dozen of clean and steam sterilized dry culture tubes were filled 5 ml of culture medium each and kept in slanting position. A small quantity of Aspergillus was transferred to the freshly prepared culture tubes then kept in an autoclave maintained at 30 ^{0}C[8,9] for 36 hrs. And then placed in refrigerator.

A small quantum of Aspergillus form culture tube was transferred to the freshly prepared inoculum medium[10–16] in a conical flask and was kept in an incubator for about 36 hours for proper growth of fungus which is finally employed for the inoculation of production medium to get the citric acid on large scale. The composition of the production medium[17] for the mycological production of citric acid by Aspergillus is as follows

Table 3:

Molasses:	20%
NH_4NO_3:	0.60%
KH_2PO_4:	0.60%
$MgSO_4.7H_2O$:	0.60%
pH:	1.8

After inoculation the fermentor flasks were placed in an incubator maintained at a constant temperature at 30 ^{0}C and were analyzed after 6, 8 and 10 days of incubation period for the production of citric acid and molasses unfermented. When the citric acid fermentation is over the fermentation broth is kept at 50 ^{0}C to avoid contamination. The broth is drained off (filtered)[18] and the mycelium mat is pressed to remove any acid contained in it. The amount of citric acid[19–27] in the filtrate was measured by titration with 0.1M NaOH against phenolphthalein as an indicator.[28,29] Total carbohydrates in the fermentation filtrate at various times was determined as glucose by anthrone-sulphuric acid method.[30,31]

3. Result and Discussion

Table 4:

Sl. No	Isolates	Yield of citric acid * in g/100 ml
1(K)	*Aspergillus oryzae* NCIM-647	5.764
2(L)	*Aspergillus flavus* NCIM-650	5.983
3(M)	*Aspergillus wentii* NCIM-661	4.985
4(N)	*Aspergillus foetidus* NCIM-511	5.236
5(O)	*Aspergillus niger* NCIM-683	6.886

* Each value represents mean of three trials.

The data recorded indicates that the fungal strain designated (O) ie *Aspergillus niger* NCIM-683 has significant yield of citric acid i.e. 6.886 g/100ml in comparison to other isolates. It is thus concluded that all the citric acid producing fungus are not of equal importance though they are morphologically similar to each other. The author therefore has selected Aspergillus niger NCIM-683 for mycological production of citric acid for further study along with different mutagen.[32–36]

4. Source of Funding

None.

5. Conflict of Interest

None.

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