

Citric Acid Production from *Aspergillus niger* using Banana Peel

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Abstract— The Citric acid is an important organic acid which is being globally consumed and produced in large numbers. In the present study, mango peel was utilized for citric acid production by *Aspergillus niger* and the fermentation parameters were optimized. Maximum yield of citric acid was obtained using banana peel 20%, inoculum 5%, potassium dihydrogen phosphate and ammonium nitrate at pH 5 and 32° C after 8 days of fermentation.

Index Terms— Citric acid; Fermentation; *Aspergillus niger*; Banana Peel.

1 INTRODUCTION

THE citric acid (2-hydroxy-propane-1, 2, 3-tricarboxylic acid) is a universal intermediate product of metabolism and is found in virtually all plants and animals. Citric acid has occupied a space in the global market due to its vast uses and applications in food, pharmaceutical, cosmetic industries and other industrial applications [1]. Citric acid can be produced mechanically, chemically and through fermentation. The mechanical and chemical production method are however, not economical.

Citric acid is naturally produced by microorganisms during TCA cycle. This natural metabolic pathway is the most feasible method for citric acid production on industrial scale. Many microorganisms have been employed for citric acid production including bacteria and fungi such as *Bacillus licheniformis*, *Arthrobacter paraffinens* and *Corynebacterium ssp.*, *Aspergillus niger*, *A. carbonarius*, *A. aculeatus*, *A. awamori*, *A. fonscaeus*, *A. foetidus*, *A. phoenicis* and *Penicillium janthinellum*; and yeasts such as *Candida tropicalis*, *C. oleophila*, *C. guilliermondii*, *C. citroformans*, *Hansenula anomala* and *Yarrowia lipolytica* [2,3]. Most of these strains, however, do not produce commercially acceptable yields of citric acid. *Aspergillus niger* is highly recommended for commercial production as it has been found to produce high yields of citric acid by utilizing a variety of substrates due to its property of citric acid accumulation [4].

Three fermentation methods have been used for citric acid production: surface fermentation, submerged fermentation and solid state fermentation. Substrate for citric acid production has also been a topic of interest for many researchers and use of a vast variety of substrates has been reported [5, 6]. Cane molasses, beet and blackstrap molasses, whey, semolina, areca husk, maize, wheat bran, rice, pumpkin [7], sweet orange pulp, sweet orange peel, sweet lime pulp, sweet lime peel, pineapple waste, wet corn distiller grains, apple pomace,

cassava bagasse, carob pod extract, date syrup, olive oil, palm oil, coffee husk, corncob, grape pomace, kiwifruit peel, okara, glycerol, date syrup and coffee husk have been reported as substrates for citric acid production [8, 9, 10].

The food and agricultural industry generate a large amount of agro industrial waste and there is a need to utilize these wastes to produce valuable products. Fruit peels are one of the wastes generated in large amount by food processing industries in Pakistan during the manufacturing of juice, jellies, jam and pickles. This waste poses an environmental problem and needs to be utilized for the production of valuable products. This study focuses on the utilization of mango, banana and sweet orange peel for the production of citric acid and optimization of fermentation parameters.

2 Materials AND METHODS

2.1 Culturing and Storage of *Aspergillus Niger*

Sterile PDA slants were inoculated with *Aspergillus niger* strain and grown at 30° C for 5-7 days. After growth was achieved, the slants were stored at 4° C.

2.2 Screening for Citric Acid Production

In a clean and sterilized environment, sucrose salt media was inoculated with *Aspergillus niger* strain and incubated at 25° C for 7 days. After 7 days, the media was assayed for citric acid production.

2.3 Pretreatment of Substrates

Banana peels were washed, air dried and then dried in a hot air oven at 70 °C for about 2-3 hours. Peels were then ground to about 1-2 mm size.

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2.4 Preparation of Starter Culture

The selected strain of *A. niger* was used to aseptically inoculate the sucrose salt media (sucrose 15%, MgSO₄.7H₂O 0.025%, NH₄NO₃ 0.25%, KH₂PO₄ 0.1%) and was incubated at 30° C for 2-3 days for preparing starter culture.

2.5 Preparation of Substrate

The fermentation media was prepared using 5 % substrate with distilled water.

2.6 Fermentation

The fermentation media of banana peel were inoculated by aseptically transferring 2% of starter culture to the fermentation media. The media was stirred and then incubated at 30° C for 7 days. The effect of phosphate sources, nitrogen sources, substrate concentration, incubation period, inoculum level and moisture content were observed. Except for the tested parameter, all others were set constant.

2.6 Assay Procedure for Citric Acid

The fermentation media was passed through a mesh of about 2 mm size to collect all the substrate and biomass particles. The resultant solution was passed through a filter paper. The filtrate then titrated against 0.1 M NaOH. The volume of NaOH used was recorded and citric acid was calculated. Percentage citric acid was then converted to g/l [11].

$$\% \text{ Citric acid} = \frac{\text{Volume of NaOH used} \times \text{Equivalent weight of citric acid} \times \text{Normality of NaOH} \times \text{Dilution factor}}{\text{Volume of sample taken} \times 1000} \times 100$$

3 RESULTS AND DISCUSSION

3.1 Effect of phosphate sources

Phosphate source affects the growth of biomass and is utilized by microbes for the production of metabolic intermediates, nucleotides and ATP. Three phosphate sources were studied for their effect on citric acid production. Figure 1 showed citric acid production increased by the addition of 0.1% phosphate source. *A. niger* produced 11.9 g/l, 24.09 g/l and 18.8 g/l of citric acid using disodium hydrogen phosphate, potassium dihydrogen phosphate and dipotassium hydrogen phosphate respectively in banana peel fermentation.

3.2 Effect of nitrogen sources

Figure 2 showed 0.25% addition of ammonium as nitrogen source had no significant effect on citric acid production. Ni-

trogen is a limiting factor and the type and concentration of this source affects citric acid production. Ammonium nitrate has more available nitrogen than ammonium sulphate required for the growth of fungi and hence proved to be a good nitrogen source as in earlier studies [13], but no significant difference of citric acid produced using the two nitrogen sources was observed in our studies.

3.3 Effect of inoculum level

In banana peel fermentation, an inoculum size of 5% was found to be good for citric acid production wherein 28.56 g/l citric acid was produced. Using 3%, 4% and 6% inoculum, 24.48, 25.48 and 27.94g/l citric acid was produced in banana peel fermentation (Figure 3). The production decreased on increasing inoculum size to 6%. The reason for this decrease can be the unavailability of sugar content to the fungi [11].

3.4 Effect of substrate concentrations

In banana peel fermentation, 48.72, 52.08, 11.76 and 0 g/l citric acid were produced using 20%, 25%, 30% and 35% substrate concentration (Figure 4). Substrate concentration 20% was selected optimum for fermentation while citric acid production decreased on increasing the substrate concentration. Citric acid production decreased on increasing substrate concentration, there is a possibility that increased concentration may decrease the activity of enzyme involved in citric acid cycle.

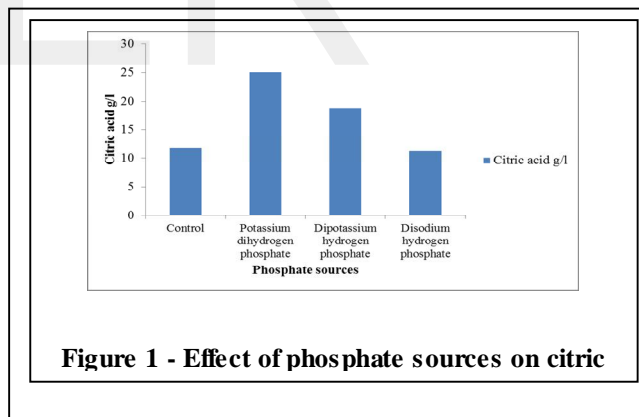


Figure 1 - Effect of phosphate sources on citric acid production

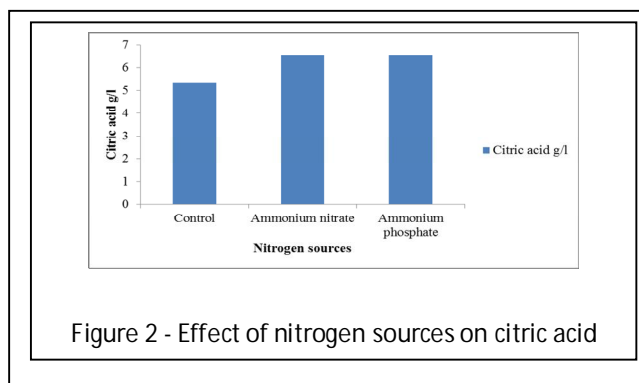


Figure 2 - Effect of nitrogen sources on citric acid production

3.5 Effect of incubation days

Citric acid produced at 6, 7, 8 and 9 days of banana peel fermentation was 41.28, 40.26, 51.68 and 24.48 g/l respectively (Figure 5). Six days and 8 days of fermentation period were found to be optimum in earlier studies as well [14]. The decrease in citric acid production after 8 days can be accounted to the decrease in sugar content and the growth phase of fungi [15].

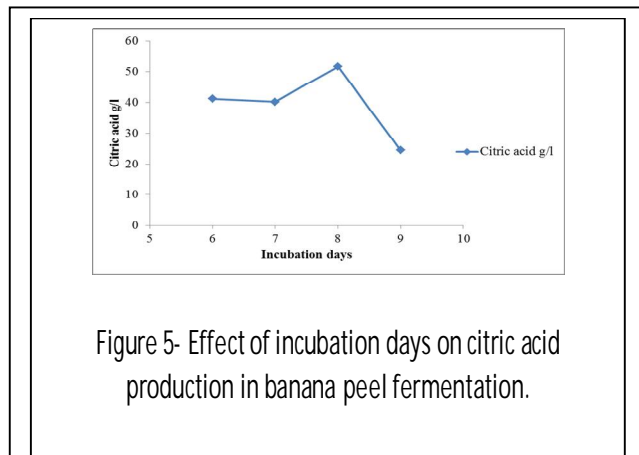


Figure 5- Effect of incubation days on citric acid production in banana peel fermentation.

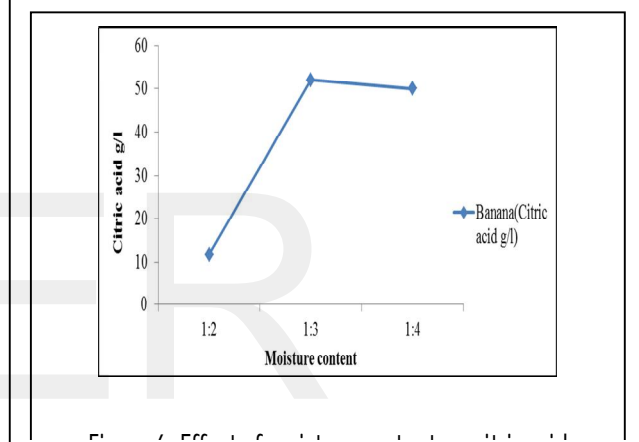


Figure 6- Effect of moisture content on citric acid production in banana peel fermentation.

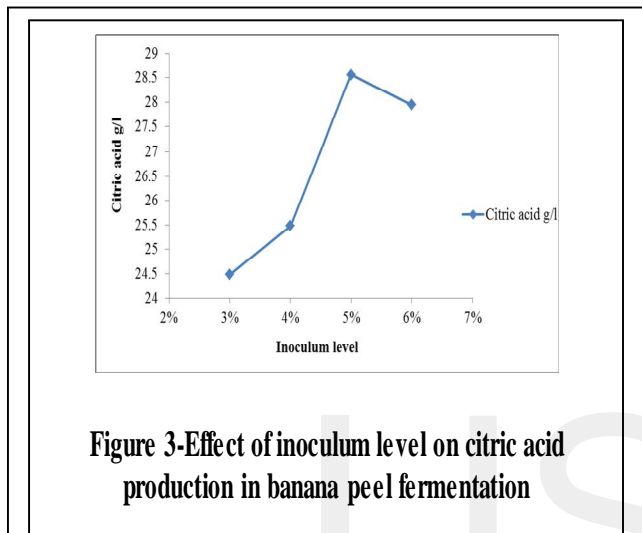


Figure 3-Effect of inoculum level on citric acid production in banana peel fermentation

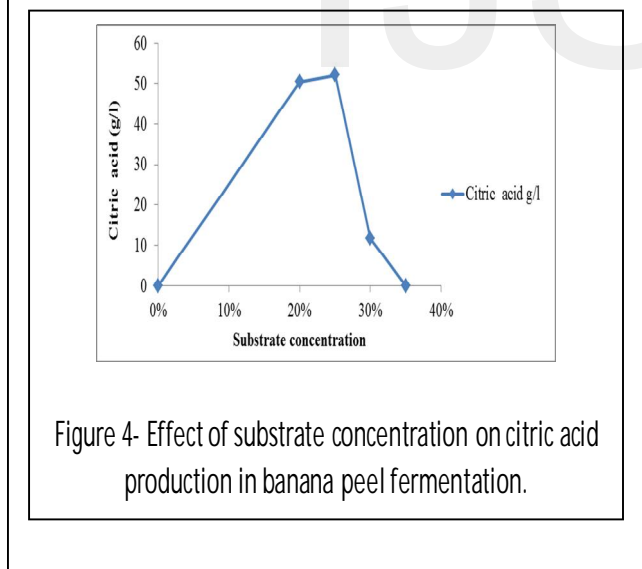


Figure 4- Effect of substrate concentration on citric acid production in banana peel fermentation.

3.6 Effect of moisture content

Citric acid produced using 1:2, 1:3 and 1:4 moisture content was 11.76, 52.08 and 50.4 g/l respectively (Figure 6). Moisture content 1:3 was found to be best for banana peel fermentation, while citric acid produced decreased when the moisture content was decreased or increased from 1:3.

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