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Optimization of Substrate Concentration for Enhanced Citric Acid Production by *Aspergillus niger* M-101

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Abstract. Studying the effect of different sugar concentration of beet molasses on citric acid accumulation in batch fermentation, 150 g/litre sugar concentration was found to be optimal for maximum citric acid production (27.25±2.35 g/litre) using *Aspergillus niger* M-101. $Y_{p/x}$ value for product formation increased with increase in sugar concentration through out the study. Q_p value for citric acid production also increased with increase in sugar concentration and reached the maximum (0.141 g/1itre/h) at 150 g/litre sugar concentration but with further increase in sugar concentration, the value decreased. When culture was grown at different substrate concentrations, the kinetic parameters monitored for $Y_{x/s}$, $Y_{p/s}$ and $Y_{p/x}$, Q_p, Q_s and q_p showed significant enhancement ($p \leq 0.05$) in citric acid production as well as biomass growth.

Keywords: *Aspergillus niger*, citric acid, kinetic parameters, beet molasses, submerged fermentation

Citric acid, the most important organic acid used in food industry is produced chiefly by fermentation. Large number of microorganisms including fungi, yeast and bacteria have been employed for citric acid production (Grewal and Kalra, 1995) but most of them are not able to produce commercially acceptable yields except the fungus, *Aspergillus niger*. Citric acid is produced by *A. niger* by submerged fermentation process (Guilherme *et al*., 2008). Main advantages of using *A. niger* are its ease of handling, its ability to ferment a variety of cheap raw materials and high yields (Soccol *et al*., 2006). Various substrates like sugar beet molasses, sugar cane molasses, inulin, kurma, date fruit syrup and carob pod (Soccol *et al*., 2006) have been used for citric acid production by *A. niger*.

Worldwide demand of citric acid is about 6×10^5 tons per year (Karaffa and Kubicek, 2003). According to an estimate, annually 500,000 tons of citric acid are produced almost exclusively through fermentation by *A. niger* and widely used in food, chemical, pharmaceutical and other industries (Wang and Liu, 1996) for applications such as acidulation, antioxidation, flavour enhancement, preservation as plasticizer and as a synergistic agent (Sarangbin *et al*., 1993).

At present, citric acid is imported into Pakistan to the tune of more than Rs. 1.5 billion, annually. Pakistan being an agricultural country is producing both cane and beet molasses approximately 2 million tons per year. About 3.5 million tons per year of the molasses are used for the production of ethanol using baker's yeast. Beet molasses, being a rich source of sucrose, is employed for citric acid production. One of the major objectives of the present study was to optimize substrate (beet molasses) concentration for maximum citric acid production using *A. niger* strain M-101 through submerged fermentation.

Approximate analysis of beet molasses was done according to the method of Ranganna (1986). Composition of molasses used in the study is given in Table 1.

The following fermentation medium was employed for citric acid production (values are in (g/litre)): beet molasses, 150.0; $NaNO_3$, 4.0; KH_2PO_4, 1.0; $MgSO_4 . 7H_2O$, 0.23; $FeCl_3$, 0.02; $ZnSO_4$, 0.0012; $MnCl_2 . H_2O$, 0.0012 (pH 4±0.2).

Table 1. Composition of beet molasses

Constituents	Dry weight basis (%)
Moisture	19.4
Dry solids	81.6
Ash 9	
Total reducing sugar	17.23
Total sugar	60.16
Sucrose (nonreducing sugar)	42.93
Nitrogen	0.38
Metal ions g (kg):	
Fe	0.076
Cu	0.012
Mn	0.019
Zn	0.122

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Pretreatment of beet molasses was carried out using sulphuric acid treatment (Mayilvahanan *et al.*, 1996), after which it was centrifuged and the supernatant was used for citric acid production. Spores of *A. niger* M-101 were grown for 192 hs and used for fermentation. Citric acid in the filtrate of the fermentation broth was estimated gravimetrically, using pyridine-acetic anhydride (Marrier and Boulet, 1958).

Study of the effect of different sugar concentrations on citric acid production by *A. niger* M-101 using beet molasses (Fig. 1) showed that the production of citric acid was in versely proportional to the biomass growth up to 15 % sugar concentration but afterwards its production was directly proportional to the biomass growth. Maximum citric acid production (27.25±2.35 g/litre) was observed in the medium containing 150 g/litre initial sugar concentration while biomass was 35.20 g/litre, percentage yield being 21.57. Sugar level of 150 g/litre (15%) is thus optimal for citric acid production. Similar results for the production of citric acid were also reported by Papagianni *et al.* (2005).

According to Pazouki *et al.* (2000), high sugar concentration leads to the accumulation of oxalic acid in the culture broth. Kubicek-Pranz *et al.* (2000) found that triggering citric acid accumulation by placing *A. niger* in high concentrations of sucrose or glucose is paralleled by a rise in the intracellular concentration of fructose- 2,6-diphosphate (Fru-2, 6-P_2), the strongest activator of PFK1. Higher concentrations of Fru-2,6-P_2 were observed in mycelia cultivated on 15 % sucrose concentration, which allows higher yields of citric acid to be obtained (Kubicek and Rohr, 1977). The regulatory osmotic effect of high sugar concentration on citric acid accumulation is due to the possible role of tyrosine kinases in regulating the ion efflux pathways induced by hyper-osmotic stimulation. These kinases are required for restoring the osmotic gradient across the cell membrane in response to increased external osmolarity (Fiedurek, 1998). Facilitated diffusion and active transport, both, are characterized by a non-linear activity response to increasing substrate concentration, whereas simple diffusion has a linear response (Wayman and Mattey, 2000).

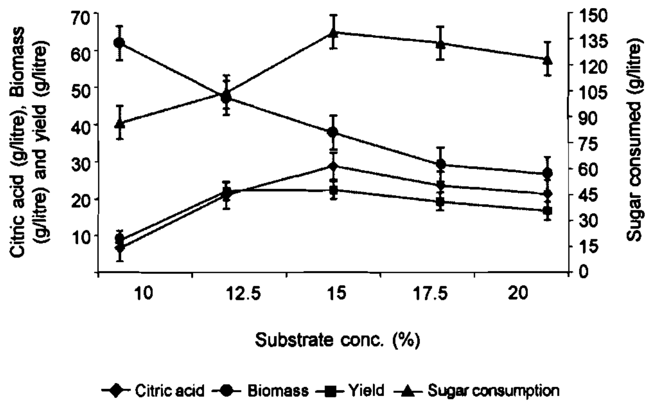

Fig. 1. Evolution of citric acid and biomass during the fermentation using different substrate concentration.

Kinetic parameters for citric acid production are shown in Table 2. Value of growth yield co-efficient ($Y_{x/s}$) tends to decrease with increase in substrate concentration and reached to its minimum value (0.225 g/g). Values of specific product yield coefficient ($Y_{p/x}$) and product yield coefficient ($Y_{p/s}$) also tend to increase with increase in substrate concentration and reached the maximum values of 0.810 and 0.215 g/g. Increase in $Y_{p/x}$ value with increase in substrate concentration was also reported by Peksel and Kubicek (2003) who obtained maximum yield (0.141 g/litre/h) of citric acid at 15 % substrate concentration.

Table 2. Kinetic parameters and coefficients of citric acid fermentation by *A. niger* at different substrate concentrations

	Substrate concentration (%)				
Kinetic parameters	10	12.5	15.0	17.5	20.0
Substrate uptake rate (Q_s)	0.419	0.498	0.657	0.628	0.586
Specific substrate uptake rate (q_s)	0.007	0.011	0.018	0.021	0.023
Product yield coefficient ($Y_{p/s}$)	0.098	0.212	0.215	0.187	0.182
Specific product yield coefficient ($Y_{p/x}$)	0.140	0.469	0.774	0.793	0.810
Growth yield coefficient ($Y_{x/s}$)	0.698	0.452	0.278	0.236	0.225
Productivity (Q_p)	0.041	0.105	0.141	0.117	0.107
Specific productivity (q_p)	0.0007	0.002	0.004	0.004	0.004

Q_s = g substrate consumed/litre/h; q_s = g substrate/g cells/litre/h; $Y_{p/s}$ = g citric acid produced/g sugar consumed; $Y_{p/x}$ = g citric acid produced/ g cells; $Y_{x/s}$ = gram cell mass/gram sugar consumed; Q_p = g citric acid produced/litre/h; q_p = g product/g cells/litre/h; all the values differ significantly at $p \leq 0.05$.

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