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Selection of Fermentation for Citric Acid in Bioreactor

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Abstract: Twenty-five strains of ***Aspergillus niger***, isolated from different soil samples were evaluated for citric acid production. Shake flask technique was employed for screening of the isolated. The best citric acid producing strain, GCB-14, was selected for further studies using a laboratory scale stirred fermentor. Cane-molasses medium and sucrose salt medium were tested for the basal fermentation medium. The results indicated that maximum amount of citric acid i.e. 55.43 gl^{-1} was produced 144 h after incubation using cane-molasses medium. The maximum amount of sugar consumed and dry cell mass produced were 126 and 27.49 gl^{-1}, respectively. Therefore, cane-molasses was optimized as the basal fermentation medium for maximal citric acid production.

Key words: ***Aspergillus niger***, citric acid, fermentation, cane-molasses, sucrose salt medium

Introduction

Citric acid (2-hydroxy propane-1, 2, 3-tricarboxylic acid) is a white solid at room temperature melts at 153°C (307°F) and decomposes at high temperature into other products (Srivasta and Kamal, 1979). It is the most important organic acid produced in tonnage and is extensively used in food and pharmaceutical industry. Because of its commercial and academic importance, the biosynthesis of citric acid by moulds has been a subject of numerous investigations (Mattey, 1992). Most of the citric acid produced commercially comes from ***Aspergillus niger*** (Fiedurek ***et al.***, 1996; Pera and Callieri, 1997; Maddox and Brooks, 1998). Carbon source is one of the factors that have been shown to exert an influence on citric acid fermentation (Papagianni ***et al.***, 1999). Various carbohydrate materials such as cane or beet molasses and crude infiltered starch hydrolysate may be used in citric acid production by ***Aspergillus niger*** in submerged fermentation (Haq ***et al.***, 1998). Workers have also investigated on the citric acid fermentation using sucrose salt media (Sanchez ***et al.***, 1970; Singh ***et al.***, 1998). However, the utilization of pure sugars as carbon source in fermentation media for large-scale is uneconomical (Islam ***et al.***, 1986). A number of reports have been published on the production of citric acid by submerged mould cultures using molasses and starch-based media (Vandenberghe ***et al.***, 1999; Kubicek and Roehr, 1977; Obaidi and Berry, 1979). Stainless steel fermentor of different working capacities can be

employed for submerged citric acid fermentation (Sanjay and Sharma, 1994; Vergano ***et al.***, 1996; Rajoka ***et al.***, 1998). The present study is concerned with the selection of fermentation medium for citric acid fermentation a stirred tank reactor.

Materials and Methods

The experiments were conducted in Biotechnology Research Laboratory, Government College University Lahore, Pakistan during the year 2001. The cultures of ***Aspergillus niger*** were isolated from different soil samples of Lahore by serial dilution method (Clark ***et al.***, 1958). Twenty-five isolates were evaluated for citric acid bio-production (GCB-1 to GCB-25) and the best isolate (GCB-14) that produced 16.04 g L^{-1} was selected for further investigations. Cane molasses obtained from Kamalia Sugar Mills were clarified according to the method of Panda ***et al.*** (1984).

Fermentation conditions

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.

Assay 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 2 h. Anhydrous citric acid was estimated colorimetrically, using pyridine-acetic anhydride method, as reported by Marrier and Boulet (1958).

Results and Discussion

In this study, cultures of ***Aspergillus niger*** isolated from various soil samples produced citric acid ranging from 0.24-16.04 g L^{-1} (Table 1). Cane molasses and sucrose salt media were evaluated for the basal fermentation media. The maximum amount of citric acid i.e. 55.43 g L^{-1} was produced using cane-molasses after 144 h of incubation (Fig. 1). The strain produced intermediate pellets when cane-molasses medium was used and it gave fine pellets when sucrose

Table 1: Screening of *Aspergillus niger* strains using molasses for citric acid production in 250 ml shake flask

Isolates of *A. niger*	Sugar consumption (g L^{-1})	Dry cell mass (g L^{-1})	Total acid (%)	Anhydrous citric acid (g L^{-1})	Anhydrous citric acid % yield*	Mycelial morphology
GCB-1	62.5	8.0	12.4	9.7	15.5	Gelatinous
GCB-2	93.0	8.5	5.4	2.4	2.6	Gelatinous
GCB-3	86.0	11.5	2.0	0.8	0.9	Gelatinous
GCB-4	72.5	9.5	1.9	1.9	2.7	Gelatinous
GCB-5	70.5	9.5	4.9	2.8	4.0	Gelatinous
GCB-6	70.0	16.0	10.1	7.1	10.1	Fine pellets
GCB-7	64.0	18.5	93.9	8.2	12.8	Fine pellets
GCB-8	69.0	14.5	16.8	12.1	17.6	Dumpy mass
GCB-9	92.5	21.5	15.2	10.7	11.5	Viscous
GCB-10	89.5	18.0	6.9	3.2	3.6	Viscous
GCB-11	52.0	17.5	10.2	6.2	12.0	Fluffy elongated mycelium
GCB-12	48.5	16.5	2.0	0.2	0.4	Fine pellets
GCB-13	98.5	8.0	5.6	2.6	2.6	Gelatinous
GCB-14	70.0	8.5	20.6	16.0	22.9	Gelatinous
GCB-15	78.5	11.5	5.0	3.1	4.0	Small round pellets
GCB-16	66.0	15.0	1.9	0.9	1.3	Viscous
GCB-17	83.0	14.2	12.4	6.8	8.3	Fine pellets
GCB-18	64.2	15.3	16.3	13.0	20.3	Viscous
GCB-19	72.3	10.9	10.4	3.6	5.1	Fine pellets
GCB-20	71.3	16.3	12.8	7.0	9.8	Gelatinous
GCB-21	120.0	31.0	3.2	1.9	1.6	Large pellets
GCB-22	100.0	34.0	8.9	5.6	5.6	Large pellets
GCB-23	85.0	28.0	21.2	14.9	17.5	Small pellets
GCB-24	91.0	23.0	12.9	10.1	11.1	Gelatinous
GCB-25	69.0	21.0	11.8	8.7	12.6	Filamentous

Initial sugar concentration 150 g L^{-1}, incubation period 148 h, temperature 30°C, initial pH 6.0, ferrocyanide concentration 200 ppm, * On the basis of sugar used

Table 2: Comparison of the mycelial morphology of *Aspergillus niger* during citric acid fermentation using cane-molasses and sucrose salt media

Incubation period (h)	Cane-molasses	Sucrose salt medium
24	Viscous	Viscous
48	Viscous	Viscous
72	Viscous	Viscous
96	Viscous	Viscous
120	Small pellets	Viscous
144	Intermediate pellets	Fine pellets
168	Large pellets	Fine pellets
192	Large pellets	Fine pellets
216	Large pellets	Gelatinous mass

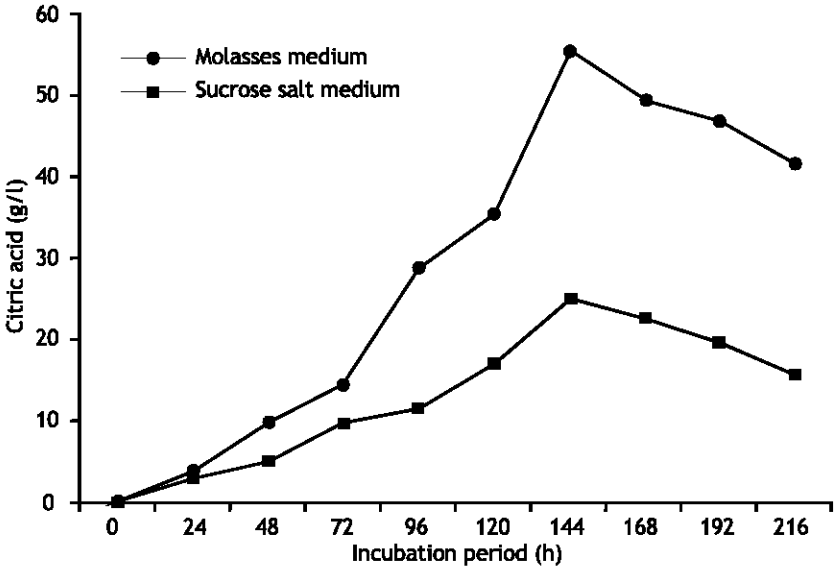

Fig. 1: Comparison of citric acid production using cane-molasses and sucrose salt media Initial sugar concentration 150 g L^{-1}, temperature 30°C, ferrocyanide concentration 200 ppm

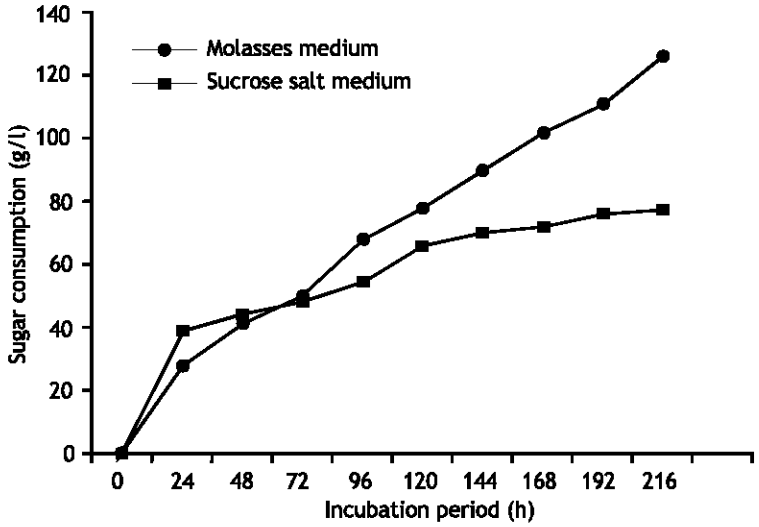

Fig. 2: Comparison of sugar consumption using cane-molasses and sucrose salt media Initial sugar concentration 150 g L^{-1}, temperature 30°C, ferrocyanide concentration 200 ppm

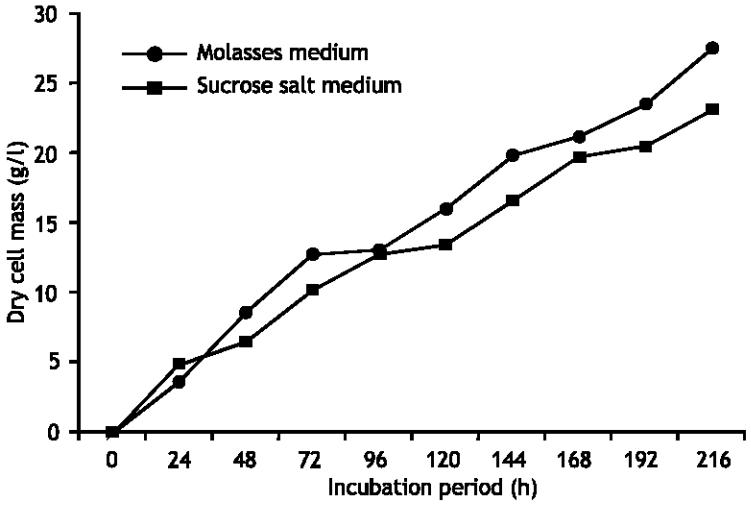

Fig. 3: Comparison of dry cell mass using cane-molasses and sucrose salt media
Initial sugar concentration 150 g L^{-1}, temperature 30°C, ferrocyanide concentration 200 ppm

salt medium was utilized after 144 h of incubation (maximum citric acid production). Fig. 2 shows the comparison of sugar consumption using cane molasses and sucrose salt media. Sugar consumption in case of cane molasses medium increases gradually with the incubation period. In the comparison of dry cell mass using the two media (Fig. 3) it increases continuously in both the cases but dry cell mass in case of molasses is more on the increase than that when sucrose salt medium was utilized as the fermentation medium. Ali ***et al.*** (2001) and Singh ***et al.*** (1998) used sucrose salt medium for citric acid fermentation and got good results. However, in this investigation, clarified cane molasses was optimized as the basal fermentation medium for subsequent studies. It was concluded that molasses medium as the best fermentation medium for enhanced and consistent yields of citric acid (Khan ***et al.***, 1970; Clark, 1962; Haq ***et al.***, 2001).

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