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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 6 (4): 334-335, 2003
ISSN 1028-8880
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Effect of Vegetative Inoculum on Submerged Citric Acid Fermentation by *Aspergillus niger*

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Abstract: The present investigation deals with the effect of vegetative inoculum on submerged citric acid fermentation by *Aspergillus niger* using stirred bioreactor. All fermentations were carried out following growth on 15% raw molasses sugars for 144 h. Ferrocyanide (200 ppm) was used to control the trace metals present in the molasses medium. The maximum production of citric acid was obtained when 1.0% vegetative inoculum was used. The dry cell mass and sugar consumption were 18.5 and 96.55 g l^{-1}, respectively. The mycelia were intermediate round pellets in their morphology. The specific productivity (qp = 0.074±0.02a g g^{-1} cells h^{-1}) was several folds higher than many other workers.

Key words: Vegetative inoculum, fermentation, *Aspergillus niger*

Introduction

Citric acid fermentation is one of the rare examples of industrial fermentation technology where academic discoveries have worked in tandem with industrial know-how. The current world market estimates suggest that upwards of 4.0×10^5 tonnes citric acid per year may be produced (Haq *et al*., 2001). The demand for this metabolite is increasing day by day which requires a much more efficient fermentation process for higher product yield. Substrate and oxygen requirements as well as biomass and product yields may only be estimated properly if material and energy balances can be applied to the bioprocess (Singh *et al*., 1998). The cultural conditions such as initial pH, temperature and metal complexing agents have direct role on the efficacy of the process (Prescott and Dunn's, 1987; Pazouki *et al*., 2000). The present investigation deals with the effect of vegetative inoculum on submerged citric acid fermentation by *Aspergillus niger* using stirred bioreactor and its kinetical basis. For this, cane-molasses was employed as the basal fermentation media.

Materials and Methods

Organism and culture media: The culture of *Aspergillus niger* NG^{GCB}-101 (obtained from the culture collection of our Labs), maintained on potato dextrose agar (Merck, Germany) was stored at 4°C. All the culture media were sterilized at 15-lbs/$inch^2$ pressure (121°C) for 15 min. Cane molasses obtained from Madina Sugar Mills, Hafizabad was clarified according to the method of Panda *et al*. (1984). The sugar content was maintained at 15% with initial pH 6.0.

Vegetative inoculum and fermentation: Hundred ml of molasses medium (Sugar 15%, pH 6.0) containing silica-gel chips (1.5 mm^2, dia), in 1-L cotton plugged conical flask was sterilized. Small amount of conidia from the slant culture was aseptically transferred with the help of inoculating needle. Flask was incubated at 30°C in an incubator shaker (Gallenkamp, UK) at 160 rpm for 24h. The inoculum was varied from 0.5-3.5%. Stainless stirred bioreactor (working volume of 9-L) was employed for citric acid fermentation. To reduce trace metals (Fe, Cu, Al, Zn) of molasses, $K_4Fe(CN)_6$ 200 ppm was added during inoculation when the medium was hot. The incubation temperature was kept at 30°C for 144h. Agitation speed of the stirrer was 200 rev/min while aeration rate was maintained at 1.0 l/l/min. Silicone oil was used to control foaming during fermentation.

Estimation methods and statistical tests: Mycelial dry weight was determined according to Haq and Daud (1995). Sugar was estimated spectrophotometrically (Cecil-700 series, UK) by DNS method Tasun *et al*. (1970). Anhydrous citric acid was estimated using pyridine-acetic anhydride method as reported by Marrier and Boulet (1958). Kinetics of research work was studied after Pirt (1975). The statistical analyses were based on Duncan's multiple range tests.

Results and Discussion

Among the factors that determine morphology and the general course of fungal fermentations, the type and size of inoculum is of prime importance. Earlier attempts have been made to standardise inocula for citric acid production in submerged culture (Martin and Waters, 1952). Fig. 1 shows the effect of vegetative inoculum

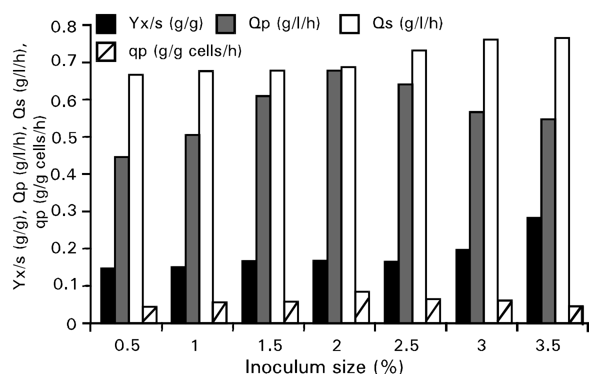

Fig. 1: Comparison of kinetic parameters for citric acid production by *A. niger* at different levels of vegetative inoculum in stirred bioreactor.
Initial sugar concentration 15%, incubation temperature 30°C, initial pH 6.0, aeration rate 1.0 l/l/min. All the values are sum mean of three parallel replicates. The values differ significantly at $P < 0.025$.

Kinetic parameters:

Yx/s = g cells/g substrate utilized
Qp = g citric acid produced/l/h
Qs = g substrate i.e., sugar utilized/l/h
qp = g citric acid produced/g cells formed/h

(0.5-3.5%) on citric acid production by *Aspergillus niger* $NG^{GCB}101$ in stirred bioreactor. Maximum citric acid production (96.86±4.0a g l^{-1}) was obtained with 1.0% inoculum. All the kinetic parameters i.e., cells yield coefficient (Yx/s = 0.153±0.03g g g^{-1}), product formation rate (Qp = 0.507±0.04d g/l/h), volumetric rate of substrate consumption (Qs = 0.680 ± 0.02c g/l/h) and specific rate constant for product formation (qp = 0.034± 0.007c g/g cells/h) revealed 1.0% vegetative inoculum to be adequate for optimal citric acid production. Lower or higher level of inoculum size did not support citrate synthase activity. It might be due to the fact that the optimum level of mycelium produced an optimum amount of enzyme for which 1.0% inoculum was sufficient. This is in accordance with the findings of Van Suijdam *et al.* (1980). With the increase in mycelial mass, the production of enzyme declined due to exhaustion of nutrients in the fermentation medium. However, the effect of inoculum on mycelial morphology in submerged culture has been assessed mainly by the presence or absence of pellets and their characteristics (Vecht-Lifshitz *et al.*, 1990). The reason for this was the lack of an adequate method to monitor mycelial morphology during fermentation.

The results indicate the manipulation of mycelial morphology to improve bioreactor performance and process yield. Citric acid production is substantial at 1.0% inoculum level. The specific productivity i.e., qp = 0.074±0.02a g g^{-1} cells/h is highly significant. Further research work is required to reveal general trends in relation to morphology of the producer organism and partial purification of citric acid from the broth culture.

Acknowledgements

The authors are thankful to the officials of Pakistan Science Foundation for financial assistance. Special thanks are due to Dr. M. I. Rajoka, PSO (NIBGE, Fasialabad) for his help in the statistical analysis of data.

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