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EFFECT OF MINERAL NUTRIENTS ON THE BIOSYNTHESIS OF CITRIC ACID BY *ASPERGILLUS NIGER* UV-6, USING SUCROSE SALT MEDIA

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Abstract

The present investigation deals with the effect of mineral nutrients on the biosynthesis of citric acid by UV-6, a mutant strain of *Aspergillus niger*. Sucrose salt media was used as the basal medium and the volume was kept at 25-ml/each 250 ml conical flask. Among the mineral nutrients tested, 0.20% NH_4NO_3 and 0.10% KH_2PO_4 were optimized as the best nitrogen and phosphate sources for maximal citric acid production (42.01 g/l). The studies also revealed that pellet formation has a great influence on citric acid biosynthesis.

Introduction

Citric acid is one of the most important organic acids. Its worldwide demand is about 6.0×10^5 tons per year (Wieczorek and Brauer, 1998). *Aspergillus niger* is the organism of choice for citric acid production (Seaton and Wales, 1994). The organisms need major elements such as carbon, nitrogen, phosphorus and sulphur in addition to various trace elements for growth. The concentration of all these elements has a profound effect on the yield of citric acid (Ali *et al.*, 2001). The nitrogen requirement for the production of citric acid is generally met by the addition of inorganic nitrogen sources such $(NH_4)_2SO_4$, NH_4NO_3 and urea. Phosphate concentration is also critical for citric acid production (Khan *et al.*, 1970). The cultural conditions markedly influence the growth pattern of filamentous fungi, which can range from a pellet to a dispersed filamentous form, affecting in this way both the growth rate and the product formation (Nielsen 1992; Pera and Callieri, 1999). In this research manuscript, we report the effect of mineral nutrients on the fungal growth pattern and citric acid-production during submerged fermentation by a mutant *Aspergillus niger* UV-6, using sucrose salt medium.

Materials and Methods

Inoculum: The mutant strain of *Aspergillus niger* UV-6, maintained on potato dextrose agar slants was used for inoculation. The conidial suspension was prepared (3-5 days old culture) by adding 10 ml of sterilized distilled water and inoculum needle was used for breaking the clumps of conidia.

Fermentation conditions: The fermentation medium contained (%, w/v); sucrose 15, KH_2PO_4 0.1, $MgSO_4.7H_2O$ 0.025, NH_4NO_3 0.25 and pH 3.5. Fermentation was rotated (200 rpm) in the rotary incubator shaker at 30°C for 7 days. All the

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experiments were run in triplicates.

Assay methods: Dry mycelial weight was determined by filtering the culture medium through weighed Whatman filter paper No. 44. The mycelium was thoroughly washed with tap water and dried at 110°C, over night and the mycelial dry weight was calculated according to Haq and Daud (1995). Sugar was estimated colorimetrically by DNS method as reported by Tasun *et at.* (1970) while anhydrous citric acid was determined by pyridine-acetic anhydride method (Marrier and Boulet 1958). The % citric acid was calculated on the basis of sugar used.

Results

Effect of different nitrogen sources: Figure I shows the effect of different nitrogen sources such as $(NH_4)_2SO_4$, NH_4NO_3, $NaNO_3$ and $(NH_2)_2CO$ on citric acid production by *Aspergillus niger* UV-6 in shake flask. Among the nitrogen sources tested, NH_4NO_3 gave maximum production of citric acid i.e., 39.01 g/l. Other nitrogen sources such as $(NH_4)_2SO_4$, $NaNO_3$ and $(NH_2)_2CO$ gave relatively less acid production i.e., 13.20, 28.62 and 26.50 g/l, respectively. Ammonium nitrate, being the best nitrogen source for mycelial growth and citric acid accumulation (49.25%), was selected for further study. Mycelial dry weight was 16.0 g/l and sugar consumed during the course of fermentation was 80.0 g/l. Mycelia were intermediate in size and rounded in shape.

Effect of different concentrations of ammonium nitrate: The effect of different concentrations of ammonium nitrate (0.10 - 0.30%) on the production of citric acid was carried out by using the mould of *A. niger* UV-6 (Figure II). With the increase in the concentration of ammonium nitrate, the production of anhydrous citric acid was also increased but up to a certain extent. Maximum production of citric acid (38.06 g/l) was achieved when 0.20% ammonium nitrate was used in the fermentation medium. Further increase in the concentration of nitrogen source, reduced the secretion of citric acid from mycelial cells. At high nitrogen concentration (0.30%), the production of acid became very low (20.50 g/l), although sugar consumption was quite high (100.0 g/l). The mycelia were small rounded pellets and their dry weight was 18.0 g/l.

Effect of different phosphate sources: Phosphate sources such as KH_2PO_4, K_2HPO_4 Na_2HPO_4 and NaH_2PO_4 were also employed during the course of study (Figure III). When KH_2PO_4 was used, the growth of mycelium was optimum resulting in greater production of citric acid. i.e., 38.90 g/l. Sugar consumption was not very high (86.0 g/l) and mycelial morphology was in the form of small pellets. The % citric acid on the basis of sugar used was observed to be 45.23% and mycelial dry weight was 16.5 g/l. The production of citric acid was extremely low (21.05 g/l) when K_2HPO_4 was used as a phosphate source while consumption of sugar was quite high (114.0 g/l). Rest of the nitrogen sources, were not found to have any impact on citric acid production. Therefore, KH_2PO_4 was optimized as the best phosphate source.

Effect of different concentrations of KH_2PO_4: Figure IV shows the effect of different concentrations (0.05-0.20%) of potassium di-hydrogen phosph

(KH_2PO_4) on the production of citric acid. When the concentration of KH_2PO_4 was low, production of citric acid was also low. Maximum amount of citric acid i.e., 42.01 g/l was achieved when 0.10% KH_2PO_4 was used as a phosphate source in the fermentation medium. Further increase in the concentration of KH_2PO_4, gradually reduced citric acid synthesis, becoming very low (31.23 g/l) at 0.2% KH_2PO_4. The maximum amount of citric acid (45.62 %) on the basis of sugar used was obtained when sugar consumption was 92.0 g/l. Mycelia were small pellets, having dry weight 17.5 g/l.

Discussion

Nitrogen constituent has a profound effect on the yield of citric acid because the type of nitrogen source and its concentration affect the development of fungus considerably. In the present study, ammonium nitrate was found to be the best nitrogen source for maximal citric acid production (39.01 g/l) due to more available nitrogen, required for the growth of fungus. Among the different concentrations tested, 0.20% NH_4NO_3 was optimized for maximal citric acid accumulation. It might be due to the fact that at this concentration of NH_4NO_3, the mycelial growth was optimum resulting in greater citric acid production. At low NH_4NO_3 concentration, the less acid production might be due to lower supply of free nitrogen for mycelial growth. On the other hand, due to larger quantity of ammonium nitrate (other than 0.20%, NH_4NO_3) the biomass grew slower and the production decreased sharply. Similar type of work has also been reported by Usami (1978). A concentration of ammonium nitrate greater than 0.25% leads to the accumulation of oxalic acid (Naguchi and Bando, 1960). This work, however, is not in good agreement with the work of Wieczorek and Brauer (1998) who reported higher yield of citric acid using 0.1% NH_4NO_3 as nitrogen source in the fermentation medium. Dhanker *et al.*, (1974) found that high concentration of nitrogen leads to a 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 maximum citric acid production under different cultural conditions.

The concentration of phosphate in the fermentation medium is also very important for the production of citric acid. The maximum production of citric acid (38.90 g/l) was obtained when KH_2PO_4 was used as phosphate source. It might be due to the fact that phosphate was readily available to the mycelium, using this phosphate source in the medium. Among different concentrations, 0.10 % KH_2PO_4 was found to be the best concentration for optimal mycelial growth which resulted in greater excretion of citric acid (42.01 g/l). Any increase or decrease in phosphate quantity reduced citric acid production due to over growth or improper growth of mould mycelium. A high concentration of phosphate in the fermentation medium promotes more growth and less acid production (Khan *et al.*, 1970). Earlier reports suggested that citric acid production begins only after the available phosphorous compounds, were assimilated by the mould (Rojas *et al.*, 1995). In general, a phosphate concentration of about 0.1 - 0.15% in the fermentation medium appears to be adequate.

Figure I. Effect of different nitrogen sources on the production of citric acid by mutant strain of *Aspergillus niger* UV-6 using sucrose salt medium.

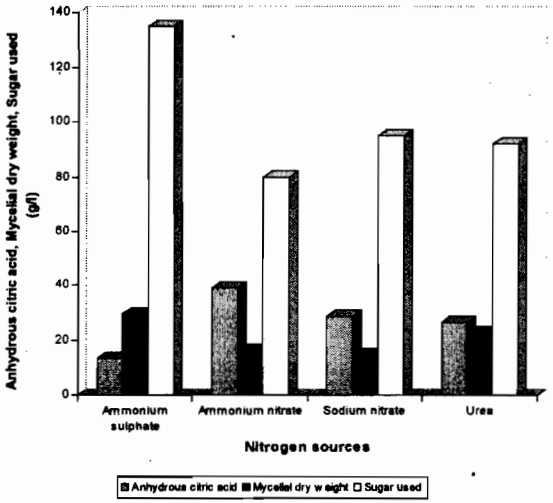


Figure II. Effect of different concentrations of ammonium nitrate on the production of citric acid by mutant strain of *Aspergillus niger* UV-6 using sucrose salt medium.

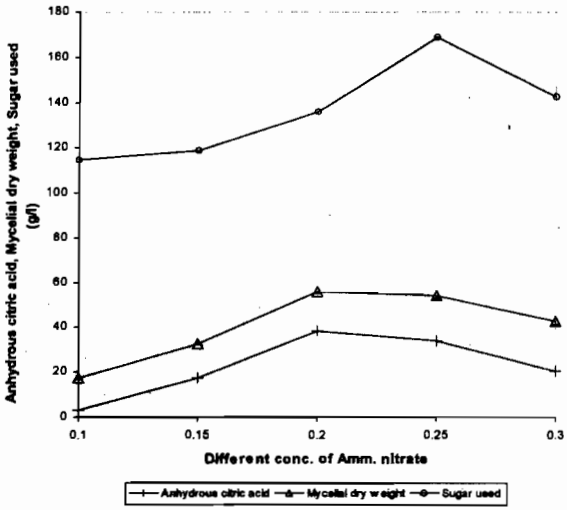


Figure III. Effect of different phosphate sources on the production of citric acid by mutant strain of *Aspergillus niger* UV-6 using sucrose salt medium.

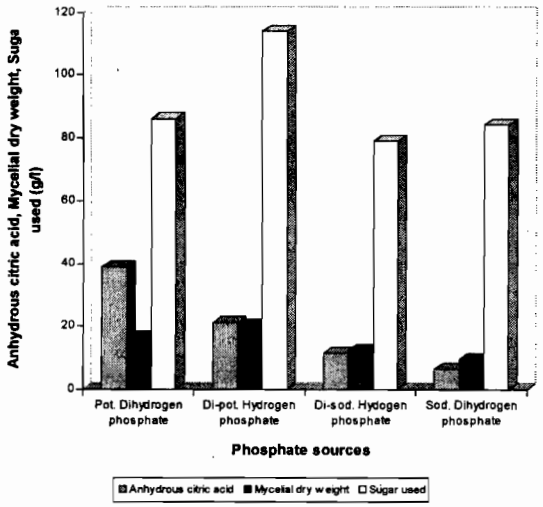
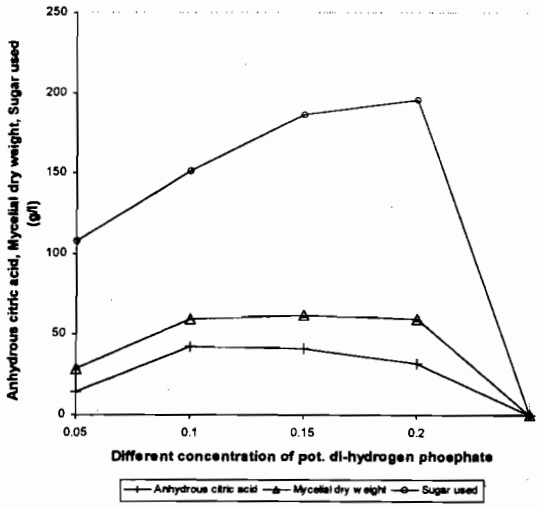


Figure IV. Effect of different concentrations of pot. di-hydrogen phosphate on the production of citric acid by mutant strain of *Aspergillus niger* UV-6 using sucrose salt medium.



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