OnLine Journal of Biological Sciences 2 (11): 760-761, 2002
ISSN 1608-4127

Time Course Profile of Citric Acid Fermentation by *Aspergillus niger* and its Kinetic Relations

Aamir Ishaq, Sikander Ali, Ikram-ul-Haq and M.A. Qadeer
Biotechnology Research Laboratory, Department of Botany, Government College Lahore, Pakistan

Abstract: The present study is concerned with the time course profile of citric acid fermentation by *Aspergillus niger* and its kinetic reletions. Maximum amount of anhydrous citric acid (70.60 g l^{-1}) obtained, 144 h after inoculation, with a sugar consumption of 88.40 g l^{-1}. The dry weight of mycelia was 17.39 g l^{-1}. On the basis of comparison of kinetic parameters namely the product and growth yield coefficients ($Y_{p/s}$, $Y_{p/x}$), volumetric rates (Q_p) and specific rate constants (q_p), it was observed that mutant strain of *Aspergillus niger* GCB47 was a faster growing organism and has the ability to hyper produce citric acid.

Key words: Citric acid, *Aspergillus niger*, fermentation, cane molasses, time course profile, kinetics

Introduction

Citric acid, i.e., 2-hydroxy propane-1, 2, 3-tricarboxylic acid ($CH_2COOHCOHCOOHCH_2COOH$) is an important organic acid produced by microbial fermentation process. It exists an intermediate in the citric acid cycle, when carbohydrates are oxidized to carbon dioxide (Haq *et al.*, 2002). Over the years, a large number of organisms including different fungi, yeast and bacteria have been screened for citric acid production. *Aspergillus niger*, however, remained the organism of choice (Fiedurk *et al.*, 1996; Maddox and Brooks, 1998). Citric acid is produced during idiophase at which the growth drops and citric acid production becomes the main cellular activity (Guebel and Torres, 2001). The optimum time of incubation for maximal citric acid production varies both with the organism and the fermentation conditions. In submerged fermentation with the current methodology, 4-6 days appear to be minimum requirement (Prescott and Dunn's, 1987). The present study deals with the kinetics of rate of citric acid fermentation by mutant strain of *Aspergillus niger* GCB47.

Materials and Methods

A mutant strain of *Aspergillus niger* GCB47 was obtained from the available stock culture of Biotechnology Research Labs, Department of Botany, Government College, Lahore.
Cane molasses obtained from Kamalia Sugar Mills was clarified according to the method of Panda *et al.* (1984).

Fermentation technique: Hundred ml of fermentation medium (clarified cane molasses; sugar 15%, pH 6.0), in 250 ml cotton wool plugged Erlenmeyer flask was sterilized at 15 lbs/inch^2 pressure (121°C) for 15 min. One ml of conidial suspension (prepared in 10 ml of sterilized 0.005% Monoxal O. T.) was used for inoculation. 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 with working volume of 9 L was used for fermentation. The fermentation medium and working vessel of the fermentor were sterilized at 121°C for 30 min using autoclave (Model: GLSC-194-100). The vegetative inoculum (5%) was transferred to the clarified molasses medium aseptically. The potassium ferrocyanide solution (200 ppm) was added in the hot medium just before inoculation. The incubation temperature was kept at 30°C throughout the fermentation period for 6 days. Agitation speed of the stirrer was kept at 200 rpm and aeration rate was maintained at 1.01 l^{-1} min^{-1}. Sterilized silicone oil (10%) was used to control foaming.

Assay methods

Residual sugar: Sugar was estimated spectrophotometrically by DNS method (Tasun *et al.*, 1970). UV/Vis double beam scanning spectrophotometer (Model: CECIL CE-7200, UK) was used for measuring colour intensity.

Dry cell mass: The culture medium was filtered through weighed Whatmann filter paper No. 44. The mycelia left upon the filter paper folds were used to determine the dry cell mass according to the method of Haq and Daud (1995).

Citric acid: Anhydrous citric acid was estimated spectrophotometrically, using pyridine-acitic anhydride method, as reported by Marrier and Boulet (1958).

Kinetic parameters: For determining kinetic parameters of batch fermentation process, the procedures of Lawtord and Rouseau (1993) and Pirt (1975) were adopted.

Results and Discussion

Rate of citric acid fermentation by mutant strain of *Aspergillus niger* GCB47 was carried out in stirred fermentor (Table 1). Twenty-four hours after incubation, citric acid production was 5.70 g l^{-1}, which increased gradually with the increase in incubation time. The maximum production was obtained, 144 h after inoculation (70.60 g l^{-1}). The yield of citric acid was 79.80%. The dry weight of mycelia was 17.30 g l^{-1} while sugar consumption was 88.40 g l^{-1}. Further increase in the incubation period resulted in the decreased production of citric acid. Different kinetic parameters such as product and growth yield coefficients ($Y_{p/s}$, $Y_{p/x}$, $Y_{x/s}$), volumetric rates (Q_p, Q_s, Q_x) and specific rate constants (q_p, q_s) were also studied (Figs. 1, 2, 3). The values for $Y_{p/s}$, $Y_{p/x}$, Q_p and q_p were more significant after 144 h of incubation than all other time periods, for citric acid production.
Citric acid production is mainly dependent on the type of strain and process used. The incubation time requirement for maximal citric acid production depend on the organism and fermentation

Table 1: Effect of different time periods on citric acid fermentation by mutant strain of *Aspergillus niger* GCB-47, using molasses based medium in stirred fermentor

Incubation period (h)	Sugar consumption (g l^{-1})	Dry cell (g l^{-1})	Anhydrous citric acid	
(h)	(g l^{-1})	(g l^{-1})	(g l^{-1})	% yield*
24	25.00	2.40	5.70	22.80
48	33.50	7.92	14.20	42.30
72	47.50	11.32	26.80	56.40
96	57.60	12.73	38.60	67.01
120	74.50	14.07	49.40	66.30
144	88.40	17.39	70.60	79.80
168	97.60	23.43	68.80	70.49
192	103.50	25.05	63.20	61.06

Initial sugar concentration 150 g l^{-1}; temperature 30°C; initial pH 6.0; ferrocyanide concentration 200 ppm, *on the basis of sugar used

Corresponding Author: Dr. Sikander Ali, Biotechnology Research Labs, Department of Botany, Government College, Lahore, Pakistan

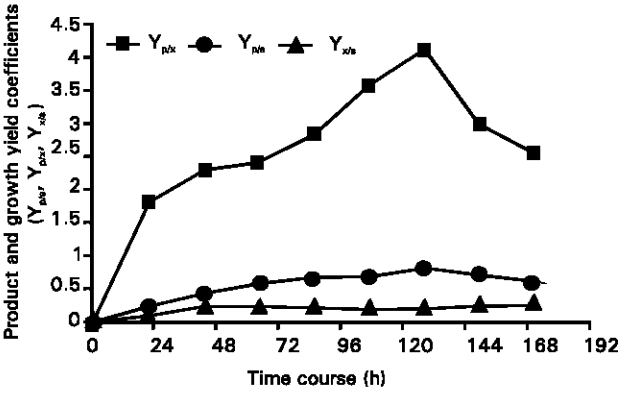

Fig. 1: Comparison of product and growth yield coefficients for citric acid fermentation.
$Y_{p/s}$ = g citric acid produced/g substrate consumed, $Y_{p/x}$ = g citric acid produced/g cells formed, $Y_{x/s}$ = g cells formed/g substrate consumed

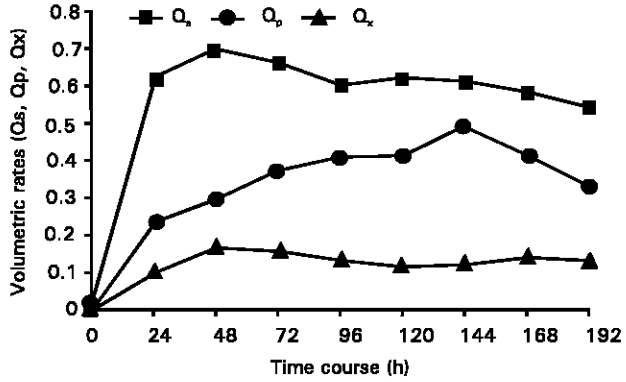

Fig. 2: Comparison of volumetric rates for citric acid fermentation.
Q_p = g citric acid produced/l/h, Q_s = g substrate consumed/l/h, Q_x = g cells formed/l/h.

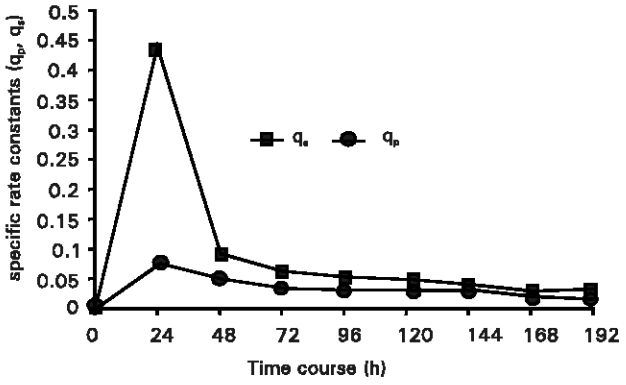

Fig. 3: Comparison of specific rate constants for citric acid fermentation.
q_p = g citric acid produced/g cells/h,
q_s = g substrate consumed/g cells/h.

conditions. Vergano *et al.* (1999) reported maximum yield of citric acid using molasses based medium, 7 days after inoculation. In submerged fermentation conditions with the current methodology, 5-6 days appear to be the minimum required. As in other fermentations, the incubation period has to be adequate for allowing growth and product formation. Since the fungi are relatively slow growing organisms, it is doubtful if incubation period less than 3-4 days will be adequate. In this study, the maximum citric acid production was achieved 6 days (144 h) after inoculation. However, the exact incubation period for the process has not been rigorously tested on a large scale to arrive at precise conclusion. The mutant strain of *Aspergillus niger* GCB47 showed improved values for $Y_{p/s}$, $Y_{p/x}$ and $Y_{x/s}$ The study is directly substantiated with the findings of Rajoka *et al.* (1998). Maximum values for $Y_{p/s}$, Q_p and q_p were several folds improved over the previous workers (Pirt, 1975; Roehr, 1998).

References

Fiedurk, J., B. Plute, J. Szezodrak and J. Jainroz, 1996. Relationship between citric acid and extracellular acid phosphate production by *Aspergillus niger*. Acta. Biotechnol., 16: 207-213.

Guebel, D.V. and N.V. Torres, 2001. Optimization of citric acid production by *Aspergillus niger* through a metabolic flux balance model. Process Biotechnol., 4: 123-127.

Haq, I., S. Ali, M.A. Qadeer and J. Iqbal, 2002. Citric acid fermentation by mutant strain of *Aspergillus niger* using molasses based medium. Process. Biochem. (accepted).

Haq, P.B. and D.A. Daud, 1995. Process of mycelial dry weight calculation for citric acid. J. Biotechnol., 9: 31-35.

Lawford, H.G. and J.D. Rousseau, 1993. Mannose fermentation by ethanologenic recombinants of *Escherchia coli*. Biotechnol. Lett., 15: 615-620.

Maddox, I.S. and J.D. Brooks, 1998. Application of a multiplayer packed bed reactor to citric acid production in solid state fermentation using *Aspergillus niger*. Process. Biochem., 33: 117-123.

Marrier, J.R. and M. Boulet, 1958. Direct determination of citric acid in milk with an improved pyridine, acetic anhtdride method. J. Dairy Sci., 41: 1683.

Panda, T., S. Kundu and S.K. Majunmdar, 1984. Studies on citric acid production by *Aspergillus niger* using treated Indian cane molasses. Microbial. J., 52: 61-66.

Pirt, S.T., 1975. Principles of Cell Cultivation. Black Wells Scientific London, pp: 115-117.

Prescott, S. and A. Dunn's, 1987. Industrial Microbiology, CBS-Publishers and Distributors. New Delhi, India, 4: 710-715.

Rajoka, M.I., M.N. Ahmad, R. Shahid, F. Latif and S. Pervez, 1998. Citric acid production from sugar cane molasses by *Aspergillus niger*. Biologia., 44: 241-253.

Roehr, M., 1998. A century of citric acid fermentation and research. Food Technol. Biotechnol., 36: 163-171.

Tasun, K., P. Chose and K. Glien, 1970. Sugar determination of DNS method. Biotech. Bioeng., 12: 921.

Vergano, M.G., N. Femndez, M.A. Soria and M.S. Kerber, 1999. Influence of inoculum oreparation on citric acid production by *Aspergillus niger*. J. Biotechnol., 12: 655-656.