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Phosphate Limitation for Enhanced Citric Acid Fermentation Using *Aspergillus niger* Mutant UV-M9 on Semi-pilot Scale

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Abstract: In the present investigation, the *Aspergillus niger* mutant UV-M9 was evaluated using phosphate limitations for enhanced citric acid production. Of the three phosphate sources, K_2HPO_4 was found to be the best yielding 75.25 gl^{-1} of anhydrous citric acid. The sugar consumption was 112.5 gl^{-1} while dry cell mass produced was 18.00 gl^{-1}. The mycelia were small pellets.

Key words: *Aspergillus niger,* citric acid, mutant, phosphate sources, semi-pilot scale, cane-molasses

Introduction

Citric acid occurs naturally in various fruits such as lemons, oranges, gooseberries, pears, figs etc. It was first isolated from lemon juice and has since been known as natural plant substance in many citrus fruits (Ranya *et al*., 1999). Citric acid obtained from fruits is known as natural citric acid as compared to synthetic produced by microbial fermentation (Prescott and Dunn's, 1987). Most of the citric acid is obtained by using the fungus Aspergillus niger as reported by various workers (Mattey and Allan, 1990; Pera and Callieri, 1997). Citric acid is used in food and pharmaceutical industries, textile printing e.t.c. These uses have placed greater stress on increasing citric acid production and search for more efficient process (Benuzzi and Segovia, 1996). The factors that have been shown to exert an effect on citric acid fermentation may include carbon, nitrogen or phosphate limitations, aeration, trace elements, morphology of the producer organism, etc (Papagianni *et al*., 1999). A part from C and N, P also has a profound effect on amount of citric acid produced (Prescott and Dunns, 1987). A high concentration of phosphate promotes more growth and the citric acid production. The present investigation deals with the phosphorous limitations for enhanced citric acid production by an *Aspergillus niger* mutant UV-M9 using cane molasses.

Materials and Methods

The experiment was conducted in Biotechnology Research Centre, Government College University, Lahore.

Fermentation conditions: The *Aspergillus niger* mutant UV-M9 was produced by giving UV dose to pre-grown mycelia. This strain was maintained on potato dextrose agar medium. 100 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 (15l $binch^{-2}$) 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. Mycelia were thoroughly washed with tap water and dried at 105°C for 2h. Anhydrous citric acid was estimated colorimetrically, using pyridine-acetic anhydride method, as reported by Marrier and Boulet (1958).

Results and Discussion

In the present study the effect of various phosphate sources on citric acid bio-production was investigated. Among the three phosphate sources, K_2HPO_4 (0.1%) yielded maximum amount of citric acid i.e. 75.25 g l^{-1} (Table 1). It might be due to the fact that phosphate was readily available to the mycelia using this phosphate

Table 1: Effect of different inorganic phosphate sources on citric acid fermentation by mutant strain of Aspergillus niger UV-M9 using molasses based medium in stirred bioreactor

				Anhydrous citric acid	
Phosphate source (0.1%)	Sugar consumption (gl^{-1})	Dry cell mass (gl^{-1})	Total acid (gl^{-1})	(gl^{-1})	*Yield (%)
KH_2PO_4	130.0	26.15	74.89	69.36	53.35
K_2HPO_4	112.5	18.00	79.60	75.25	66.80
Na_2HPO_4	96.0	15.50	69.10	61.30	63.85

Initial sugar concentration 150 gl^{-1}, Incubation period 144 h, temperature 30°C, initial pH 6.0, ferrocyanide concentration 200 ppm, *On the basis of sugar used

Table 2: Comparison of mycelial morphology using different phosphate sources in the fermentation medium

	Mycelial morphology		
Conc. of phosphate source (%)	K_2HPO_4	KH_2PO_4	Na_2HPO_4
0.05	Fine pellets	Gelatinous	Viscous
0.10	Small pellets	Dumpy mass	Viscous
0.15	Large pellets	Dumpy mass	Fine pellets

Initial sugar concentration 150 gl^{-1}, Incubation period 144 h, temperature 30°C, initial pH 6.0, ferrocyanide concentration 200 ppm

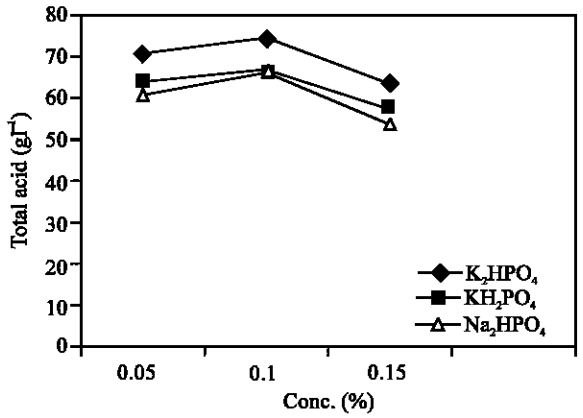

Fig. 1: Comparison of total acid using different phosphate sources in the fermentation medium

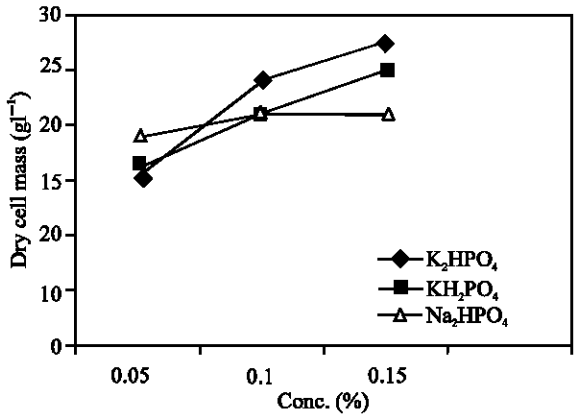

Fig. 3: Comparison of Dry cell mass using different phosphate sources in the fermentation medium

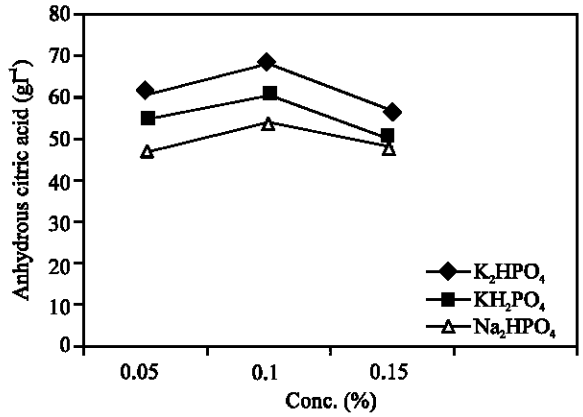

Fig. 2: Comparison of anhydrous citric acid using different phosphate sources in the fermentation medium

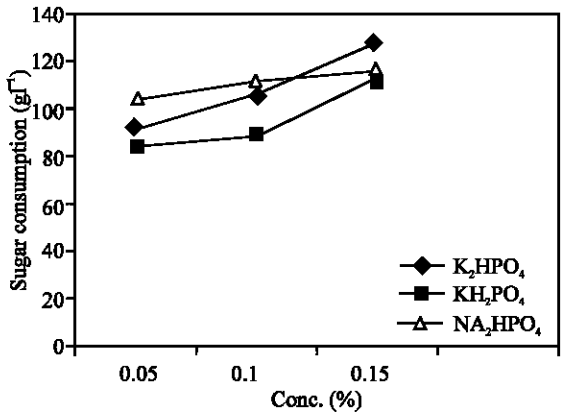

Fig. 4: Comparison of sugar consumption using different phosphate sources in the fermentation medium

source in the medium. The mycelia were small pellets (Table 2). Any increase or decrease in the phosphate quantity reduced citric acid production due to improper growth of mould mycelia. 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 phosphorus compounds

were assimilated by the mould (Joseph, 1944). The maximum amount of total acid was also obtained using 0.1% K_2HPO_4 (Fig. 1 and 2). The maximum amount of dry cell mass produced was 27.55 gl^{-1} using 0.05% K_2HPO_4 (Fig. 3). According to Fig. 4 sugar consumption was also highest at 0.15% of K_2HPO_4. Although there are not too many reports on this aspect, in general, a phosphate concentration of about 0.1-0.15% in the fermentation medium was appeared to be adequate.

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