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Citric acid production: Surface culture versus submerged culture

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Surface and submerged fermentation methods were used to produce citric acid by *Aspergillus niger* using chemically defined media or cane molasses. Fermentation process parameters were optimized in pilot scale tower and tray fermenters. Fermentation was running from 10 - 20 days with pH controlled at 3 - 6.5. Citric acid concentrations varied from 60 - 100 g/L depending on the strain used, the substrate, the fermentation system and the general conditions under which fermentation took place (initial sugar concentration, aeration rate, inoculum size, pH and temperature). Some essential criteria such as lower process sensitivity to short interruptions or breakdowns in aeration, expenses for equipment, consumption of electrical energy and higher yield and productivity, showed that surface fermentation is superior to submerged fermentation. However, the yield and productivity obtained in submerged fermentation using indigenous strains of fungi is not yet high enough for commercial production.

Key words: Citric acid, surface, submerged indigenous strains, yield, productivity.

INTRODUCTION

The State of Khuzestan, Iran, is a large producer of sugar cane molasses. Thousands of tons of sugar cane molasses are produced daily by the sugar processing industries. Thus, there is an urgent need to find suitable applications of this byproduct. One alternative for its economic utilization is to use it as substrate in fermentation processes for the production of value added products like citric acid. The aim of this study was to compare citric acid production in submerged and surface fermentation systems using indigenous strains of fungi.

Citric acid is one of the most commonly used organic acids in food and pharmaceutical industries. The food industry is the largest consumer of citric acid, using almost 70% of the total production, followed by about 12% for the pharmaceutical industry and 18% for other applications (Milsom, 1987; Meers and Milsom, 1987; Briffaud and Engasser, 1979a, b; Roehr et al., 1981; Berovic and Cimerman, 1982). Its pleasant taste, high solubility and flavor-enhancing properties have ensured its dominant position in the market. Although citric acid can be obtained by chemical synthesis, the cost is much higher than using fermentation. It is mainly produced by submerged fermentation by the filamentous fungus *Aspergillus niger*.

In order to decrease the cost of citric acid production using *A. niger*, surface fermentation has been studied as a potential alternative to submerged fermentation. Surface fermentation was the first fermentation system used for the production of citric acid on an industrial scale (Allen and Robinson, 1989; Blain et al., 1979; Anderson et al., 1980; Jernejc et al., 1982) . Today, almost all citric acid produced by fermentation is manufactured by strains of *A. niger* in submerged and surface culture.

The production of citric acid depends strongly on an appropriate strain and on operational conditions such as aeration, type and concentration of the carbon source, nitrogen and phosphate limitation, pH, concentration of trace elements and the morphology of the producer orga-

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nism (Khan and Shaukat, 1990; Sakurai and Imai, 1992; Qazi et al., 1990; Papagianni, 2007).

In this study some initial experiments were carried out with four strains of fungi in liquid culture and, based on the productivity of citric acid, two strains of *A. niger*, EP1 and EP4, were selected for the further studies of submerged and surface fermentation. Main factors affecting productivity and yield were optimized in both systems and results showed that submerged fermentation had higher sensitivity to trace metal concentration and short interruptions or breakdowns in aerations, which resulted not only in losses of yield, but also in a total breakdown of respective batches.

An order of magnitude estimate was made using generalized assumptions about the project to estimate its costs. Some essential criteria such as expenses for equipment, consumption of electrical energy, and higher yield and productivity showed that surface fermentation is superior to submerged fermentation.

MATERIALS AND METHODS

Microorganisms

Four strains of *A. niger* were screened for citric acid production in liquid culture which contained sucrose (g/l) 120 - 300, $NaNO_3$ 0 - 5, KH_2PO_4 0 - 2, $MgSO_4.7H_2O$ 0 - 1, $CuSO_4.7H_2O$ 0 - 0.02, $FeSO_4.7H_2O$ 0 - 1, $ZnSO_4.7H_2$ O 0 - 1. The initial pH of medium was adjusted to 6.5 without control during fermentation.

Inoculum

A. niger mycelia pellets were used, they were grown at the temperature of 27°C in 500 ml shake flasks and aerated 2 L stainless steel tanks for 2 - 3 days in medium that has the same composition as the production medium. After 2 days of fermentation the Inoculum pH was around 3 - 3.5. The production medium is then inoculated at a concentration of 5 - 10% (v/v).

Substrate

Sugar cane molasses which contained $K_4Fe(CN)_6$ and a synthetic media which contained sucrose, KH_2PO_4, $MgSO_4.7H_2O$, $CuSO_4.7H_2O$, $FeSO_4.7H_2O$, $ZnSO_4$. $7H_2O$ were tested. The initial pH is dependent on the medium employed but during the fermentation it was controlled to 3.0 - 5.5 by addition of lime (Jernejc et al., 1982; Qazi et al., 1990; Clark and Lentz, 1963; Chaudhary et al., 1978; Berovic and Cimerman, 1982; Ilczuk, 1983; Kundu et al., 1984).

Fermentation

In surface fermentation *A. niger* is cultivated on the liquid surface of stainless steel trays. These trays are stacked in fermentation rooms supplied with filtered air which serves both to supply oxygen and to control the temperature of fermentation (Milsom, 1987; Meers and Milsom, 1987). The air supply for the chambers was sterilized by passage through a 2 inch thick cotton filter impregnated with salicylic acid, then passed through a water spray and heaters to bring it to 40% humidity at 25 - 30°C (Dawson, 1989; Choe and Yoo, 1991; Szewczyk and Myszka, 1994; Roukas and Alichanidis, 1988).

The air supply was 0.25 - 3 vvm. The trays are 1 ∗ 1.5 m in size and 10 cm deep. They were sterilized and filled to a depth of 3 - 7 cm with the Liquid medium which was sterilized at 121°C for 15 min and inoculated with prepared inoculum at a concentration of 5 - 20% (v/v) and incubated 9 - 20 days (Sodeck et al., 1981; Johnson, 1949; Sakurai and Imai, 1991; Shierholt, 1977; Roukas and Alichanidis, 1991) . After the maximum concentration of citric acid was reached, the mycelium was separated from the fermentation broth by filtration. The biomass was washed with water to remove citric acid and the washings were added to the main liquor (Milsom, 1987; Meers and Milsom, 1987; Kapoor et al., 1983; Atkinson, 1985). In submerged fermentation, the microorganism was grown in the fermentation broth. Fermentation was carried out in aerated stainless steel tank as a tower fermenter. This fermenter had a proportion from 1:6 and total volume of 150 L. Air was supplied from the bottom of the column via a distribution system. Oxygen transfer by rising air bubbles also ensures a thorough mixing in the fermenter. Thus, an agitator, which needs additional energy and makes the fermenter system much more complicated, was not necessary (Briffaud and Engasser, 1979a, b). Despite aeration, other factors affecting citric acid production were the same in both cases.

Analytical methods

Samples (5 ml) were mixed well with 50 ml of distilled water and filtered. The filtrate so obtained was subjected to high performance liquid chromatograph analysis using a Shimadzu LC-10AD HPLC. A temperature of 60°C and 5 m MH_2SO_4 as the mobile phase at a flow-rate of 0.6 ml/min were used. Citric acid and total sugars were detected in the column evaluated by differential refractometer (Shimadzu RID-10A). Moisture, pH and total sugars content were determined (Soccol, 1992).

RESULTS AND DISCUSSION

Initial experiments were carried out with four strains of *A. niger* in 500 ml shake flasks for 10 days in medium that has the same composition as the production medium.

The initial pH of medium was adjusted to 6.5 without any control during fermentation. Results were shown in Figure 1

As shown in Figure 1 it will inferred that two strains of *A. niger* , EP2 and EP3 have the same manner and despite the increase of citric acid production, concerning the time, the rate of production is not expected to be suitable for industrial application. On the other hands, strains EP1 and EP4 have the equal maximum productivity after a 10 days period, but EP1 had more productivity in middle stages during this fermentation period. Consequently strain EP4 has the maximum productivity and production rate at lower pH when fermentation system is not vulnerable to infection. Based on this results and the nature of surface and submerged culture, two strains of *A. niger*, EP1 and EP4, were respectively selected for the further studies of submerged and surface fermentation.

Citric acid production in surface and submerged culture

After 10 days of fermentation with initial sucrose concen-

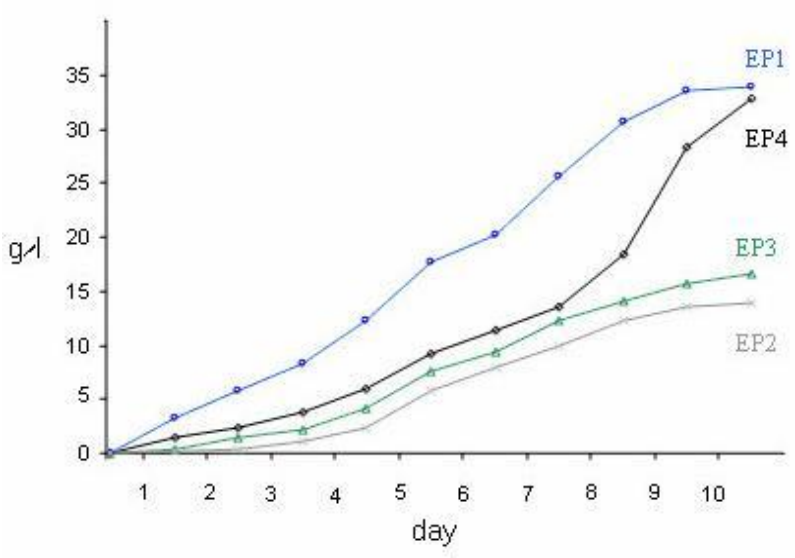

Figure 1. Citric acid productivity of four strains of *A. niger* in shake flask

Table 1. Comparison of fermentation results in surface and submerged culture.

Fermentation system	Submerge culture on molasses	Submerge culture on synthetic media	Surface culture on molasses	Surface culture on synthetic media
Strain	EP1	EP1	EP4	EP4
pH	5.5	4	3	3
Temperature(°C)	25-26	25-26	28-30	27-30
Initial Sugar (g/L)	150	150	300	300
FeSO4.7H2O(mg/L)	-	100	-	400
$K_4Fe(CN)_6$(mg/L)	600	-	200	-
$NaNO_3$ (g/L)	0.8	1	1	1.5
KH_2PO_4(g/L)	0.2	0.3	0.5	0.7
CuSO4.5H2O(mg/L)	10.0	20.0	20.0	30.0
Stability time(h)	960	2000	960	2000
Aeration rate(vvm)	1	1.2	0.2	0.2
Yield	66%	74%	80%	74%
Productivity g/(L.h)	0.44	0.46	0.5	0.46

concentration of 150 g/L the productivity and yield of citric acid production for strain EP1 in submerged culture were respectively around 66% and 0.44 g/(L.h) for sugar cane molasses and around 74% and 0.46 g/(L.h) for synthetic medium. Results are shown in Table 1.

As shown in Table 1 in surface fermentation, there was a high yield of sugar consumption and productivity by strain EP4 in both molasses and synthetic medium. The productivity and yield of citric acid production by strain EP4 in surface culture were respectively around 80% and 0.5 g/(L.h) for sugar cane molasses and around 74% and 0.46 g/(L.h) for synthetic medium. In submerged fermentation the stability of citric acid biosynthesis by recycled mycelia pellets were constant around a period of 960 h but in surface fermentation the mycelia stability was around 2000 h.

Feasibility study

An order-of magnitude estimate is made using generalized assumptions about the project to estimate its costs. The method used for the estimate was developed by some skilled estimator. This estimate was approached in two areas, process and architectural. The process and utility support were estimated from preliminary pricing of operating (production, purification, waste water treatment and wages) and equipment cost for an industrial scale plant with normal capacity of 10000 tons and Payback Period of 5 year. It should be noted that all data and

Table 2. Total cost of citric acid production with surface and submerged fermentation.

Fermentation system	Submerged culture	Surface culture
Strain	EP1	EP4
Medium	Synthetic Medium	molasses
Variable production cost($/kg)	2.5	0.51
Fixed production cost ($/kg)	0.05	0.25
Direct materials cost($/kg)	0.1	0.5
Direct labor cost($/kg)	0.1	0.3
Total cost($/kg)	2.75	1.55

results are just valuable for Iran and third world Middle East countries.

Some essential criteria such as lower process sensitivity to short interruptions or breakdowns in aeration, expenses for equipment, consumption of electrical energy, and higher yield and productivity showed that surface fermentation is superior to submerged fermentation. However, the yield and productivity obtain in submerged fermentation using indigenous strains of fungi were not yet high enough for commercial production. As shown in Table 2 Surface fermentation of fungi on molasses is the best choice for citric acid production. With considering price of citric acid in market and its added value it was concluded that industrial scale production of citric acid is completely economic and beneficiary.

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