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Temperature Optima for Citric Acid Accumulation by *Aspergillus niger*

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Abstract: The effect of different temperatures on citric acid fermentation by *Aspergillus niger* ANABt was studied in a 15 L stirred fermentor. Ferrocyanide treated (200 ppm) cane molasses medium containing sugar 150 g l^{-1}, was used as the basal fermentation medium. The citric acid yield ranged from 48.58-65.96 g l^{-1}. Maximum amount of anhydrous citric acid obtained during the course of study was 65.96 g l^{-1} at 30°C. The sugar consumption was 98 g l^{-1} while mycelial dry weight was 14.55 g l^{-1}. Mycelia were intermediate round pellets.

Key words: *Aspergillus niger*, biosynthesis, cane molasses, citric acid, fermentation, temperature

Introduction

Citric acid occurs naturally in various fruits such as lemons, oranges, pears, figs etc. It was first isolated from lemon juice and has since been known as natural plant substance in many citrus fruits (Ali *et al*., 2001; Ranya *et al*., 1999). Until the early part of this century, citric acid was produced mostly from lemon juice. The fungi were reported to produce citric acid from a nutrient solution containing sucrose as a carbon source. Today, most of the citric acid used in food and other industries comes from fungal fermentations. Although chemical synthesis of this organic acid is possible, as yet there is no competitive synthetic process developed that is superior to fungal fermentations. *Apergillus niger* and other fungi used in citric acid fermentation have an optimum temperature between 25 and 32°C (Srivasta and Kamal, 1979; Hang and Woodams, 1998; Amer *et al*., 1999). Nowakowska *et al*. (1978) showed that temperature above 35°C inhibited the citric acid production and increased the production of by-product acids and also inhibit cultural development. Cane-molasses is the commonly available raw material for submerged fermentation of citric acid. Pakistan, being an agricultural country, is producing molasses more than $1.5x10^5$ tons, annually. The present communication deals with the temperature optima for citric accumulation by *Aspergillus niger* ANABt in a stirred fermentor.

Materials and Methods

The experiments were conducted in Biotechnology Laboratory, Government College University, Lahore.

Fermentation conditions: The *Aspergillus niger* strain ANABt was maintained on PDA slants. Cane molasses obtained from Kamalia Sugar Mills was clarified according to the method of Panda *et al.* (1984). Hundred ml of fermentation medium (clarified cane molasses; sugar 15%, pH= 6.0) containing glass beads, in 250 ml cotton wool plugged Erlenmeyer flask was sterilized at 121°C for 15 min. One ml of conidial suspension (prepared in 10 ml of sterilized distilled water) was added. The flask was incubated at 30°C in a rotary incubator shaker at 200 rpm for 24 h. Stainless steel stirred fermentor of 15 L capacity (GLSC-AF-199-10, Pak made) with working volume of 9 L was used for fermentation. The fermentation medium and working vessel of the fermentor were sterilized at 121°C (15 lb/inch^2) for 35 min using autoclave (GLSC-194-100). The vegetative inoculum was transferred at a level of 4% (v/v). $K_4Fe(CN)_6$ solution (200 ppm) was added during the time of inoculation. Agitation speed of the stirrer was kept at 200 rpm and aeration rate was maintained at 1.0 vvm. Sterilized silicone oil was used to control the foaming.

Analytical methods: Sugar was estimated gravimetrically by DNS method (Tasun *et al.*, 1970). Photoelectric colorimeter (Model: AE-11M Erma, Japan) was used for measuring colour intensity. Dry cell mass was determined by filtering the culture medium through weighed Whatmann filter paper No. 44. Mycelium was thoroughly washed with tap water and dried at 105°C for two hours. Anhydrous citric acid was estimated colorimetrically, using pyridine-acetic anhydride method, as reported by Marrier and Boulet (1958).

Results and Discussion

The temperature of fermentation medium is one of the critical factors that have a profound effect on citric acid production. The data (Table 1) highlights the effect of different temperatures (26-36°C) on citric acid production by *Aspergillus niger* ANABt, using molasses based medium. The culture gave maximum production of citric acid at 30°C temperature. The sugar consumption was 98 gl^{-1} and dry cell mass was 14.55 gl^{-1}. The mycelia were intermediate pellets. As the temperature increased above 30°C or decreased below this, citric acid accumulation in the medium was also decreased. When temperature of the medium was low, the enzyme activity was also low giving no impact on enhancement of citric acid. A temperature of 30°C was found to be the best for citric acid fermentation in present studies. Workers have also optimized a temperature of 30°C for citric acid production (Amer *et al.*, 1999). A temperature of 40°C was the most favourable for oxalic acid production while citric acid accumulation was completely inhibited at this temperature as reported by Srivasta and Kamal (1979). When temperature of the medium was increased above 30°C, the biosynthesis of the citric acid was decreased. It might be due to that; high temperature can cause denaturation of the enzyme citrate synthase. An increase in the temperature of incubation beyond 30°C has been found to

Table 1: Effect of different temperatures on citric acid fermentation by *Aspergillus niger* strain ANABt, using molasses based medium

Temperature (°C)	Sugar consumption (g l^{-1})	Mycelial dry wt. (g l^{-1})	Total acid (%)	Anhydrous citric acid (g l^{-1})	Anhydrous citric acid % yield*	Mycelial morphology
26	110	11.76	58.88	48.58	44.16	Small pellets
28	102	12.05	63.92	59.52	58.35	Viscous
30	98	14.55	75.29	65.96	67.29	Intermediate pellets
32	118	18.50	65.70	58.80	49.83	Small pellets
34	126	16.79	63.84	52.53	41.69	Large pellets
36	122	15.40	59.99	49.56	40.62	Mixed pellets

Initial sugar concentration 150 gl^{-1}; incubation period 144 hours; initial pH 6.0; ferrocyanide concentration 200 ppm; *On the basis of sugar used Citric acid production.

decrease the citric acid yield due to catabolite repression and increase in the oxalic acid accumulation (Doelger and Prescott, 1934). Thus it was concluded that 30°C is the most suitable temperature for mycelial growth and fungal physiology and subsequently citric acid production on a semi-pilot scale plant.

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