# CITRIC ACID PRODUCTION BY SSF PROCESS USING SUGAR CANE BAGASSE

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**ABSTRACT**

*This study comprises of Citric acid production using novel technique Solid State Fermentation. Citric acid was produced by sugar cane bagasse (waste) and Aspergillus niger activated culture via solid state fermentation process. All samples were cut, dry, per treatment and sieved for less than 0.85 mm and 0.85 mm to 1.40 mm particle size. The moisture contain was regular maintained which played very important in fermentation process. The sugar from the sample can be estimated using dinitrosalicylic acid colorimetric method and Anthrone colorimetric method. The produced citric acid estimated by acetic anhydride and pyridine. In present study, citric acid production, sugar utilization and analysis 150 mg/gm of citric acid produces.*

**Keywords**: *Citric acid production, Aspergillus niger, solid state fermentation(SSF)*

## 1. INTRODUCTION:

Citric acid fermentation was first observed as a fungal product by Wehmer in 1893 by a culture of *Penicillium glaucum* on sugar medium. After a few years, he isolated two new fungal strains with the ability to accumulate citric acid, which were designated *Citromyces (Penicillium)*. However, industrial trials did not succeed due to contamination problems and long duration of fermentation. It was the work of Currie which opened up the way for successful industrial production of citric acid. In 1916, he found that numerous strains of *Aspergillus niger* produced significant amounts of citric acid. The most important finding was that *Aspergillus niger* grew well at pH values around 2.5–3.5 and high concentrations of sugars favour citric acid production. [1]

The first citric acid fermentations were carried out in surface cultures. In the 1930s, some units were implanted in England, in Soviet Union, and in Germany for the commercial production. In general, citric acid is commercially produced by submerged microbial fermentation of molasses; the fermentation process using *Aspergillus niger* is still the main source of citric acid worldwide. Although methods were well developed to synthesise citric acid using chemical means, better successes were achieved using microbial fermentations, and over the period of time, this technique has become the method of ultimate choice for its commercial production over chemical synthesis. Despite that, the introduction of submerged fermentation presented several problems, including the choice of productive strains with low sensitivity to trace elements. It was necessary to consider raw material much more carefully. Several works were dedicated to the optimization of the conditions for the utilization of cheap material like sugar cane molasses, beet molasses, starch and hydrolysate starch. Various processes for treating and purifying molasses were developed, especially for the removal of trace metals. Moreover, it was found that a small excess of copper ions was beneficial to achieve high yields of citric acid. [5]

There are annual growths of 3.5–4.0 % in demand/ consumption of citric acid. In the last years, a considerable interest has been shown in using agricultural products as alternative sources of carbon and their wastes such as maize, apple and grape pomace, pineapple, mandarin orange and brewery wastes, citrus and kiwi fruit peel for citric acid production by Aspergillus niger. The industry is seeking newer cheap and economic process technology. [11]

## 2. MATERIAL AND METHOD:

### 2.1. Micro-Organism

A citric acid producing strain of *Aspergillus niger* 1055, was procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory Pune. It was maintained on Sabouraud Dextrose Ager (SDA) slants and subculture periodically which were stored at 4 ℃.

### 2.2. Media Preparation

Bagasse was sun dried, cut into small pieces, grounded and screened to collect two fractions of different particle sizes, less than 0.85 mm and 0.85mm to 1.40 mm. Bagasse (3 g) of sired particle size was taken in 250 ml Erlenmeyer flasks and moistened with medium (20% sucrose, 0.25%, $NH_4NO_3$, 0.1%, $KH_2PO_4$, 0.025%, $MgSO_4$ and 0.004%, $CuSO_4$, pH 4.0) to set the 75% moisture level. Media were sterilised at 121 ℃ for 60 min to provide proper cooking of the substrate and to increase its susceptibility to microbial attack.

### 2.3. Inoculum

Activated culture was inoculated in sterilized SDA broth and incubated at 120 rpm for 48 hr. which was then used as inoculums for SSF. Spore count in activated culture was calculated used counting chamber and find spore count ($1\times10^7$) was maintained.

### 2.4. Solid State Fermentation

Each flask, containing medium was inoculated with 1 ml of spore suspension followed by mixing and incubation at 30±2 ℃, inside the humidity-controlled incubator. 4% (w/v) Methanol was added to the medium before inoculation. One flask was harvested every day for the estimation of citric acid produced and sugar consumed, till whole sugar consumed. Sampling was continued until all the sugar of the medium was consumed.

### 2.5. Analytical Methods

There are two methods used for sugar estimation calorimetric determination of glucose by the 3,5-dinitrosalicylic acid method and anthrone method [42] and citric acid estimation by acetic anhydride and pyridine method [43].

## 3. RESULTS AND DISCUSSION:

The present work consisted of comparison and selection of suitable carrier bagasse for citric acid production by SSF. The effects of different particle size of bagasse, sugar level, and citric acid concentration in the fermentation medium, have been studied.

### 3.1. Reducing Sugar Estimation by DNS Method

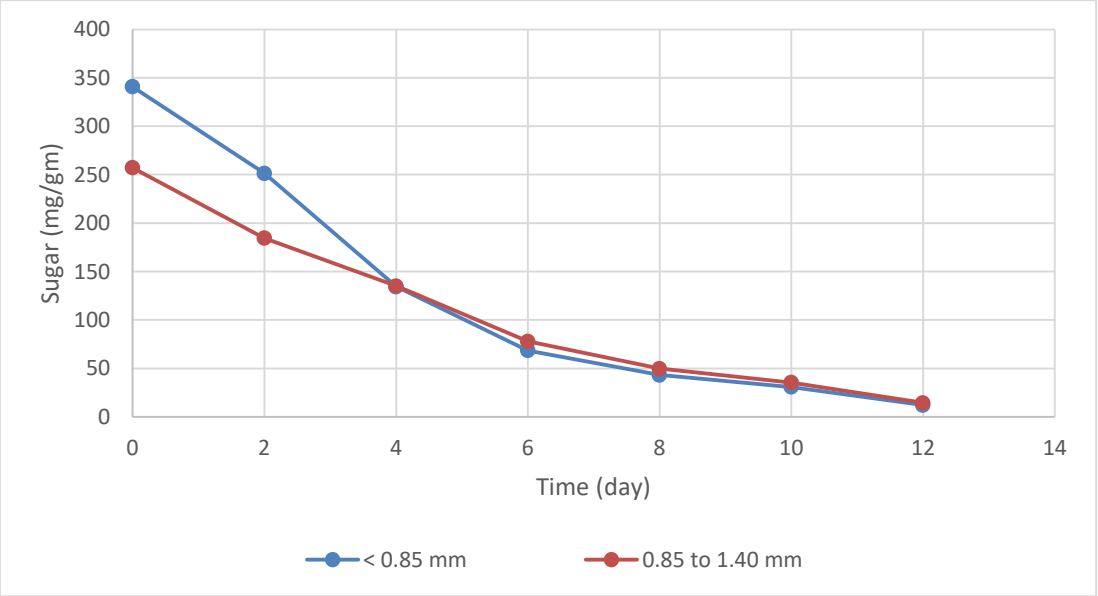


**Chart 1**: Comparison of substrates for reducing sugar consumption DNS method

### 3.2. Total Sugar Estimation by Anthrone Method

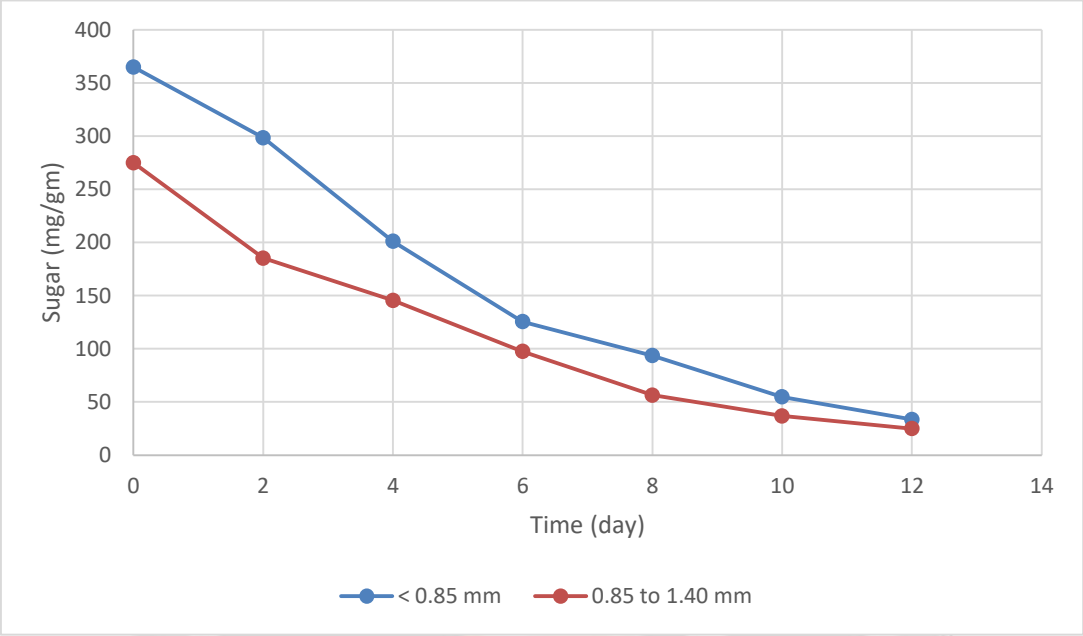


**Chart 2**: Comparison of substrates for total sugar consumption Anthrone method

### 3.3. Citric Acid Production

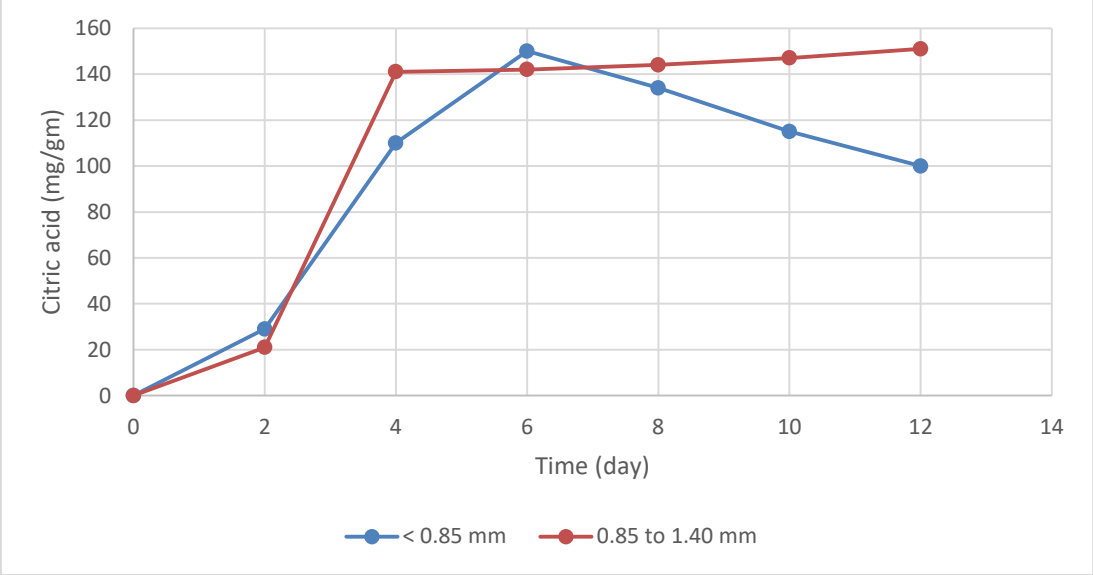


**Chart 3**: Comparison of substrates for citric acid production

## 4. CONCLUSION:

From the experiments it is evident that fermentation is the route to be taken if cheap production of citric acid is to be carried out. The sugar estimation and citric acid estimation have been carried out successfully. The sugar estimation experiments showed that not all the sugar gets consumed in the fermentation process. This is because of the inhibition caused by the citric acid produced. The maximum citric acid production was obtained from media

0.85 to 1.40 mm particle size. The less than 0.85mm particle size sugar concertation is high but media create agglomeration form then citric acid production is less.

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