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EFFECT OF DIFFERENT NITROGEN SOURCES ON CITRIC ACID PRODUCTION BY *ASPERGILLUS NIGER*

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ABSTRACT

The effect of four different nitrogen sources, ammonium nitrate, ammonium sulphate, urea and ammonium phosphate on citric acid production by Aspergillus niger was studied. Ammonium nitrate was found to be the best nitrogen source followed by urea, ammonium phosphate and ammonium sulphate, the highest citric acid yields obtained from these nitrogen sources are 13.66g/l, 10.25g/l, 8.97g/l, 8.11g/l respectively.
***Keywords**: Citric acid, Aspergillus niger, Submerged fermentation, Nitrogen source.*

INTRODUCTION

Citric acid is one of the most versatile industrial organic acids that are used in foods, cosmetics and pharmaceuticals. About 70% of citric acid is utilized in food industry, confectionery and beverages as an accidulant, flavour enhancer, preservative, chelator, buffer, emulsifier, stabilizer and antioxidant. About 10% is used in cosmetics and pharmaceuticals (Kubicek and Rohr, 1985; Lodhi *et al.*, 2001). Citric acid is colourless, odourless and easily soluble in water and alcohol with a pleasant taste, solid at room temperature and melts at 153^{0}C. It exists as an intermediate in the Krebs cycle (Haq *et al.*, 2003). Citric acid production is directly influenced by the concentration and nature of the nitrogen source. Physiologically, ammonium salts are preferred; these include ammonium nitrate (NH_4NO_3), ammonium sulphate (NH_4SO_4), peptone and malt extract. Acid ammonium compounds are more preferrable because their consumption leads to pH decrease which is essential for the citric acid fermentation (Lodhi *et al.*, 2001). This study was aimed at improving citric acid yield by the isolate through the use of different nitrogen sources.

MATERIALS AND METHODS

Organism and culture maintenance

Aspergillus niger isolate was obtained from the department of Microbiology, Ahmadu Bello University, Zaria, Kaduna state. The isolate was maintained on potato dextrose agar slants.

Inoculum Preparation

The spores of the isolates were harvested from slant bottles of 4-6 days old cultures by washing with sterile distilled water containing 0.8% Tween 80(Poolyoxyethylene- sorbitanmonooleate) and enumerated using a haemocytometer (Lasure *et al.*, 2003).

Nitrogen Sources

The following nitrogen sources were used at the same concentration of 0.31%, NH_4NO_3, ammonium phosphate (NH_4PO_4), ammonium sulphate (NH_4SO_4) and Urea. The *Aspergillus niger* was screened for citric acid production using submerged fermentation in shake flask. The fermentation medium also consisted of, 0.15g of KH_2PO_4/litre, 2.2g of MgSO4.7H2O/litre and glucose concentration of 15%. Other cultural conditions include initial pH of 6.0, temperature of between 26^{0}C-29.5^{0}C, 450 rpm and length of fermentation of 168h (7 days). Throughout the work the fermentations were carried-out in 500ml Erlenmeyer flasks and the working volume was 60% (300ml) of the flask capacity. The inoculum size was 10ml of the spores suspension corresponding to 10^7spores per ml.

Analytical Methods

Citric acid was determined daily titrimetricaly as described by the British Pharmacopea, 1973 & Al-Delaimy and El-Holi, 2003. Dry mycelia weight was determined as described by Al – Delaimy and El-Holi, (2003), Haq *et al.*, (2003). Residual sugar was determined from the filtrate obtained above using the 3, 5- Dinitrosalycilic acid (DNS) method (Miller, 1959).

RESULTS

Figures 1, 2, 3 and 4 depicts citric acid yield, residual sugar concentration and mycelia weight of *A. niger* using ammonium nitrate, ammonium sulphate, urea and ammonium phosphate as nitrogen sources respectively. Ammonium nitrate gave the highest citric acid yield of 13.66g/l, followed by urea (10.25g/l), ammonium phosphate (8.97g/l) and ammonium sulphate (8.11g/l). In all cases the highest citric acid yield was obtained after 144h (6 days) of fermentation except in the case of ammonium sulphate in which the highest citric acid yield was obtained after 120h (five days) of fermentation. Residual sugars concentration decreased progressively for all the four nitrogen sources, from 117.76g/l to 6.30g/l, 120.00g/l to 4.10g/l, 123.00g/l to 9.80g/l and 118.30g/l to 5.30g/l with respect to ammonium nitrate, ammonium sulphate, urea and ammonium phosphate respectively while mycelia weight increased progressively over the seven days of fermentation, from 2.98g/l to 15.04g/l, 2.02g/l to 11.05g/l, 2.45g/l to 11.02g/l and 2.34g/l to 10.10g/l for ammonium nitrate, ammonium sulphate, urea and ammonium phosphate respectively.

DISCUSSION

Citric acid has been known to be formed when nitrogen is the limiting factor (Ikram-Ul *et al.*, 2005). Of the four nitrogen sources studied ammonium nitrate at the concentration of 0.2% gave the highest (13.66g/l) amount of citric ac[illegible] after 144h ([illegible]. This tally with the finding[illegible] al., 2002, in [illegible] ammonium nitrate at the same concentration of 0.2% gave the highest citric acid yield of 89.64g/l after 144h. Studies by El-aasar (2006), on the effect of different concentrations of ammonium nitrate, ammonium sulphate and urea showed that the *A. niger* strain used in the study preferred 0.3% ammonium nitrate as a nitrogen source. Among the other three nitrogen sources, urea gave the highest citric acid yield of 10.25g/l after 144h of fermentation. Studies by Ghosh and Banik, (1998) and Lodhi *et al.*, (2001) showed that urea at a concentration of 0.4% and 0.25% respectively was the best nitrogen source. There was significant difference in the mean citric acid produced between ammonium nitrate(11.04g/l) and the other three nitrogen sources, but there was no significant difference in the mean citric acid produced from urea(7.07g/l), ammonium sulphate(6.95g/l) and ammonium phosphate(6.60g/l) ($P<0.05$). The difference in substrates used, organism and optimum conditions may be the source of variation between their findings and the findings of this study. El-aasar (2006), reported that the best nitrogen source among others for citric acid production are ammonium nitrate and urea.

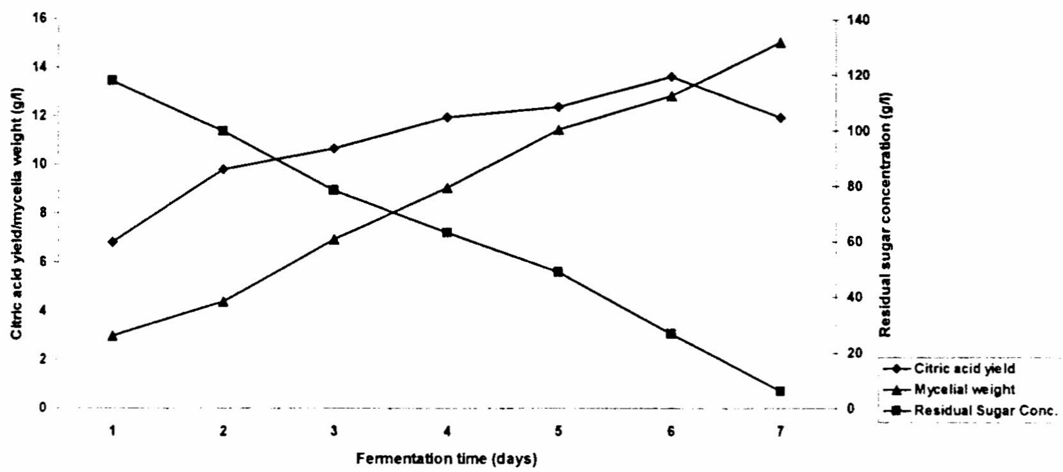

Figure 1: Citric acid yield, residual sugar concentration and mycelia weight of *A. niger* using ammonium nitrate as nitrogen source

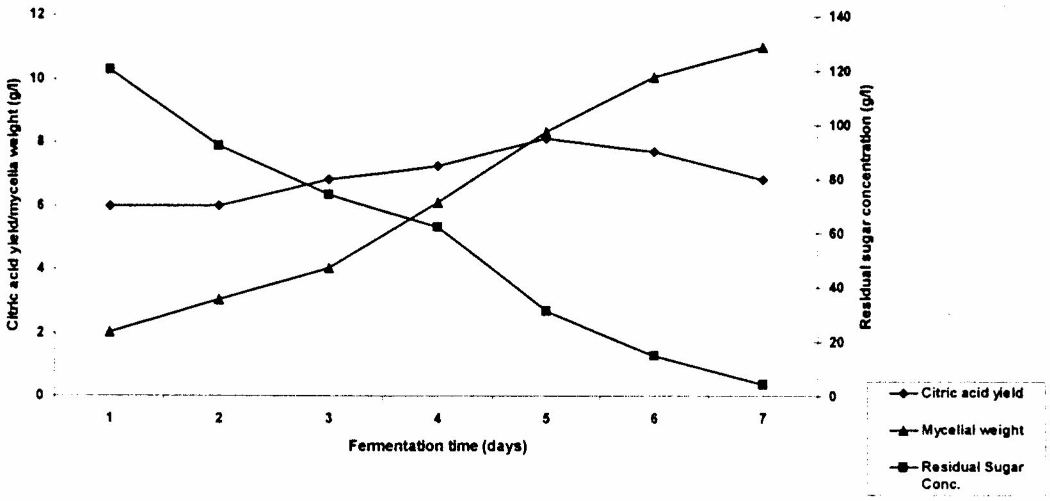

Figure 2: Citric acid yield, residual sugar concentration and mycelia weight of *A. niger* using urea as nitrogen source

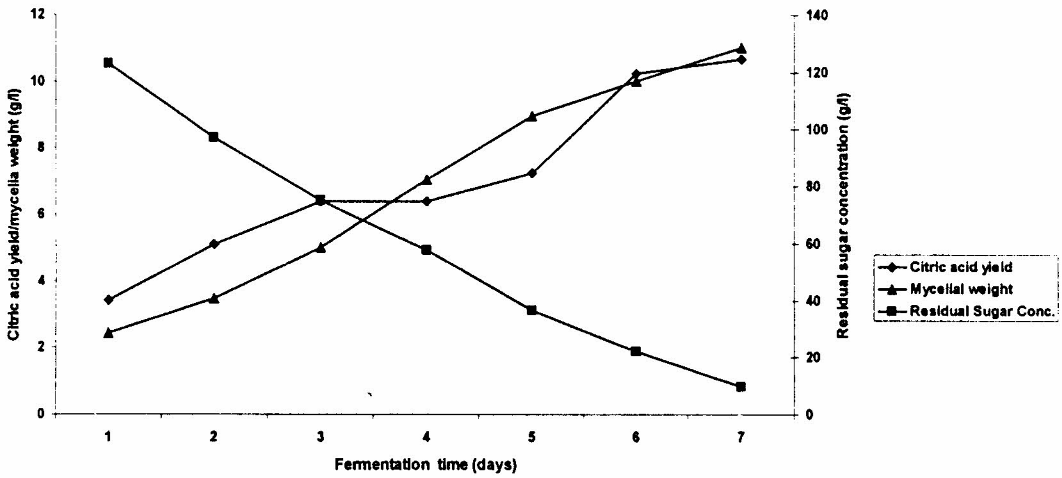

Figure 3: Citric acid yield, residual sugar concentration and mycelia weight of *A. niger* using ammonium phosphate as nitrogen source

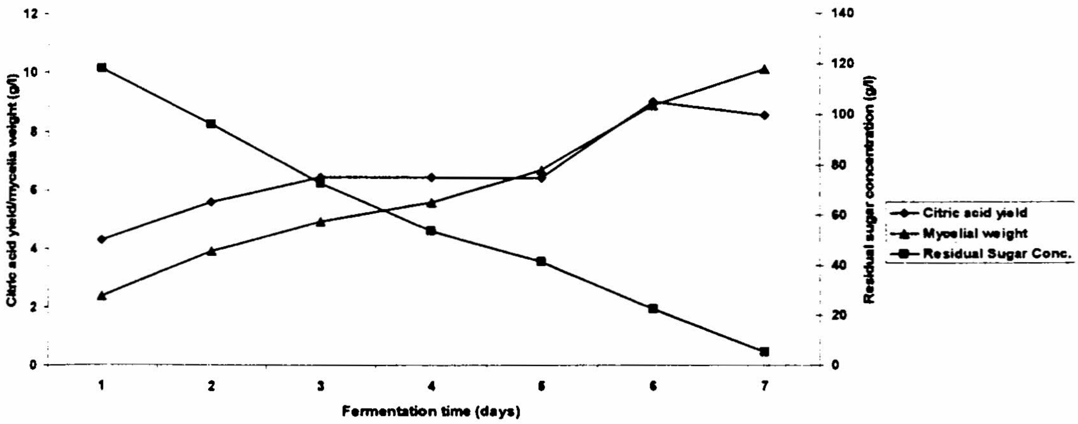

Figure 4: Citric acid yield, residual sugar concentration and mycelia weight of *A. niger* using ammonium sulphate as nitrogen source

CONCLUSION

Nutrients, especially nitrogen source have a marked influence on citric acid productivity because it is an essential constituent of basal proteins. In general, a high concentration of nitrogen leads to greater vegetative growth and delays the onset of the production phase. It is therefore, necessary to correctly determine the nitrogen source and the concentration essential for maximal citric acid production by different fungal strains under different fermentation conditions.

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