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CITRIC ACID PRODUCTION BY ASPERGILLUS NIGER ETGP12, ETGP18 ON SOLID STATE FERMENTATION AND EFFECT OF INITIAL TEMPERATURE ON YIELD

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ABSTRACT

Citric acid is mainly produced by solid state fermentation by the filamentous fungus Aspergillus niger. Production of citric acid depends strongly on an appropriate strain and on operational conditions such as aeration, type and concentration of carbon source, nitrogen and phosphate limitation, pH, temperature, concentration of trace elements and morphology of the producer organism. The yield of citric acid increased with the increase in the initial temperature of the fermentation upto 30°C by *A. niger* ATCC 9142, thereafter a gradual decrease in the citric acid yield was noticed for all the days of fermentation on both the substrates. The highest yield of citric acid (90.23 g/kg and 96.38 g/kg from sesamum oil cake and rice chaff respectively) was observed at 30°C at 72 hrs, whereas the lowest yield (50.23 g/kg and 53.61 g/kg from sesamum oil cake and rice chaff respectively) of citric acid was observed at 40°C at 72hrs. It infers that the increase in the temperature increases the yield of citric acid to certain level and at higher temperatures the yield of the citric acid will be less.

KEYWORDS

Aspergillus niger, Temparature, standardization, ETGP12, ETGP18, Control culture

INTRODUCTION

Citric acid is one of the most commonly used organic acids in food and pharmaceutical industries. The food industry is the largest consumers of citric acid, using almost 70% of the total production, followed by about 12% for pharmaceutical industry and 18% for other applications. Although citric acid can be obtained by chemical synthesis, cost is much higher than using fermentation . It is mainly produced by solid state fermentation by the filamentous fungus *Aspergillus niger* .In order to decrease the cost of citric acid production using *Aspergillus niger*, solid state fermentation has been studied as a potential alternative to solid state fermentation. Production of citric acid depends strongly on an appropriate strain and on operational conditions such as aeration, type and concentration of carbon source, nitrogen and phosphate limitation, pH, temperature, concentration of trace elements and morphology of the producer organism. To develop a process for the maximum production of citric acid, standardization of media and fermentation conditions is crucial. Media optimization by the classical method- a single dimensional search involving changing one variable while fixing others at a certain level is laborious and time consuming, especially when number of variables are larger. In this study, some initial experiments were carried out with 2 strains of *A.niger,* ETGP12 & ETGP18 were selected for the further studies of solid state fermentation using *A.niger* ATCC9142 as control culture on sesamum oil cake and rice chaff. Main factors affecting productivity and yield were optimized in both systems and results showed that solid state fermentation had higher sensitivity to temperature variations and short interruptions which resulted in low yield of citric acid fermentation.

MATERIALS AND METHODS

Spore suspensions were prepared from 168 h old cultures grown on PDA slants by adding 10 ml sterile distilled water containing 0.01% Tween–80 and suspending the spores with a sterile loop. One ml of the spore suspension containing about $1x10^7$ spores/ml were used to inoculate experimental media in the flasks in all the solid state fermentation studies (Shankaranand 1995). Sesamum oil cake was ground and dried at 52°C for 6 hours (Tran & Mitchell 1995). The powders thus obtained were analysed for fermentable sugars and adjusted to pH 3.0 (Hang & Woodams 1985). The samples thus prepared were taken out separately into 250 ml Erlenmeyer flask and rehydrated to required moisture content by adding sterile water as per Hang and Woodams 1987. The cotton plugged flasks were autoclaved at 121°C for 15 minutes followed to cool to about 35°C. The contents of flasks were inoculated with 1ml of inoculum ($1x10^7$ spores/ml). Then the flasks were mixed thoroughly by gently beating on the palm of the hands and incubated in a slanting position at 35°C in an incubator with 65-70% relative humidity for 7 days (Ramesh and Lonsane, 1990).About 40 g of the ground substrates were taken in 250 ml Erlenmeyer flasks and thus prepared flasks were inoculated at different temperatures like 20, 25, 30, 35 and 40°C.

RESULT AND DISCUSSION

The effect of different temperature levels on the yield of citric acid by *A. niger* strains from sesamum oil cake and rice chaff are represented in figures 1 to 6.

The yield of citric acid increased with the increase in the initial temperature of the fermentation upto 30°C by *A. niger* ATCC 9142, thereafter a gradual decrease in the citric acid yield was noticed for all the days of fermentation on both the substrates. The highest yield of citric acid (90.3 g/kg and 96.4 g/kg from sesamum oil cake & rice chaff respectively) was observed at 30°C at 72 h, whereas the lowest yield (50.2 g/kg and 53.6 g/kg from sesamum oil cake & rice chaff respectively) of citric acid was observed at 40°C at 72hrs. It infers that the increase in the temperature increases the yield of citric acid to certain level and at higher temperatures the yield of the citric acid will be less.

Similarly, in case of *A. niger* ETGP12, the yield of the citric acid on both the substrates increased as temperature increased upto 30°C, thereafter a decrease in the yield was observed. It has yielded the highest amount of citric acid (95.43 g/kg and 105.39 g/kg from sesamum oil cake & rice chaff respectively) at 30°C. In case of *A. niger* ETGP18 (89.52 g/kg and 95.42 g/kg from sesamum oil cake & rice chaff respectively at 30°C), the similar kind of observation was made, but the quantity of the citric acid was found less compared to the yield produced by ETGP12.

The content of the residual sugars decreased gradually as citric acid accumulation occured during the fermentation on both the substrates by ATCC 9142, ETGP12 and ETGP18. Though all the strains produced different quantities of citric acid using the above said substrates, there was very less difference in their sugar conversion efficiencies. *A. niger* ATCC 9142 has converted sugars into citric acid (62.5 & 64.7% sugars respectively from sesamum oil cake and rice chaff), while ETGP12 has converted 67.7 and 68.4% from sesamum oil cake and rice chaff respectively and ETGP18 has converted 65.3 and 66.35% from sesamum oil cake and rice chaff respectively at 30°C.

For the growth of *A. niger* and for the production of high yield of citric acid there is a need of optimum temperature. Hence, any increasing temperature has been found to decrease in citric acid yields and the increase in oxalic acid accumulation . Since various temperature levels have been reported as optimum for the fermentation, it seemed desirable to check the influence of incubation temperature upon the fermentation. With this context, the yield of citric acid by *A. niger* ETGP18 and *A. niger* ETGP12 have been analyzed.

The yield of citric acid increased with the increase in the initial temperature of the fermentation upto 30°C by *A. niger* ATCC 9142, thereafter a gradual decrease in the citric acid yield was noticed for all the days of fermentation. The highest yield of citric acid (90.23 g/kg & 96.38 g/kg from sesamum oil cake & rice chaff respectively) was observed at 30°C at 72 hrs, It infers that the increase in the temperature increases the yield of citric acid to certain level and at higher temperatures the yield of the citric acid will be less. Similarly, in case of *A. niger* ETGP12, the yield of the citric acid on both the substrates increased as temperature increased upto 30°C, thereafter a decrease in the yield was observed . In case of *A. niger* ETGP18 (89.5 g/kg and 95.4 g/kg from sesamum oil cake & rice chaff respectively at 30°C), the similar kind of the observation was made, but the quantity of the citric acid was found less compared to the yield produced by ETGP12. Similar type of observations at the optimum 30°C temperature was found to be better for the yield of citric acid production by *A. niger* strains (Khare *et al* 1995).

In this study, the content of the residual sugars decreased gradually as citric acid accumulation occurs during the fermentation on both the substrates by ATCC 9142, ETGP12 and ETGP18. Though all the strains produced different quantities of citric acid using the above said substrates, there was very less difference in their sugar conversion efficiencies. *A. niger* ATCC 9142 has converted sugars into citric acid (62.5 and 64.7% sugars respectively from sesamum oil cake & rice chaff), while ETGP12 has converted 67.7 and 68.44% from sesamum oil cake & rice chaff respectively and ETGP18 has converted 65.32 and 66.35% from sesamum oil cake & rice chaff respectively at 30°C.

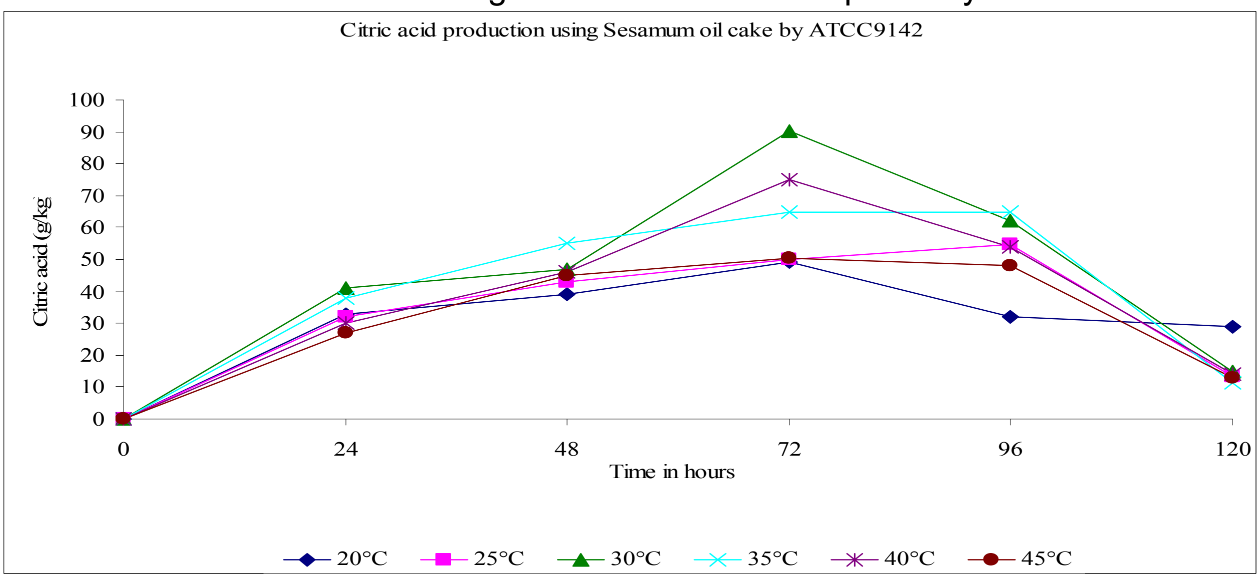

Fig. 1 Production of citric acid at various temperatures using Sesamum oil cake as the substrate using

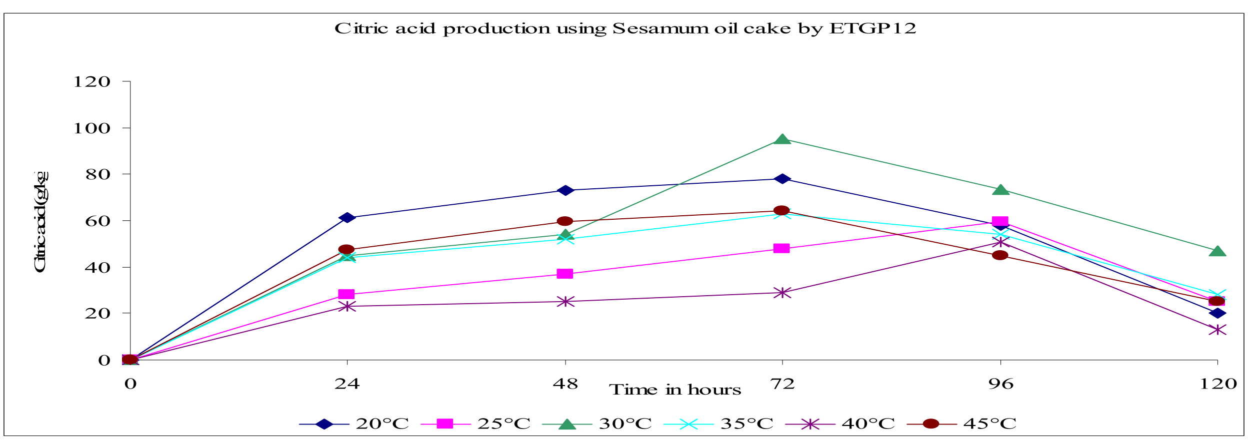

Fig. 2 Production of citric acid at temperatures using Sesamum oil cake as the substrate using ETGP12 strain.

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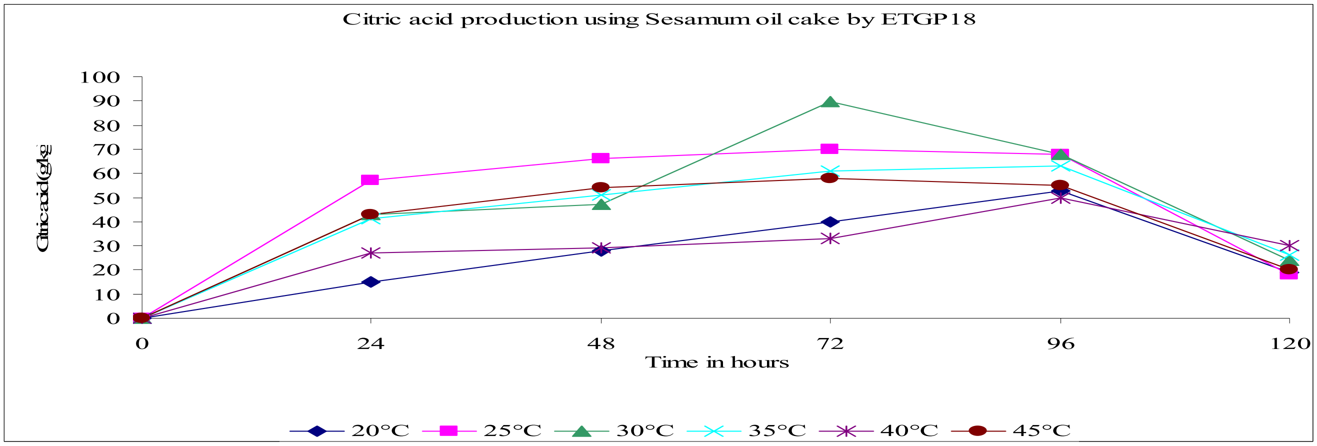

Fig. 3. Production of citric acid at various temperatures using Sesamum oil cake as the substrate using ETGP18 strain.

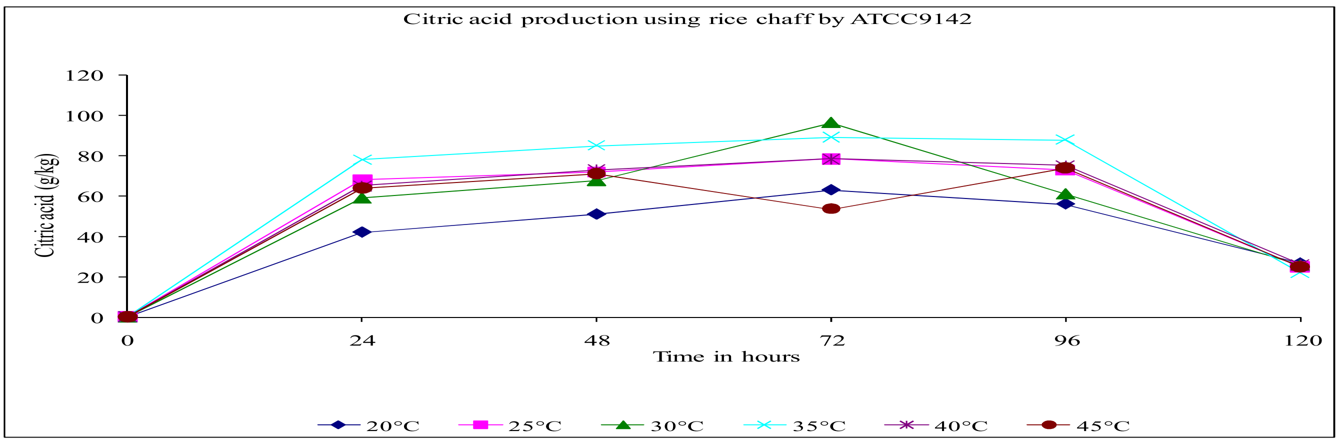

Fig. 4 Production of citric acid at various temperatures using rice chaff as the substrate using ATCC9142 strain.

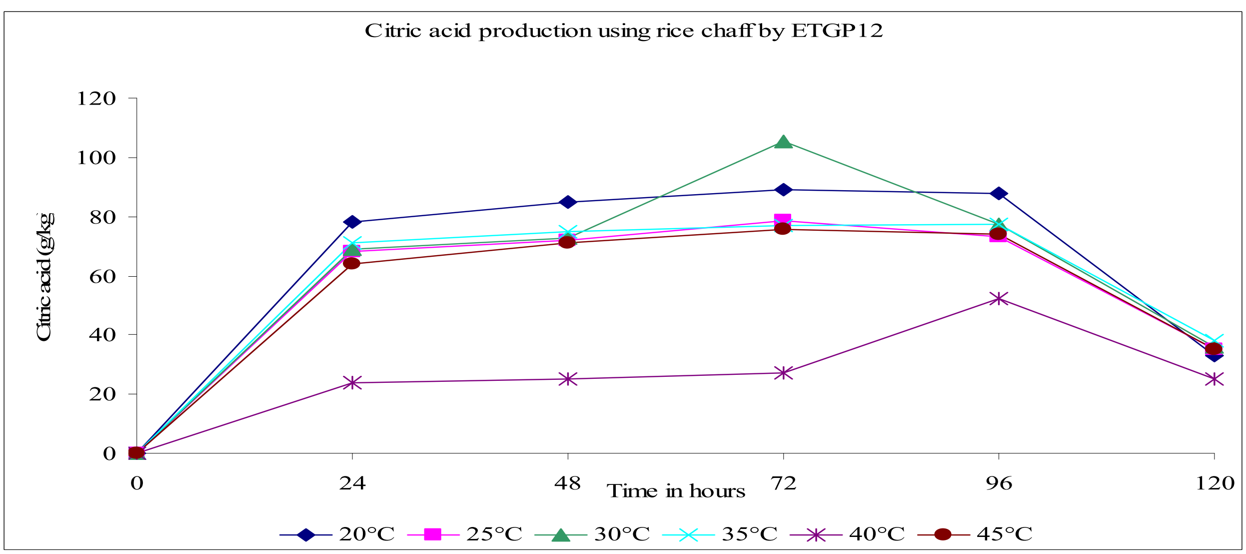

Fig. 5 Production of citric acid at various temperatures using rice chaff as the substrate using ETGP12 strain.

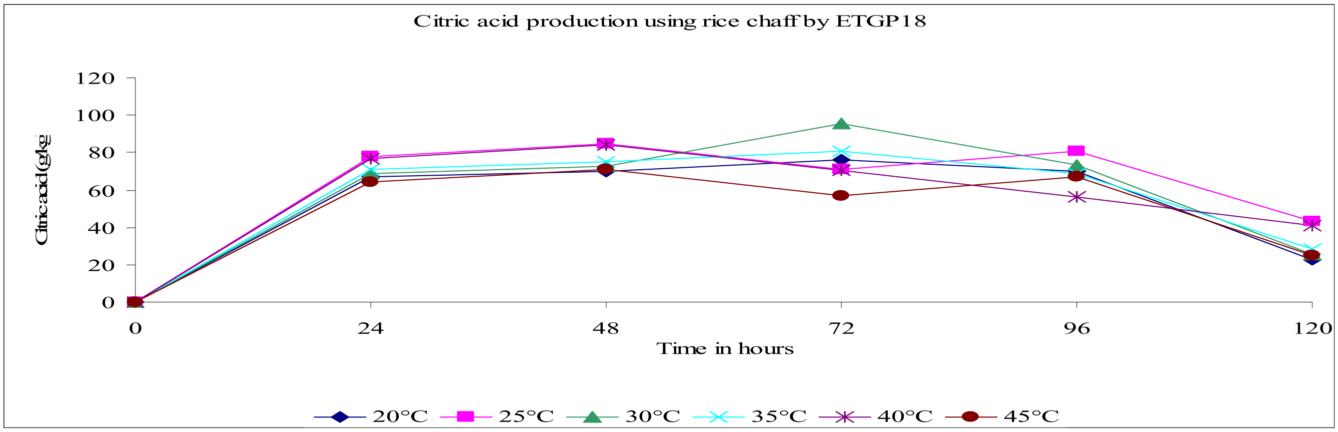

Fig. 6 Production of citric acid at various temperatures using rice chaff as the substrate using ETGP18 strain.

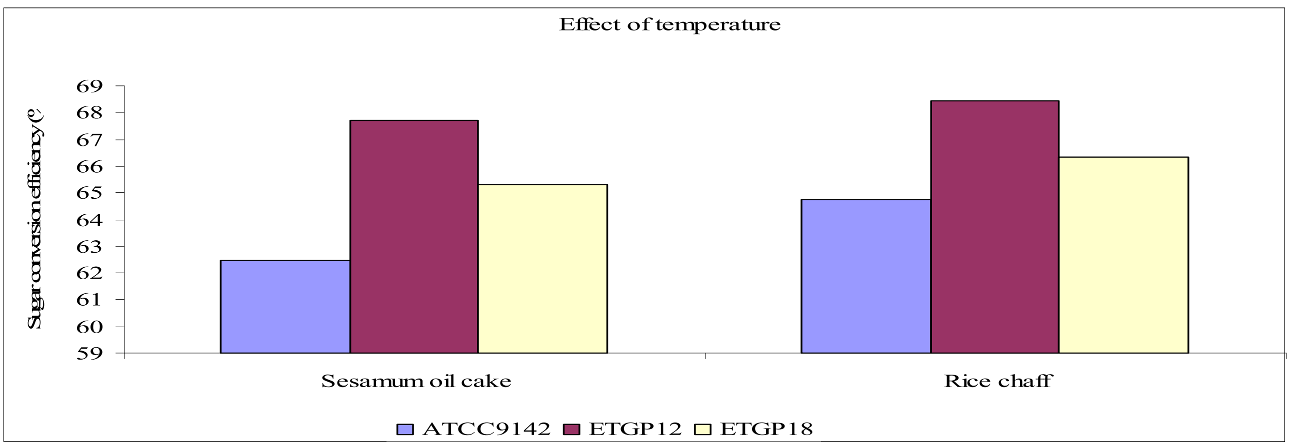

Fig. 7 The temperature at which the sugar conversion efficiency observed for standard and experimental

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