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EFFECT OF INITIAL MOISTURE CONTENT ON THE YIELD OF CITRIC ACID BY *ASPERGILLUS NIGER* UNDER SOLID STATE FERMENTATION

***LINGAPPA K, CHANDRASHEKHAR NAIK, VIVEK BABU C.S., VENKATA REDDY**

Food and Fermentation Laboratory, Department of Studies in Microbiology, Gulbarga University, Gulbarga-585 106, Karnataka, India

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Abstract–Under solid state fermentation (SSF) conditions the yield of citric acid by strain *Aspergillus niger* MTCC 281 and KLCN 2 varied considerably with initial moisture content of the sunflower seed cake. The yield of citric acid was highest at 55% moisture level by strain MTCC 281, on the other hand strain KLCN 2 yielded highest at 35% initial moisture content of the substrate. Among two strains tested, strain KLCN 2 was found to be the superior for citric acid production under solid state fermentation.

INTRODUCTION

The requirement of water for growth and metabolic activities of microorganisms are well established (Hahn Hagerabal 1986; Gildayal 1991). A high moisture content of the substrate in solid state fermentation (SSF) results in decreased porosity, lower oxygen diffusion, reduction in gas volume increased risk of bacterial contamination and decreased gas exchange (Silman *et al.*, 1979). Similarly the lower moisture levels lead to sub optimal growth and a low degree of substrate swelling (Gildyal 1991; Mishra 1991). Thus the concept of water availability in a substrate becomes very important and it has been widely studied under quantitative aspects (Wang *et al.*, 1975; Nishio *et al.*, 1979; Raimbalut & Alazard 1980; Narahara *et al.*, 1982, Hoel Kim *et al.*, 1985).

Under SSF condition, the yield of citric acid by strains of *Aspergillus niger* varied considerably with initial moisture content (Wang 1975; Hang & Woodams 1986; Shankaranand & Lonsane 1995). Hence, the investigations were carried out to determine the effects of initial moisture content on the yield of citric acid by using sunflower seed cake as a novel substrate (Lingappa & Chandrashekhar Naik 1998) with two different strains of *Aspergillus niger* through the SSF.

MATERIALS AND METHODS

Two strains of *Aspergillus niger* MTCC 281 (procured from IMTECH Chandigarh) and KLCN 2 (isolated from spoiled orange and identified as *Aspergillus niger* by IARI, New Delhi) were employed to produce Citric Acid using sunflower seed cake as substrate, in SSF system during the present study.

Standardization of moisture content of substrate

40 g of sample was collected and dried at 100°C for 6 h, cooled and weighed until constant weight was achieved. Then different moisture levels in the substrate were adjusted by rehydrating with required quantities of distilled water (Hang & Woodams 1986; Kamini *et al.*, 1998).

Spore inoculum preparation

Culture grown on Potato dextrose agar slants were flooded with 3 ml distilled water and shaken vigorously for one minute. The spore suspension was diluted to concentrate 1×10^7 spores/ml (Fernandez Vergano *et al.*, 1996).

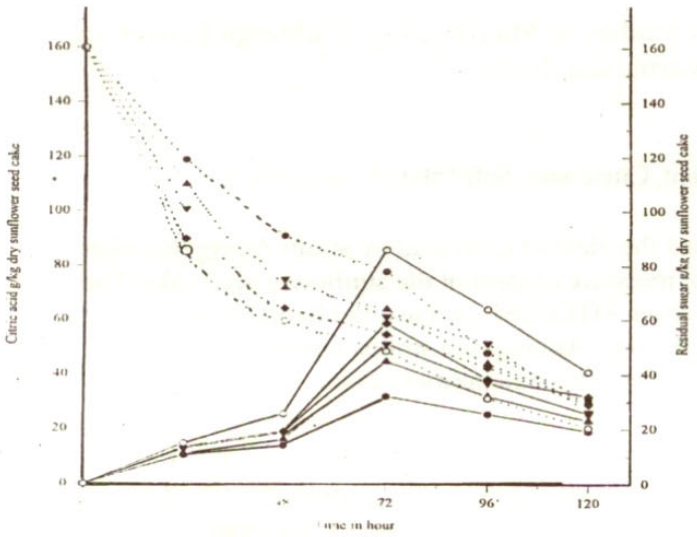
Inoculation and solid state fermentation of flasks

The flasks containing substrate with different initial moisture content were autoclaved at 121°C for 60 min, allowed to cool to about 30°C and inoculated with 1 ml spore suspension, contents were mixed thoroughly gently beating the flasks on the palm of the hand. The flasks were incubated in slanting position at 30°C for 7 days (Ramesh & Lonsane 1989; 1990).

*** Author for correspondence** : E-mail :lingappakl@rediffmail.com, cknaik@rediffmail.com

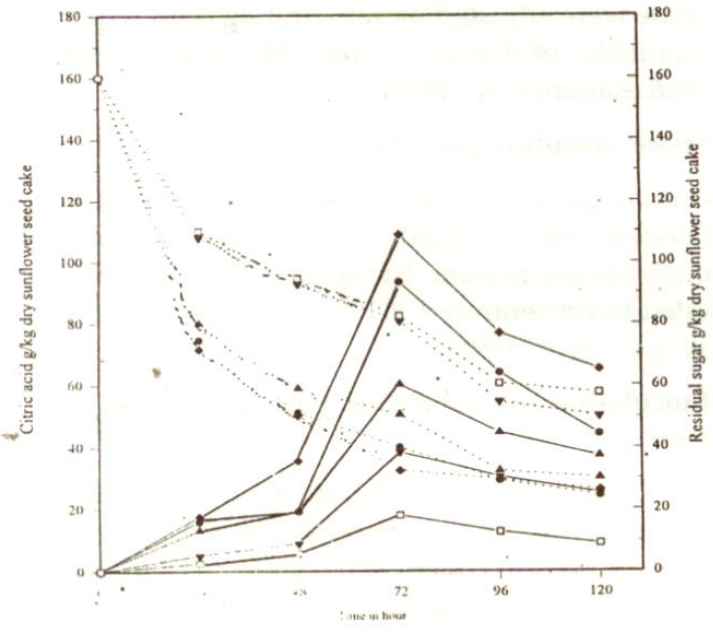
Estimation of Citric acid and Residual sugars

The samples were withdrawn periodically for every 24 h in aseptic conditions, and extracted with distilled water (Lakshminarayana *et al.*, 1975). The extracts were analyzed for citric acid (Marier & Boulet 1958) and residual sugars (Dubois *et al.*, 1956).



Moisture levels 30 (●), 35 (◆), 45 (◆) & 50 (O)

Fig. 1a. Effect of initial moisture content on the yield of citric acid by *A. niger* MTCC 281 on sunflower seed cake.

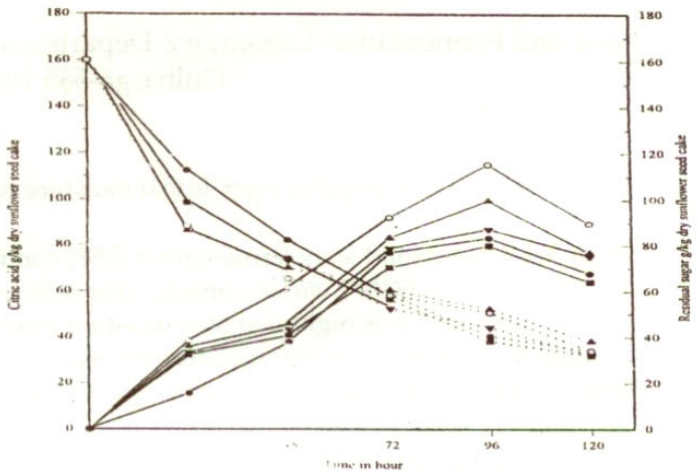


Moisture levels 55 (◆), 60 (●), 65 (◆), 70 (◆) & 75 (❑)

Fig. 1b. Effect of initial moisture content on the yield of citric acid by *A. niger* MTCC 281 on sunflower seed cake.

RESULTS & DISCUSSION

The results obtained in the present study are presented in Fig. 1a to 2b. In case of *Aspergillus niger* MTCC 281 strain, yield of citric acid increased with moisture levels upto 55% and thereafter decreased at higher moisture levels on all the days of study, the least being observed at 75% moisture level (Fig. 1a



Moisture levels 30 (●), 35 (O), 4o (◆), 45 (◆) & 50 (■)

Fig. 2a. Effect of initial moisture content on the yield of citric acid by *A. niger* KLCN 2 on sunflower seed cake.

and 1b) and maximum yield of citric acid at any moisture level was at 72 h of fermentation. On the other hand, citric acid yield by *Aspergillus niger* KLCN 2 (Fig. 2a and 2b) was higher at 35% moisture level on all the days of analysis, maximum being observed at 96 h and thereafter it decreased sharply at more than 35% initial moisture level. The increase in the yield of citric acid during the early stage of growth of *Aspergillus niger* appears to be due to higher initial moisture content of the substrate, consequent faster growth and early entry into the productive state (Eric *et al.*, 1988; Ramesh & Lonsane 1990). With both strains, as citric acid increases correspondingly decrease in the residual sugar content was observed (Fig. 1a-2b). Therefore, utilization of sugars and citric acid yield are seems to be closely related phenomena. The strain MTCC 281 yielded maximum citric acid (108.80 g/kg) using 74.62% of residual sugars at 72 h of fermentation. Whereas, strain KLCN 2 yielded maximum citric acid (115.20 g/kg) utilizing 76.12% of residual sugars at 96h of fermentation.

The optimum moisture levels as well as optimum fermentation period differed to a great extent. While maximum citric acid yield was observed at 55% moisture level at 72 h in case of *A. niger* MTCC 281,

the same was obtained at 35% moisture level at the end of 96 h fermentation period by *Aspergillus niger* KLCN 2. It is known that at lower and higher initial moisture levels than the optimals, the metabolic activities coupled with consequent product synthesis are adversely affected in both bacterial and fungal system during SSF (Beckrod *et al.*, 1945; Feniksova *et al.*, 1960; Gildayal *et al.*, 1981; Hang *et al.*, 1985; Ramesh & Lonsane 1986; 1987a; 1990). As such the substrate moisture level of 55% and 35% appears to be required for the two strains of *Aspergillus niger* , MTCC 281 and KLCN 2 to carryout their optimum metabolic activity.

Just as the optimal moisture levels vary for the strains of same species (Hang *et al.*, 1985; Ramesh & Lonsane 1990). The present study also reveals that the optimum fermentation period too varies for different strains of the same species.

The present study indicates the superiority of strain *Aspergillus niger* KLCN 2 over the strain MTCC 281, in that the former requires lower substrate moisture content than the latter and yet produces more citric acid, though delayed by 24 h. Since moisture level is less, more concentrated citric acid can be harvested by employing strain KLCN 2. This observation also suggest that larger quantities of the sunflower seed cake can be economically fermented in small fermentors to yield concentrated citric acid, as opined by Ramesh and Lonsane (1990) for α-amylase production by *Bacillus licheniformis* in SSF. SSF itself is regarded as better economical tool than Submerged fermentation and in such a situation the employment of strains requiring lower substrate moisture levels would further economize the process.

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