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Time Course Study of Citrate Fermentation by *Aspergillus niger* in Stationary Culture

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Abstract: The present investigation deals with the time course study of three different mutant strains of *Aspergillus niger* (IFS-5, IFS-6 & IFS-17) during citric acid production by surface culture technique. The mutant IFS-17 was found to be the best producer of citric acid (76.22 g l^{-1}, 7 days after inoculation) having high production yield coefficients (i.e., $Y_{p/s}$ 0.492 g g^{-1} & $Y_{p/x}$ 1.992 g g^{-1}). The product is low volume and high cost product. Thus the use of mutant IFS-17 in citric acid fermentation is economically more feasible due to larger citric acid production vs. shorter incubation period.

Key words: Citric acid, biosynthesis, fermentation, *Aspergillus niger*, time course study, surface culture study.

Introduction

Citric acid fermentation by *Aspergillus niger* is an aerobic process and the organism needs a fairly high and constant oxygen supply for its growth (Hang 1988; Haq *et al*., 2001). Surface culture technique (SCT) is a conventional method of citric acid production. Most of the pilot plants are using this technique due to low energy consumption and manpower involved (Singh *et al*., 1998). In SCT, the substrate remains stationary and organism form mycelial mat on the surface of medium. The relation between constitution of the fermentation medium and rate of citric acid production has been investigated (Elimer and Ewaryst, 1995). Sucrose salt medium as synthetic while cane or beet molasses as natural fermentation media have long been employed as usual routine basal media (Ali *et al*., 2001). Clark *et al*. (1965) obtained 80% conversion of available sugar, 12 days after incubation. Farouk *et al*. (1977) pointed out that the age of culture also affect the yield of citric acid. The mutant strains have greater ability to produce citric acid. The present investigation deals with the time course study during citric acid productivity by stationary culture using different strains of *Aspergillus niger* and their comparison on kinetic basis.

Materials and Methods

Organism: In the present study, 3 different mutant strains of *Aspergillus niger* (hyper producers of citric acid in submerged culture) were used. These strains (IFS-5, IFS-6 & IFS-17) have already been developed by mutation in *Biotechnology Labs, Government College University, Lahore* during the year 2000 and maintained on potato dextrose agar medium. The cultures were stored at 4°C in a refrigerator.

Sterilization: All the culture media were sterilized in an autoclave at 15 lbs/inch^2 pressure (121°C) for 15-20 minutes.

Culture medium: Sucrose salt medium containing (g l^{-1}); Sucrose 150.0, $MgSO_4.7H_2O$ 0.25, KH_2PO_4 2.5, NH_4NO_3 2.5 at pH 3.5 was used as the basal fermentation medium.

Fermentation technique: Citric acid was produced by surface culture technique, following the method of Singh *et al*. (1998). Conidial inoculum, prepared in sterilized distilled H_2O was used as the inoculum. The optimum conditions for citric acid production were investigated in 250 ml Erlenmeyer cotton wool plugged conical flasks, containing 25 ml fermentation medium. The flasks were incubated at 30°C for 1-11 days. The results are sum mean of three parallel replicates.

Analysis and comparison: Dry cell mass was determined according to Kirimura *et al*. (1992). Residual sugar was estimated by DNS method (Tasun *et al*., 1970) while pyridine acetic anhydride method was employed for the determination of anhydrous citric acid as reported by Marier and Boulet (1958). A scanning spectrophotometer (Cecil-700 series, UK) was used for the determination of colour intensity at 420-546 nm wavelength. Anhydrous citric acid, sugar consumption and dry cell mass were determined with reference to time course and calculated in g l^{-1}. Statistical analysis and kinetics of time course was also under taken for data comparison (Pirt, 1975).

Results and Discussion

Time course study is one of the most critical factors, which determines the efficacy of the process along with product formation. The data of Table 1 shows the biosynthesis of citric acid at different intervals of time.

Table 1: Time course study during citric acid fermentation by *Aspergillus niger*

Fermentation period (Hours)	Dry cell mass (g l^{-1})			Sugar consumption (g l^{-1})			Citric acid (g l^{-1})		
	IFS-5	IFS-6	IFS-17	IFS-5	IFS-6	IFS-17	IFS-5	IFS-6	IFS-17
24	2.50	3.72	4.00	46.5	24.5	35.0	1.56	2.13	0.67
48	5.05	9.10	7.50	54.2	56.6	49.5	2.10	6.25	1.26
72	6.55	16.25	10.61	69.5	79.1	56.2	6.26	9.55	4.50
96	9.00	24.80	12.31	79.5	88.5	73.0	9.55	16.12	16.80
120	13.25	26.45	17.22	87.4	96.0	86.0	13.18	31.52	20.04
144	16.40	29.05	20.11	96.0	100.8	89.5	16.52	26.27	38.55
168	21.15	32.46	23.20	98.5	114.5	94.0	23.55	21.05	76.22
192	24.52	36.62	26.19	104.6	121.0	99.5	32.16	18.66	37.50
216	28.90	37.15	29.27	121.0	126.2	100.6	39.25	18.50	29.53
240	34.62	40.65	31.12	126.5	132.5	106.5	48.14	17.16	27.62
264	39.46	41.20	31.50	128.5	135.6	118.0	42.66	17.05	19.40

Sugar added 150 g l^{-1}, Initial pH 3.5, Temperature 30°C

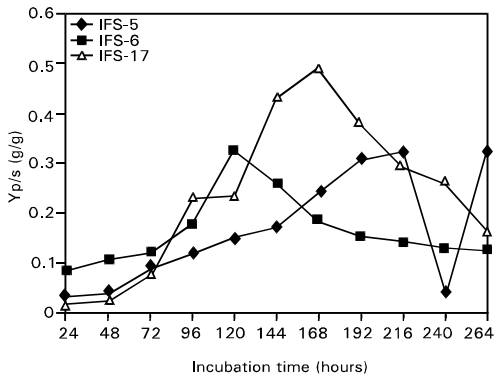

Fig. 1: Comparative study of product yield coefficient ($Y_{p/s}$, g g^{-1}) during citric acid fermentation by *Aspergillus niger* strains (IFS-5, IFS-6 & IFS-17). The value of $Y_{p/s}$ was determined by product (g l^{-1}) /substrate utilized (g l^{-1})

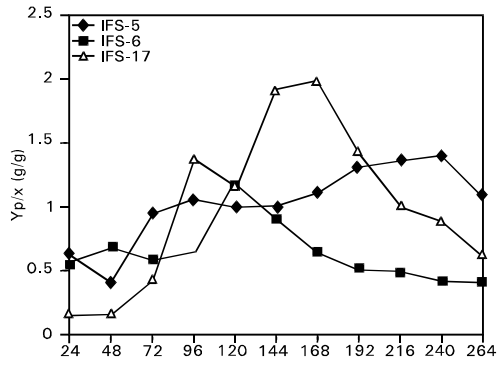

Fig. 2: Comparative study of product yield coefficient ($Y_{p/x}$, g g^{-1}) during citric acid fermentation by *Aspergillus niger* strains (IFS-5, IFS-6 & IFS-17). The value of $Y_{p/x}$ was determined by product (g l^{-1}) / cell mass (g l^{-1})

Three different mutant strains of *Aspergillus niger* were used for their time course comparison. These cultures were incubated at 30°C for 1-11 days. The maximum production of citric acid with mutant IFS-5 was obtained, 10 days after incubation, which seems to be uneconomical due to longer fermentation period. When IFS-6 was used for inoculating the culture medium, a maximum citric acid production of 31.52 g l^{-1} was obtained with a high degree of consumable sugars. Although the fermentation period became very short (5 days only) as compared to mutant IFS-5 but the yield of product was too low as required for an economical process. The maximum production of citric acid by mutant strain of *A. niger* IFS-17, was achieved, 7 days after the inoculation. The dry cell mass and sugar consumed were 23.20 and 94.0 g l^{-1}, respectively. Further increase in the incubation period did not enhance, the production of citric acid, rather it was decreased. It might be due to the reduction of sugar contents in the fermentation medium and accumulation of other by-products such as oxalic acid and different toxins, etc. Age of the fungi is an additional factor for less acid productivity. Thus incubation period of 7 days was found to be optimum for maximal citric acid biosynthesis. Our results are in agreement with the findings of many workers (Jaszwry *et al*., 1971; Singh *et al*., 1998). For maximum citric acid production, the optimum time of incubation varies from organism to organism depending on fermentation medium provided (Elimer and Ewaryst, 1995). The kinetic time course study of citric acid fermentation by mutant strains of *Aspergillus niger* was also worked out (Fig. 1 & 2). There was a marked difference of product yield coefficients ($Y_{p/s}$ and $Y_{p/x}$) among different mutant strains i.e., maximum $Y_{p/s}$ value in case of IFS-17 was much higher as compared to mutants IFS-5 and IFS-17 and the maximum $Y_{p/x}$ value in case of mutants IFS-5 and IFS-6 was lower in comparison with IFS-17. So, the mutant *Aspergillus niger* IFS-17 is better producer of citric acid as compared to others. Meyrath and Ahmed (1989) have attempted to reduce the fermentation time by vermiculite

addition and found that time is reduced from 0-9 days. Lakshminarayan *et al.* (1975) incubated cultures of *Aspergillus niger* for 7 days and achieved good results. Shamrai and Orlow, (1986) described that the optimum period of fermentation was dependent on the intensity of fermentation and got better citric acid production, 8 days after incubation. So, our finding (46.22 g l^{-1} citric acid, 7 days after inoculation by mutant IFS-17 is more encouraging and significant as compared to Shamrai & Orlow (1986).

Conclusion: The mutant strain of *Aspergillus niger* IFS-17 is better citric acid producer due to optimum growth rate and higher enzyme activity. Time required for maximal citric acid production depends mainly on the fermentation design, type of the strain and composition of basal medium. Suitable depth and Cu^{++} ions addition in the molasses medium might increase the mycelial branching level and subsequently citrate productivity.

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