A study of citric acid production from *Aspergillus niger* using different substrates in Solid State Fermentation (SSF) and Submerged Fermentation (SmF)

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Abstract

This study was undertaken to estimate and characterize citric acid produced by the *Aspergillus niger* using submerged and solid state fermentation methods. The isolated pure cultures of *A. niger*, was inoculated into the fermentation medium in both Submerged Fermentation (SmF) and Solid State Fermentation (SSF). After incubating 7-8 days at room temperature, the fermented medium was used for further procedures. Medium was filtered to remove the spores and mycelia, and the filtrate was used for further estimation of the amount of citric acid production and reducing sugar concentration in the fermented media. Estimation of citric acid was carried out by titration. From the titre value, the amount of Citric acid produced was determined.. Concentration of reducing sugar was detected by DNS method. From this work, we concluded that SSF is better than that of SmF for the improved production of citric acid.

Key words: citric acid, *Aspergillus niger*, SSF, SmF, substrates.

INTRODUCTION

There is a great worldwide demand for Citric acid consumption due to its low toxicity when compared to other acidulates used mainly in the pharmaceutical and food industries. The introduction of submerged fermentation presented several problems, including the choice of productive strains with low sensitivity to trace elements.(Kamal *et al.*, 1999). Several works were dedicated to the optimization of conditions for the utilization of cheap materials like sugarcane molasses, beet molasses, starch and hydrolyzed starch. The various fermentation processes used in the manufacture of citric acid are surface culture process, submerged fermentation (SmF), and solid state fermentation (SSF).

The first process proposed for the production of Citric acid by solid state fermentation using different absorbing materials like beet pulp, sugar cane molasses, pineapple pulp, apple and grape, orange peel, kiwi fruit peel, etc., can reduce the cost of citric acid production. Most of the microorganisms, however, are not able to produce commercially acceptable yields due to the fact that citric acid accumulation rises in appreciable amounts only under conditions of drastic imbalances (Arzumanov *et al.*,2000). Among the different species of fungi, *A. niger* has remained the organism of choice for commercial production because it produces more citric acid per unit time. (Mattey *et al.*,1990) It is most commonly used for citric acid production. This is because of the fact that this organism has the capacity to utilize variety of substrates due to its well developed enzymatic system. This work gives insights for future possibilities of cost effective fermentation process for citric acid production from several cheap raw materials.

MATERIALS AND METHODS

Isolation of *Aspergillus niger*

Aspergillus niger was isolated from bread, orange peel, molasses and pomegranate . They were confirmed by studying the morphology and the spore morphology such as shape, size, colour, echinulation, etc.. The improved Citric acid producing strain was detected by culturing the four isolates on Czapek-Dox broth. After sterilization of the broth, *A. niger* inoculum was added into the broth and incubated at room temperature for 7 days. After that, the best strain producing maximum amount of citric acid was determined by titration.

The industrial citric acid production can be carried in two different ways: by submerged fermentation and

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solid state fermentation or Koji's process, all of these methods require raw materials and inoculums preparation. In submerged fermentation the sources used were molasses, pineapple and SDB media. Pure culture of *Aspergillus niger*_was inoculated into the medium and incubated for 7 days at room temperature. After fermentation, the medium was filtered and then filtrate was used for subsequent analysis.

Substrates used in SSF included orange peel, pumpkin and sugarcane bagasses. Small amount of sterile distilled water was added into the flask for providing moisture. After sterilization *A. niger* was inoculated into the flask and incubated at room temperature for 7 days. After fermentation, the flask was dried in an oven at 50°c and extracted. The mixture was agitated on a rotary shaker for 2 hours and then filtered. Supernatant was used for the estimation of sugar and citric acid.

Product analysis

The amount of citric acid in the fermented medium was determined by titration, the filtrate obtained was 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 was used to find the percentage of acid in the sample. The amount of sugar was estimated by DNS (3,5-Dinitrosalicylic acid) method. The total amount of reducing sugars in the culture filtrate was determined by dinitrosalicylic acid (DNS) method which gives a rapid and simple estimation of the extent of saccharification in the supernatant. (Truong and Nyugen,1970).

RESULTS AND DISCUSSION

Aspergillus niger from the four sources, molasses, bread, orange and pomegranate, was inoculated on the Czapek-Dox media, to find out their ability to produce Citric acid. Among this *Aspergillus niger* from bread showed better result, (Table 1) and so the strain from bread was selected for performing further experiments.

Estimation of Citric acid

Both submerged and solid state fermentation processes were used for the production of citric acid from *A niger*. The amount of citric acid produced was estimated by titration using phenolphthalein as the indicator. From the titre value, the amount of citric acid in the sample was estimated using the formula $N1V1 = N2V2$

Table 1. Production of citric acid over different substrates

Czapek-Dox Media	wt/l of citric acid(in g/ml)
Molasses	0.369
Pomegranate	0.528
Orange	0.648
Bread	0.984

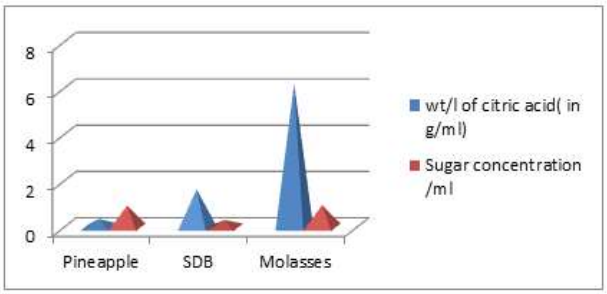

Fig. 1. submerged Fermentation

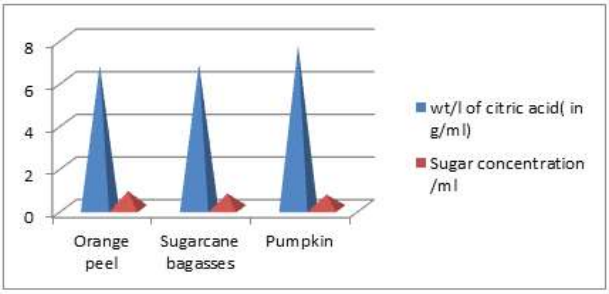

Fig. 2. Solid state fermentation

Estimation of reducing sugar.

Reducing sugar formed in the fermentation medium was detected by DNS method. From the O.D of unknown sample (fermented medium), the concentration of reducing sugar/ml was determined using standard graph.

Citric acid, a tricarboxylic acid cycle (TCA cycle) intermediate, is one among the foremost important metabolic products produced commercially by fermentation with specific molds but *Aspergillus niger* remains the organism of choice for citric acid fermentation (Pera and Callieri, 1999). In the present study, we were examining citric acid production by the isolates of *Aspergillus niger* was determined by using the substrates from various sources. Incubation period of 7-8 days was found to be best for citric acid fermentation because, further increase in incubation time did not enhance citric acid production. (Arzumanov *et al*., 2005)

The present study the production rate of citric acid in both submerged and solid state fermentation processes was determined. In submerged fermentation process, citric acid production was more in the molasses medium (Fig 1). Molasses is a saccharine material, widely used in many fermentation processes. It is used as a carbon substrate in the fungal production of citric acid.

Important fact was that, the amount of iron in these media were minimum or absent in the case of SmF, there is no need for the addition of nutrients in the case of SSF.(Narayanamurthu *et al.*, 2007).Because, for the efficient production of citric acid it is necessary to reduce the amount of iron, which is an activator of aconitase enzyme in TCA cycle.

Among the submerged and solid state fermentation, the SSF gives the better result (Figs 1 and 2). It is due to the fact that in SSF the increased volume and aeration helps to improve the production rate. When the volume increases, the fungi can ferment more and more. Also better aeration helps to the increased production rate. Aeration can be provided by agitating the flasks in both processes. *Aspergillus niger* is a filamentous fungus which remained the organism of choice for citric acid production due to ease of handling, and its ability to ferment a variety of cheap raw materials with higher yields. A cost reduction in citric acid production can be achieved by using cheap agricultural wastes such as orange peel, pine apple peel, sugarcane bagasse and cane molasses.

In solid substrate fermentation, the substrate itself acts as a carbon source and occurs in the absence or near absence of free water. However, in solid-state fermentation, the process occurs in absence or near absence of free water by employing a natural substrate or inert substrate as solid support (Kumar *et al*., 2003). The aim of SSF is to bring cultivated fungi or bacteria in tight contact with the insoluble substrate and to achieve the highest nutrient concentration from the substrate for fermentation. This technology thus far is run only on a little scale, but has an advantage over submerged fermentation. Substrates like agro-industrial residues are proved by many researchers to be better for filamentous fungi. The morphology of filamentous fungi supports them to penetrate the hardest surface due to the presence of turgid pressure at the tip of their mycelium.(Raimbault *et al*., 1997; Bhargav *et al*., 2008S). Hence, the raw materials considered as waste might be used for production of value added fine products and reducing pollution problems. Formation of citric acid as an industrial by-product will help to tackle waste disposal issue and also reduce the dependency of industry over other acid producers. Thus, the industry would be benefitted ecologically and economically.

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