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RE-USE OF FUNGAL MYCELIUM FOR THE PRODUCTION OF CITRIC ACID BY *ASPERGILLUS NIGER*

IKRAM-UL-HAQ, SIKANDER ALI AND ASAD-UR-REHMAN

Biotechnology Research Laboratories,
Department of Botany, Government College University, Lahore, Pakistan.

Abstract

The present study deals with the re-use of fungal mycelium for citric acid fermentation. Among the 16 – different isolates of *Aspergillus niger*, IS-6 was optimised for citric acid production. The re-use of mycelium was found to be economical due to decrease in fermentation period and high volumetric productivity (Yp/x = 3.233 & 2.914 gg^{-1}) but in the third batch, the production of citric acid was markedly decreased due to the mycelial age factor.

Introduction

Citric acid has a wide range of applications in food, pharmaceutical and beverage industries. The entire worldwide demand for citric acid is met by fermentative production, mainly by the moulds involving the filamentous fungus *Aspergillus niger* (Usami & Fukutomi, 1977; Steinboch *et al.*, 1991; Suzuki *et al.*, 1996). Authors have used sucrose salt medium (Begum *et al.*, 1990; Singh *et al.*, 1998) and raw materials such as cane and beet molasses (Mattey 1992; Maddox & Brooks, 1995; Haq *et al.*, 2000) as well as starch hydrolysate (Takatomi & Usami, 1960; Crueger & Crueger, 1984) for citric acid fermentation. The re-use of fungal mycelia may also increase citric acid production, regarding the consistency of fermentative product (Kirimura *et al.*, 1988; Suzuki *et al.*, 1996). The present report describes the production of citric acid by re-using the fungal mycelium, from synthetic medium like sucrose salt medium in shake flask.

Materials and Methods

Sixteen different cultures of *Aspergillus niger* were isolated from soil samples of Lahore, by serial dilution method (Clark *et al.*, 1958). These cultures were maintained on potato dextrose agar (PDA) slants, incubated at 30°C and stored at 4°C in a refrigerator. The conidial suspension was prepared in 10 ml of 0.005 % Monoxal O. T. (Dioctyle ester of sodium sulpho succinic acid) from a 3-6 days old slant culture. The inoculum needle was used for breaking clumps of conidia. The fermentation medium contained (%age, w/v); Sucrose 15.0, KH_2PO_4 0.1, $MgSO_4.7H_2O$ 0.025 and NH_4NO_3 0.25. The optimal conditions for citric acid fermentation were investigated in 250 ml Erlenmeyer flask containing 25 ml of the medium having pH 3.5. One ml of the conidial suspension (1.2 x 10^6 conidia) was added to each flask. The flasks were rotated (160 rpm) in the rotary incubator shaker (Model: GLSC. OSIR-195-0.004, Pak made) at 30°C for 8 days. All the experiments were run parallel in triplicates.

Sugar was determined colorimetrically by DNS method as reported by Tasun *et al.* (1970) while citric acid monohydrate was estimated by the method of Marrier & Boulet (1958). The colour intensity was determined by photoelectric colorimeter (Model: AE-11B, ERMA, Japan) using green wratten filter of 530 nm. Dry cell mass was determined according to Haq & Daud (1995).

Table 1. Screening of *Aspergillus niger* isolates for the production of citric acid using sucrose salt media.

Isolates of *Aspergillus niger*	Sugar (g/l)		Dry cell mass (g/l)	Citric acid monohydrate (g/l)	% age citric acid*
	Used	Residual			
IS – 1	66.5	83.5	8.5	4.56	6.86
IS – 2	81.0	69.0	11.0	9.21	11.37
IS – 3	68.0	82.0	9.0	6.55	9.63
IS – 4	57.0	93.0	6.9	7.62	13.37
IS – 5	75.0	75.0	10.5	12.50	16.67
IS – 6	87.5	62.5	13.5	36.45	41.66
IS – 7	90.0	60.0	18.6	26.52	29.47
IS – 8	72.5	77.5	12.5	11.27	15.54
IS – 9	62.0	88.0	8.8	6.72	10.84
IS – 10	52.8	97.2	7.5	5.42	10.26
IS – 11	60.5	89.5	11.5	14.21	23.49
IS – 12	55.5	94.5	12.0	19.12	34.45
IS – 13	83.0	67.0	19.5	20.25	24.40
IS – 14	64.0	86.0	14.2	23.56	36.81
IS – 15	50.5	99.5	9.0	3.12	6.18
IS – 16	71.0	79.0	13.0	25.76	36.28

Sugar added = 150 g/l
Incubation temperature = 30°C
Initial pH = 3.5
Fermentation period = 8 days
Agitation rate = 160 rpm

*On the basis of sugar used.

Results and Discussion

Citric acid is one of the most important organic acids produced commercially by fermentation with specific moulds, mostly strains of *Aspergillus niger* (Mattey, 1992). The present investigation deals with the isolation of *A. niger* strains and then re-use of the fungal mycelium for hyper production of citric acid in shake flask, using sucrose salt media. Among the 16 different isolates of *A. niger* screened for citric acid production (Table 1), isolate IS-6 gave maximum production of citric acid i.e., 36.45 g/l (41.66 % on the basis of sugar used). The sugar consumption was 87.5 g/l while the mycelium were round pellets having dry cell mass 13.5 g/l. Rest of the *A. niger* strains produced relatively lower yields of citric acid monohydrate. This highlighted the idea that it is the type of the strain, which is responsible for high degree product formation. Similar observation has been reported by Maddox & Brooks (1995).

To study the effect of re-use of fungal mycelia on the production of citric acid by *A. niger* IS-6, the mould mycelium after fermentation was separated from fermented broth under aseptic conditions, using sterile centrifuge tubes. The mycelium thus obtained from previous batch was transferred to fresh sterile medium contained in shake flasks. Each fermentation was run for 4-8 days. The experiments were performed in triplicates. In the first batch, the maximum amount of citric acid produced was 48.50±0.12 g/l and glucose consumed was noted as 72.0 g/l (Table 2). The dry cell mass, citric acid production on the basis of sugar used and kinetic parameter, Yp/x values were 15.0 g/l, 67.36 % and

3.233 gg^{-1}, respectively. Further use of the mould mycelium resulted in lowering the consumption of glucose and subsequently the production of citric acid as 88.5 g/l and 52.45±0.34 g/l, respectively (59.26 % citric acid on the basis of sugar used having Yp/x = 2.914 gg^{-1}). The re-use of mycelium for the next batch, greatly reduced both glucose consumption and citric acid excretion (second and third batch). But the incubation period decreased from 8 to 6 days (first to second batch) and then only 4 days in the third repeated batch. The decreased incubation period and a fairly high yield of citric acid monohydrate makes the process economical. While in the third fermentative batch, the formation of the product was totally unsatisfactory (12.16 – 18.25 g/l citric acid, 4-5 days after incubation). This might be due to the fact that with the increase in age of mycelium, the metabolic pathways loose their efficiency to secrete the enzyme citrate synthase and also the substrate (sugar) consumption capability, resulting in the lower product formation and finally reaching to about nill in the fourth batch (The data is not given). In a similar study Kirimura *et al.*, (1988) obtained consistent yields of citric acid production, many folds than the first batch. Haq *et al.*, (2000) got a high yield of citric acid in fed-batch culturing of *A. niger* fermentatation in SS-stirred fermentor.

The present study could suggest that the re-use of fungal mycelia in citric acid fermentation is economical due to consistent yield of the product and decrease in incubation period but one has to find out a potent strain for all this.

Table 2. Effect of Re-use of fungus mycelium on the production of citric acid by *Aspergillus niger* IS – 6.

Mycelia (g/l)	Sugar (g/l) Used	Sugar (g/l) Residual	Incubation period (Days)	Dry cell mass (g/l)	Citric acid monohydrate (g/l)	% age citric acid**	Yp/x*** (gg^{-1})
FIRST BATCH							
5.0	64.0	86.0	8	9.0	16.24±0.20*	25.38	1.804
7.5	69.8	80.2	8	10.3	25.55±0.16	36.60	2.480
10.0	73.5	76.5	8	16.5	34.62±0.08	47.10	2.098
12.5	72.0	78.0	8	15.5	48.50±0.12	67.36	3.233
15.0	81.5	68.5	8	17.0	40.26±0.02	49.40	2.368
17.5	88.0	62.0	7	22.5	32.15±0.26	36.53	1.429
20.0	89.5	60.5	7	25.0	29.96±0.29	33.47	1.198
SECOND BATCH							
10.0	91.4	58.6	7	16.5	34.56±0.16	37.81	2.094
12.5	88.5	61.5	6	18.0	52.45±0.34	59.26	2.914
15.0	88.0	62.0	6	22.0	39.12±0.31	44.45	1.778
THIRD BATCH							
10.0	48.0	102.0	5	13.5	12.16±0.07	25.33	0.901
12.5	67.4	82.6	4	14.0	18.25±0.10	27.08	1.304
15.0	59.5	90.5	4	17.5	18.04±0.23	30.32	1.031

Sugar added = 150 g/l
Incubation temperature = 30°C
Initial pH = 3.5
Agitation rate = 160 rpm

*standard deviation (SD) between the replicates ranging from ±0.02 to ±0.34.
**on the basis of sugar used.
***Product yield coefficient (Yp/x, gg-1) = Citric acid produced. g / Dry cell mass. G

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