



## Original Research Article

### Isolation and characterization of citric acid producing *Aspergillus niger* from spoiled coconut

V. Maharani<sup>\*</sup>, D.Reeta<sup>2</sup>, A. Sundaramanickam, S. Vijayalakshmi and T. Balasubramanian

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608 502, Cuddalore, Tamil Nadu, India

<sup>2</sup>Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar-608002, Cuddalore, Tamil Nadu, India

*\*Corresponding author*

## A B S T R A C T

### Keywords

Citric acid;  
*Aspergillus niger*;  
optimum condition.

Citric acids are industrially important molecules which are play potent role in preprocessing, bioremediation and brewing industries. Hence, our study focuses on production of citric acid by optimizing the conditions. *Aspergillus niger* was isolated from the spoiled coconut and it was studied for citric acid production. The citric acid production was optimized by screening different fermentation parameters which gives high yield. pH 3.5 gives higher production of citric acid (82.65 g l<sup>-1</sup>) and the temperature 30 °C showed maximum yield 80.68 g l<sup>-1</sup>. However, inoculum size and time course playing main role by producing maximum amount of citric acid at 5% inoculum (83.24 g l<sup>-1</sup>) and 192 hrs (85.18 g l<sup>-1</sup>) of incubation respectively. Our study reveals the optimum conditions for citric acid production are pH - 3.5, temperature 30 °C, inoculum level - 5% and the time course - 192 hrs.

## Introduction

Citric acid is an essential organic acid naturally presents in various citrus fruits. The citric acid has been first isolated and crystallized by Swedish chemist Carl Wilhelm Scheele. From this incident, the importance of citric acid has been understood and has utilized by various industries like food, cosmetics and pharmaceuticals. The estimated global production of citric acid is about 7, 36,000 tons per year. More than 70% of the citric acid is used in the food and fermentation

stabilizers, flavor enhancers and chelators. Less than 10% is used in cosmetic and pharmaceutical industries.

The citric acid is being produced by microorganisms during fermentation through their metabolic citric acid cycle (Haq et al., 2002). When microbes concern, there are many organisms which are producing citric acids. However, fungus is considered to be suitable producer of citric acid. Especially, *Aspergillus niger* is utilized as much as it

produces. Citric acids can be produced by well-developed methods in using chemicals. However, microbial fermentations are been used for long time for industrial scale citric acid production. The citric acids can also be used in remediation aspects. Citric acids are used as chelating agents to remove heavy metals in soils (Sayer and Gadd, 2001). Fungal organic acids usually consisting one or more carboxylic groups which is responsible for the chelating properties. The citric acids can effectively remove zinc, chromium and nickel from the soil (Elliott and Shastri, 1999; Wasay et al., 2001).

Keeping this in mind, we have used agricultural waste for the cheaper and effective production of citric acid. The present study deals with production and optimization of citric acid by using economically feasible and easily available natural substrates.

## **Materials and Methods**

### **Chemicals and Media**

All the chemicals and reagents used in this study were of analytical grade (AR). Media and constituents were purchased from HiMedia Laboratories (Mumbai)

### **Organism and culture condition**

*Aspergillus niger* was isolated from spoiled coconut and it was cultured in Czapek's dox medium (NaNO<sub>3</sub> 3.0; K<sub>2</sub>HPO<sub>3</sub> 1.0; MgSO<sub>4</sub> 0.5; KCl 0.5; FeSO<sub>4</sub> 0.01; Sucrose 30.0; Agar 15.0 (g/L) and the pH 7.2) and maintained in slants for 5 to 7 days at room temperature. Spore suspension was prepared using 5 mL of sterile distilled water per slant and mixed with conidia and the suspension was used for further fermentation studies.

### **Effect of different pH on citric acid production**

The Czapek's dox broth was prepared in 1000 ml quantities in a conical flask and pH of the broth was adjusted with 0.1 N HCl to obtain the pH level of 1.5, 2.5, 3.5 and 4.5 separately and 5 per cent of the prepared spore suspension was inoculated and incubated for 3-5 days at 30 °C

### **Effect of different temperature on citric acid production**

The Czapek's dox broth was prepared in 1000 ml quantities in a conical flask and 5 per cent of prepared spore suspension was inoculated separately and incubated for 3-5 days at different temperatures viz., 10, 20, 30 and 40 °C in a BOD incubator separately.

### **Effect of different inoculum levels on citric acid production**

The Czapek's dox broth was prepared in 1000 ml quantities in a conical flask prepared spore suspension was inoculated at different inoculum levels of 1, 3, 5 and 7 per cent separately. The flasks were incubated for 3-5 days at 30 °C.

### **Time course of citric acid production**

The Czapek's dox broth was prepared in 1000 ml quantities and 5 per cent of the prepared spore suspension was inoculated. And the flasks were incubated for different fermentation periods viz., 96, 144, 192 and 240 hrs respectively.

### **Citric acid estimation**

The broth culture was filtered through cheese cloth to separate mycelia, and the filtrate was used for estimation. The citric acid estimation was done by

gravimetrically using the method of Marier and Boulet (1958). In brief, 1 ml of sample was added with 1.3 ml of pyridine and mixed well. The solution was added with 5.7 ml of acetic anhydride and placed incubated at 32 °C for 30 minutes in water bath. The OD values of the samples were recorded at 405 nm.

## Results and Discussion

### Effect of different level of pH on citric acid production

The *Aspergillus niger* produced higher concentration (82.65 g l<sup>-1</sup>) of citric acid at the pH 3.5 (Fig. 1). Followed by pH 2.5 (70.58 g l<sup>-1</sup>). Mostly, the fungal strains are seemed to be grown well under acidic conditions ranging from 3 to 6 (Fawole and Odunfa, 2003). Even for solid state and submerged fermentation the pH range 2 to 6 is commonly used (Adham, 2002; Lesniak et al., 2002). Ali et al (2005) reported the maximum concentration of citric acid production (140 g l<sup>-1</sup>) was observed at the pH 3.5.

### Effect of different temperature on citric acid production

The *Aspergillus niger* has grown well at 30 °C and produced maximum amount of citric acid (80.68 g l<sup>-1</sup>) followed by 20 °C with 74.21 g l<sup>-1</sup> of citric acid (Fig. 2). Similarly, Ali (2002) have reported maximum amount of citric acid production (99.56 ± 3.59 g l<sup>-1</sup>) achieved by *Aspergillus niger* GCBT7 at 30°C. Anand Kishore (2008) has also reported that maximum citric acid production was recorded at 30°C using *Aspergillus niger* NCIM 705. The effect of temperature plays an important role in the production of citric acid by *Aspergillus niger* (Grewal and Kalra, 1999).

### Effect of different inoculum levels on citric acid production

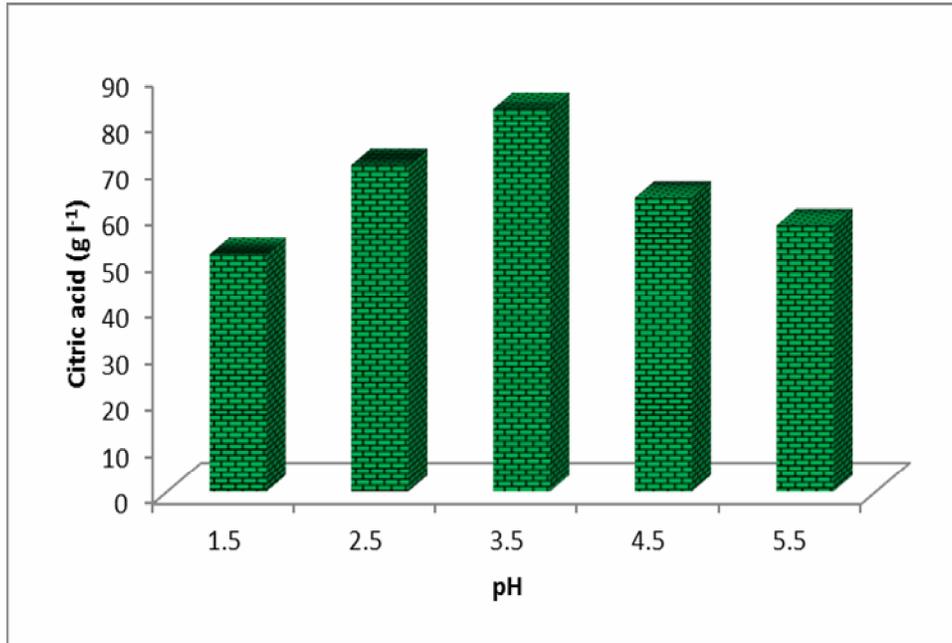
Among different concentrations of inoculum used, 5% conc. seems to be optimum and produced 83.24 g l<sup>-1</sup> of citric acid during the fermentation period (Fig. 3). Similarly, Ali et al (2003) reported that, the 5% conc. of inoculum was optimum for maximum citric acid production (96.55 g l<sup>-1</sup>) using *Aspergillus niger* under submerged fermentation condition. The 5% conc. of the inoculum is seems to be optimum for citric acid production.

### Time course of citric acid production

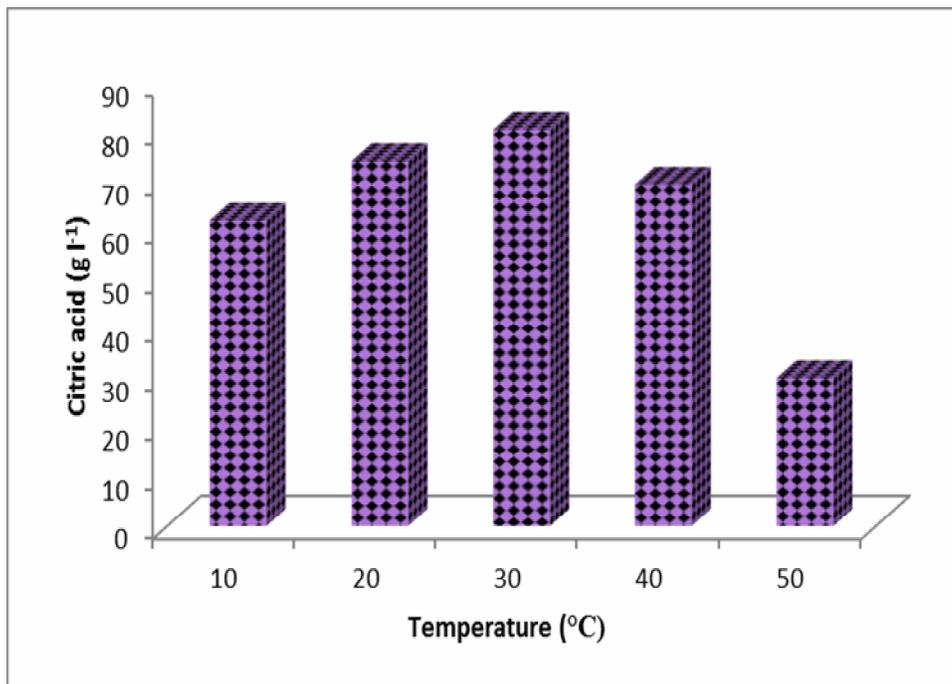
The *Aspergillus niger* has incubated for several hours. It was produced the maximum amount of citric acid 85.18 g l<sup>-1</sup> on 192 hrs, followed by 79.32 g l<sup>-1</sup> on 144 hrs of incubation (Fig. 4). Similarly, Sanchez et al. (1990) reported the maximum amount of citric acid production occurred at 192 hrs of incubation. The time course of citric acid production may vary for wild strains, however, our study revealed that the optimum time course is 192 hr for citric acid production.

From the above results, the following fermentation parameters viz., pH - 3.5, temperature - 30°C, inoculum level - 5% and the time course - 192 hrs were selected as optimum for the production of citric acid using *Aspergillus niger*. The present study showed that *Aspergillus niger* have the potential to produce citric acid in large quantity at the optimized fermentation conditions.

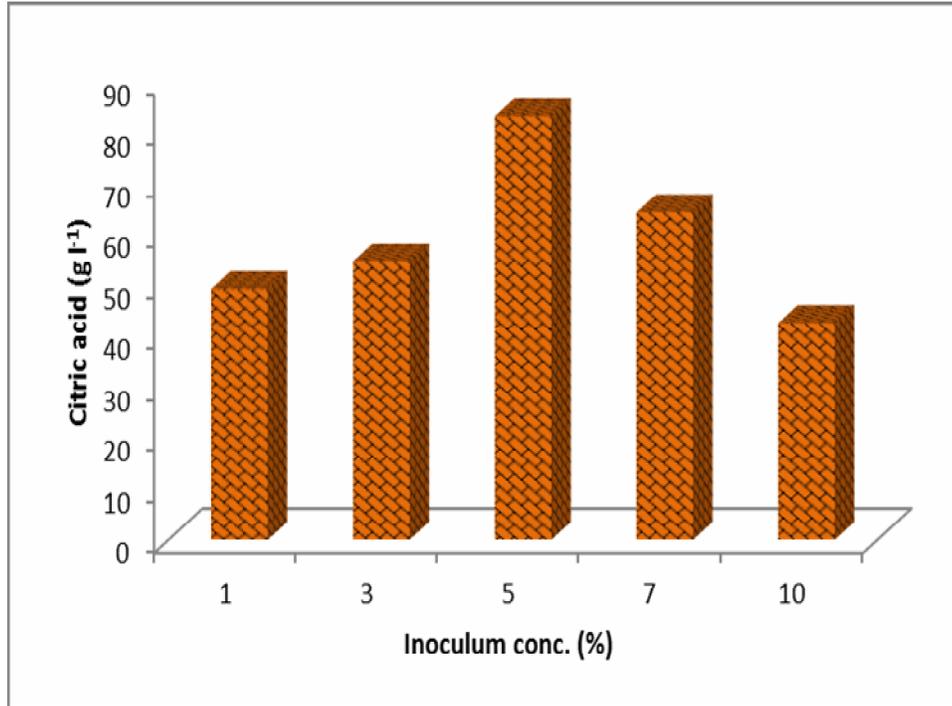
**Figure.1** Effect of pH on citric acid production



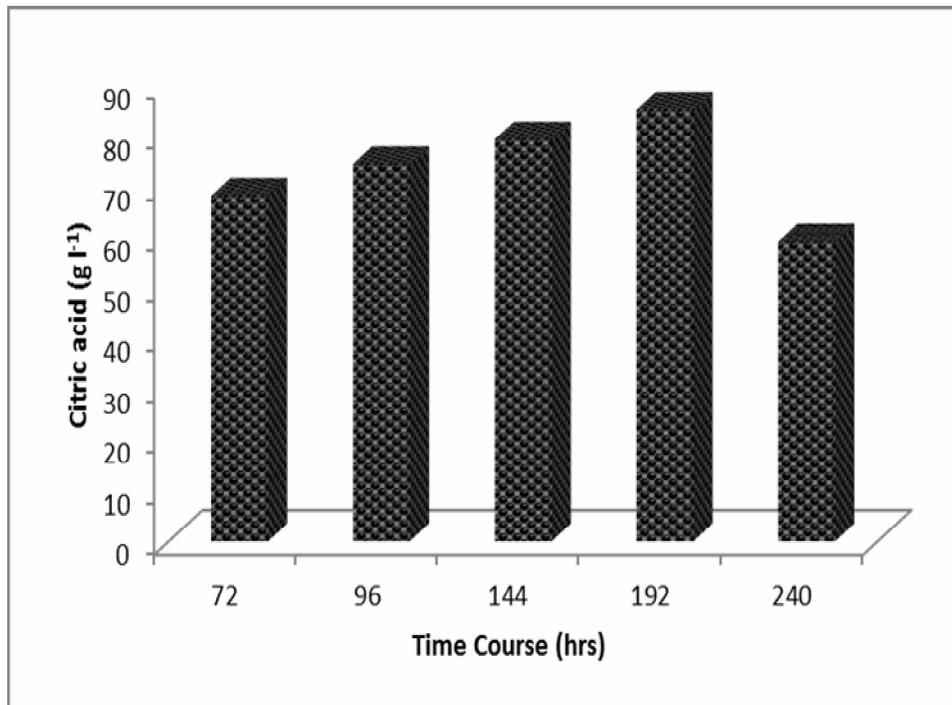
**Figure.2** Effect of Temperature on citric acid production



**Figure.3** Effect of inoculum on citric acid production



**Figure.4** Time course of citric acid production



## Acknowledgement

The authors are thankful to the Annamalai University authority for providing permission and necessary facilities to carry out this study.

## References

- Adham, N.Z., 2002. Attempts at improving citric acid fermentation by *Aspergillus niger* in beet-molasses medium. *Bioresource Technology*. 84 (1): 97-100.
- Ali, S., I. Haq, M.A. Qadeer and J. Iqbal. 2002. Production of citric acid by *Aspergillus niger* using cane molasses in a stirred fermentor. *Electronic Journal of Biotechnology*. 5 (3): 258-271.
- Ali, S., I. Haq and J. Iqbal. 2005. Effect of Low pH on continuous citric acid fermentation by *Aspergillus niger*. *Pakistan Journal of Botany*. 37 (4): 981-987.
- Kishore, K.A., M.P. Kumar, V.R. Krishna, V. Ravi and G.V. Reddy. 2008. Optimization of process variables of citric acid production using *Aspergillus niger* in a batch fermentor. *Engineering Letters*. 16 (4): p572.
- Elliott, H.A., and N.L. Shastri. 1999. Extractive decontamination of metal-polluted soils using oxalate. *Water, Air, and Soil Pollution*. 110 (3-4): 335-346.
- Fawole, O.B., and S.A. Odunfa. 2003. Some factors affecting production of pectin enzymes by *Aspergillus niger*. *International Biodeterioration & Biodegradation*. 53 (4): 223-227.
- Grewal, H.S., and K.L. Kalra. 1995. Fungal production of citric acid. *Biotechnology Advances*. 13 (2): 209-234.
- Haq, I., S. Khurshid, S. Ali, H. Ashraf, M.A. Qadeer and M. Ibrahim Rajoka. 2002. Mutation of *Aspergillus niger* for hyperproduction of citric acid from black strap molasses. *World Journal of Microbiology & Biotechnology*. 17: 35-37.
- Lesniak, W., J. Pietkiewicz and W. Podgorski. 2002. Citric acid fermentation from starch and dextrose syrups by a trace metal resistant mutant of *Aspergillus niger*. *Biotechnology Letters*. 24 (13): 1065-1067.
- Marier, J.R., and M. Boulet. 1958. Direct determination of citric acid in milk with an improved pyridine-acetic anhydride method. *Journal of Dairy Science*. 41 (12): 1683-1692.
- Ali, S., A. Rehman and I. Haq. 2003. Time course study of citrate fermentation by *Aspergillus niger* in stationary culture. *Pakistan Journal of Biological Sciences*. 6: 331-333.
- Sanchez-Marroquin, A., R. Carreno and M. Ledezma. 1970. Effect of trace elements on citric acid fermentation by *Aspergillus niger*. *Applied Microbiology*. 20 (6): 888-892.
- Sayer, J.A., and G.M. Gadd. 2001. Binding of cobalt and zinc by organic acids and culture filtrates of *Aspergillus niger* grown in the absence or presence of insoluble cobalt or zinc phosphate. *Mycological Research*. 105 (10): 1261-1267.
- Wasay, S.A., S. Barrington and S. Tokunaga. 2001. Organic acids for the *in situ* remediation of soils polluted by heavy metals: Solid flushing in columns. *Water, Air, and Soil Pollution*. 127 (1-4): 301-314.