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STUDIES ON SOME BIOCHEMICAL CHANGES DURING CITRIC ACID PRODUCTION IN A SYNTHETIC MEDIUM BY A MUTANT *ASPERGILLUS NIGER* AB1801

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ABSTRACT

An experimental study was carried out to examine the biochemical changes in the fermentation broth during citric acid production by a mutant *Aspergillus niger* AB1801. The production of citric acid was increased upto 8th day of fermentation, and then declined. pH decreased continuously, dry cell weight followed continuous increment pattern. Amino nitrogen and urea nitrogen decreased gradually, but ammonical nitrogen increased steadily.

INTRODUCTION: The role of utilization of carbon and nitrogen sources by an organism from the growth medium can be calculated from the estimation of these compounds in the medium of different stages of growth. The growth of the microorganism as well as the quantitative and qualitative yield of the desired products is dependent on the availability of the nutrient and its utilization and physico-chemical environmental in the medium.

So, the studies on the biochemical changes occurring in the medium during the citric acid fermentation process is essential before standardizing the large scale production method.

Many reviews are available on different aspects of biochemical changes in the fermentation broth during citric acid production [1-11].

Considering the facts, our present study was intended to examine the pattern of sugar, ammonical nitrogen, amino nitrogen, urea nitrogen, citric acid concentration, cellular nitrogen, pH changes in the fermentation broth at different time intervals.

MATERIALS AND METHODS

Microorganism: A mutant Aspergillus *niger* AB1801 developed in our laboratory from a regulatory mutant Aspergillus *niger* AB100 by induced mutation was used for this study [12].

Composition of synthetic medium for citric acid fermentation : Composition of synthetic medium for citric acid production was as follows: Sucrose, 10%; urea, 0.25%; KH_2PO_4, 0.15%; $MgSO_4.7H_2O$, 0.03%; boric acid, 0.05% mg/L; Ni^{2+}, 10 µg/ml; Co^{2+}, 5 µg/ml; thiamine- HCl, 10 µg/ml; and pH was adjusted to 3.

Physical conditions for the Fermentation: The fermentation was carried out using 1 L. Roux bottle for 9 days with 150 ml fermentation medium with 8 days

old 10 ml spore suspension (2.6 X 10^7 spores/ml) at pH 3.

Determination of pH: pH of the medium was determined with the aid of a previously standardized pH meter (Unicam 9450 model).

Estimation of Residual Sugar: Residual sugar was determined by DNS method as proposed by Miller (1969)[13].

Estimation of Residual, Ammonia and Urea Nitrogen: Nitrogen was measured by the micro Kjeldahl method of Allen (1931) [14].

Estimation of Dry Cell Weight (DCW): Dry cell weight was estimated by the method as proposed by Shah *et al* (2002).

Estimation of Citric Acid: Citric acid was measured according to the method of Marrier and Boulet (1958) [16].

Measurement of Aconitase Activity: Aconitase activity in different stage of fermentation was measured by the method of Kubicek and Rohr (1985) [17].

Estimation of Amino Nitrogen: Amino nitrogen was estimated by descending paper chromatographic method (1961) [18].

Statistical Analysis: Data were analysed by one way ANOVA followed by Dunett's post hoc multiple comparison test (using Graph Pad Inc., USA).

RESULTS AND DISCUSSION:

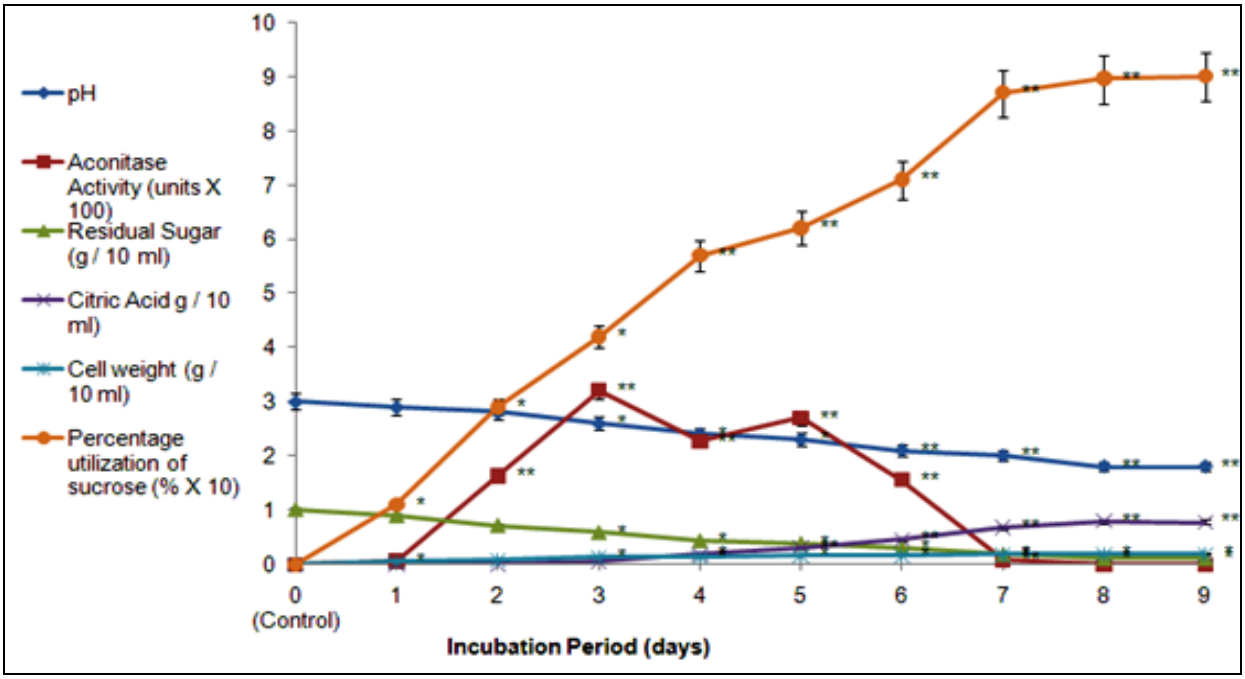

FIG. 1: RATE OF ELABORATION OF CITRIC ACID BY *ASPERGILLUS NIGER* AB1801 IN RELATION TO CHANGES IN pH, SUCROSE UTILIZATION AND CELLULAR GROWTH

(Values were express as mean ± SEM, where n = 6; *p<0.05, **p<0.01 when compared to control)

Fig. 1 showed that there was an initial accelerated growth with high sugar consumption and lowered production followed by rapid sugar utilization with high acidity rate reaching an efficiency of 90% conversation with 9th day. It is also evident from the Fig.1, that the rate of citric acid production increased upto the 8th day of fermentation when it attained the peak value and then decreased again. Maximum cell growth was obtained on 9th day of incubation. pH decreased from 3 to 1.8. Sucrose was utilized upto 90%.

Hossain *et al.,* (1985) said that when large amount of citric acid are lost from the cell, the activity of aconitase increases as a response to decreased intracellular supply of its substrate [19].

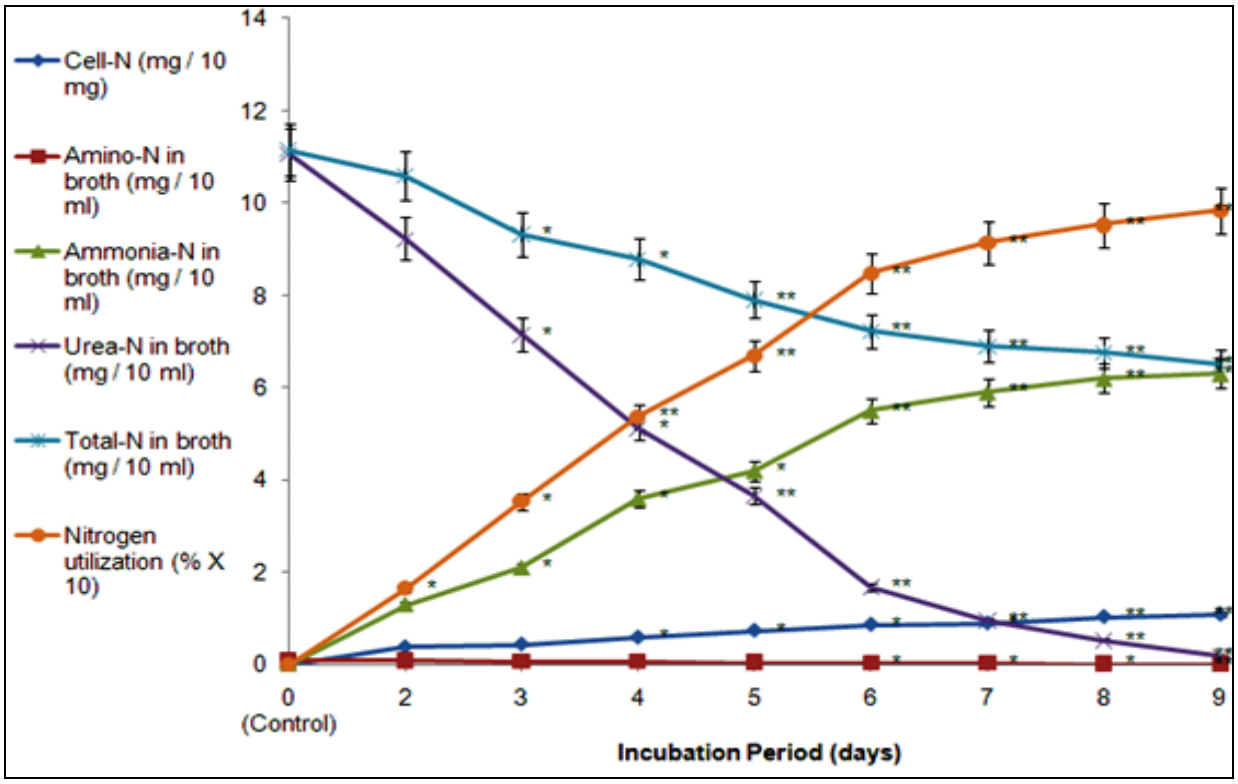

FIG. 2: NITROGEN BALANCE DURING CITRIC ACID FERMENTATION BY *ASPERGILLUS NIGER* AB1801.

(Values were express as mean ± SEM, where n = 6; *p<0.05, **p<0.01 when compared to control)

Fig. 2 indicates that cell nitrogen increased till the end of fermentation. Amino nitrogen decreased gradually. Ammonia nitrogen increased steadily. Urea nitrogen declined rapidly. Total nitrogen content of the broth decreased. Maximally, 98.3% nitrogen was utilized with 9th day of fermentation.

So, it may be inferred from this work that, both carbon and nitrogen are well balanced with respect to their amounts provided and utilized.

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