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EFFECT OF TOXIC CHEMICALS ON CITRIC ACID PRODUCTION BY *ASPERGILLUS NIGER*

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Citric acid fermentation of beet molasses by locally isolated cultures of *Aspergillus niger* was studied by surface culture method in one litre conical flasks. Effect of the addition of metabolic inhibitors (0.5-4% v/v) such as methanol, ehtanol, n-propanol. n-butanol, chloroform orcarbon tetrachloride, was studied on spore germination, mycelial growth and citric acid production. Of all the toxic chemicals, methanol showed least inhibition. The citric acid formation decreased in the order of ethanol, chloroform, n-propanol, n-butanol and carbon tetrachloride, The morphological changes occurring in the mould were very senstive to alcohols. The mould morphology was chagned into gelatinous mat of mycelium without black sporulation in the presence of n-propanol. However, a beaded mycelial mat with little black sporulation with n-butanol. The addition of ethanol slightly influenced the mould growth. In control cultures, however, the mycelial mat was uniform with white base and sufficient black sporulation on the surface.

INTRODUCTION

The prodction of citric acid by *Aspergilus niger* is extremely sensitive to trace metal spresent in the fermentation media[1,2]. The level of trace metals is generally decreased by treatment of fermentation medium with lime and phosphoric acid[3], ion exchange resins [4,5] and chelating agents such as EDTA and morpholine[6]. Peanut oil[7] also improves the yield of citric acid significantly in the presence of high concentration of trace metals particularly iron and copper. Another important observation in the technology of citric acid fermentation was made by Moyer [8,9] that the addition of alcohols or esters to the media reduced the inhibitory effect of trace metals such as iron, copper, zinc and managese on citric acid production. The stimulatory effects of methanol thus permits its application in the commercial production of citric acid. The present paper describes the effect of various aliphatic alcohols, chloroform, and carbon tetrachloride on the growth process of *A.niger* and citric acid production in beet molasses medium.

METHOD

The strain of *Aspergillus niger* E.U-1, locally isolated, was used in the present investigations.

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Inoculum Preparation

Spore incoulum was used in the present study. A simple synthetic agar medium containing (g/1) suerose. 150; agar 20; $NH_4\ NO_3$, 2.5; $KH_2\ PO_4$, 1.0; $MgSO_4 . 7H_2O$ 0.25; and trace metals (mg/l): Fe^{+++} ($FeCl_3$) 2.20: $CuSO_4\ 5H_2O$) o.48 and Zn^{++} ($ZnSO_4$) 3.8 was used for both sporulation and culture maintenance. 15 ml, of agar medium was sterlized in a cotton wool plugged test tube and the cultures were inoculated at 30°. Spores from 7 days old cultures were wetted with 5ml. of 0.05% solution of sodium lauryl sulphate. The plate was washed with sterile distilled water. The combined washings were made up to 50 ml and shaken with glass beads to break up clumps of spores.

Fermentation Media

Beet Molasses (crop 1978) obtained from Charsadda sugar mills was used for citric acid fermentation. Sugar content of the molasses was about 50%. For medium preparation the molasses were diluted to 15% sugar concentration with tap water. The pH of the medium was kept 5.0. All media were autoclaved at 121° for 15 min.

Conditions of Cultivation

100 ml of fermentation medium including 5 ml of

spore inoculum was placed in one liter conical flask plugged with cotton wool. The flasks were incubated at 30° for eleven days.

Analytical methods

Mycelial weight was determined by filtering 25 ml of culture through weighed Whatman No: 41 washed 3-4 times with tap water, and the mycelial mat was dried at 100° overnight, before weighing. Citric acid was estimated spectrophotemetrically by the method of Marrier and Baulet[10]. A spectrophotometer Unicam, SP600, Series 2 was used to measure colour intensity. Sugar was estimated by the ferricyanide reduction method, a modification of Fugita and Iwateke[11].

RESULTS

Mould Morphology. The mould morphology in the presence of methanol at pH 5.0 of the medium was like control culture i.e. uniform mycelial mat with white base and sufficient black sporulation. The mould growth at pH 5.0 in the presence of 0.5-1.5% v/v ethanol, was like control culture. The aaddition of n-propanol at pH 5.0 had no effect on spore germination and mould morphology. The effect of n-butanol on mould growth was quite different than that of ethanol or n-propanol. The mucelial mat was like heads and sporulation was also greatly affected at the level of 0.5% v/v n-butanol at pH 5.0. Further increase in the concentration of n-bitanol to 1.0% v/v resulted in the inhibition of spore germination.

Effect of Toxic Chemical

Methanol: The effect of methanol was determined on the production of citric acid by adding 0.5 to 3.5% v/v alcohol at pH 5.0 (Table 1). There was significant increase in ctiric acid formation as the concentration of methyl alcohol increased. The optimum production of citric acid (87.5 g/l) was achieved at 3.5% alcohol. The mycelial dry weight was influenced slightly by the addition of methanol. Sugar utilization remained about the same as in control culture.

Ethanol. Effect of various concentrations of ethanol (0.5 to 3.5% v/v) on mycelial growth, sugar consumption and citric acid production was comparable to that of methanol. the percentage citric acid yield found improved by increasing ethanol concentrations.

n-Propanol. At pH 5.0 n-propanol showed toxic effects even in concentrations as low as 0.5-1.0%. Mycelial formation, sugar utilization and citric acid production by the mould was greatly affected. Further increase in the alcoholic concentration inhibited spore germination,

n-Butanol. Effect of n-butanol is shown in Table 4. There was slight increase in mycelial dry weight and citric acid production at a concentration of 0.5% v/v butanol. Further increase in concentration of n-butanol completely inhibited the growth of the fungus.

Chloroform. The data of Table 5 shows the effect of chloroform up to a concentration of 4.0% v/v.

Chloforom was added just before the spore inoculation. The mycelial dry weight was greatly affected by CH Cl_3, but the effect was variable when the amount of chloroform was further increased. The formation of citric acid, however, was increased by the addition of chloroform up to 1% v/v concentration after which it gradually decreased with the increase in the concentration of the solvent.

Carbon Tetrachloride. From Table 6 it is evident that the mycelial formation was affected at 0.5% v/v of CCl_4 i.e. 42.74 g/l. Furhter, increase in the concentration of carbon tetrachloride, however, showed no effect on mycelial dry weight.

The consumption of sugar, remained about the same as compared with the control culture. The conversion of sugar into citric acid, however, was decreased with the increase in the concentration of carbon tetrachloride.

DISCUSSION

The results given above show that the addition of metabolic inhibitors like methanol, ethanol, etc, to culture medium greatly influenced the spore germination, mould morphology and citric acid synthesis by *A. niger* surface culture method. Methanol, however, gave better results of citric acid formation. The optimum level of methanol was 3.5% and the amount of citric acid produced was 87.5% g/l. Citric acid production was slightly decreased when 3.5% ethanol was used, as compared with that of methanol at equal concentration. Further increase in the concentration of methanol and ehtanol affected mycelial dry weight, sugar consumption and citric acid production. It, therefore, follows that molecular weight of the alcohols has direct relationship with the stimulatory effect, that is alcohols with low molecular weight can penetrate in the mycelial cells easily as compared with alcohols with higher molecular weight.

Table 1. Effect of methanol on mycelial dry weight, sugar consumption and citric acid production by *A.niger* (E.U.78-1).

pH: 5.0

S. No.	Mech % v/v	Mycelial dry wt. g/l	Sugar consumed g/l	Citric acid g/l	% Yield of citric acid
1.	0.0	30.5	143.0	50.0	34.96
2.	0.5	29.5	133.0	51.0	38.34
3.	1.0	28.6	131.5	53.3	40.68
4.	1.5	28.0	132.5	53.8	40.50
5.	2.0	27.3	131.0	54.0	41.22
6.	2.5	27.0	136.5	55.5	40.65
7.	3.0	28.5	138.7	73.5	53.14
8.	3.5	32.3	146,67	87.5	59.65

Table 2. Effect of ethanol on mycelial dry weight sugar consumption and citric acid production by *A. niger* (U.E. 78-1).

S. No.	EtOh % v/v	Mycelial dry wt. g/l	Sugar consumed g/l	Ctiric acid g/l	% Yield of citric acid
1.	0.0	28.45	139.75	50.0	35.77
2.	0.5	27.54	138.0	55.0	39.85
3.	1.0	27.0	141.0	58.0	41.13
4.	1.5	27.5	143.0	61.0	42.65
5.	2.0	27.67	144.5	62.0	42.90
6.	2.5	28.43	144.0	63.0	43.75
7.	3.0	31.15	144.5	65.0	44.98
8.	3.5	35.19	144.5	76.5	52.95

Table 3. Effect of n-propanol on mycelial weight, sugar consumption and citric acid production by *A. niger* (E.U. 78-1).

S. No.	n-Proponal % v/v	Mycelial dry wt. g/l	Sugar consumed g/l	Citric acid g/l	% Yield of citric acid
1.	0.0	35.9	139.0	45.0	32.37
2.	0.5	17.30	120.0	8.0	6.66
3.	1.0	9.30	110.0	7.0	6.36
4.	1.5	Growth inhibited	Nil	Nil	Nil

Table 4. Effect of n-Butanol on mycelial dry weight sugar consumption and citric acid production by *A. niger* (E.U. 78-1).

S. No.	n-Butanol % v/v	Mycelial dry wt. g/l	Sugar consumed g/l	Citric acid g/l	% Yield of citric acid
1.	0.0	31.95	142.0	50.0	35.21
2.	0.5	32.0	140.0	60.0	42.85
3.	1.0	Growth inhibited	Nil	Nil	Nil
4.	1.5	Growth inhibited	Nil	Nil	Nil

Table 5. Effect of chloroform on mycelial dry weight sugar consumption and citric acid production by *A. niger* (E.U. 78-1).

pH: 5.0

S. No.	$CHCl_3$ % v/v	Mycelial dry wt. g/l	Sugar consumed g/l	Ctiric acid g/l	% Yield of citric acid
1.	0.0	36.5	142.0	51.0	35.91
2.	0.5	34.0	146.0	65.0	44.52
3.	1.0	33.25	147.5	76.5	51.5
4	1.5	33.65	145.5	60.0	41.23
5.	2.0	32.17	138.0	30.0	21.73
6.	2.5	25.62	132.0	28.0	21.21
7.	3.0	24.0	135.0	26.3	19.48
8.	3.5	23.7	134.0	25.5	18.88
9.	4.0	24.4	133.0	20.0	15.03

Table 6. Effect of Carbon tetrachloride on mycelial dry weight, sugar consumption and citric acid production by *A. niger* (E.U. 78-1).

pH: 5.0

S. No.	CCl_4 % v/v	Mycelial dry wt. g/l	Sugar consumed g/l	Citric acid g/l	% Yield of citric acid
1.	0.0	51.17	140.0	45.0	32.14
2.	0.5	42.74	138.0	33.0	23.91
3.	1.0	43.0	130.0	28.8	22.15
4.	1.5	42.0	131.0	26.0	21.37
5.	2.0	42.5	131.0	27.0	20.61
6.	2.5	36.3	139.0	22.0	15.82
7.	3.0	39.0	137.0	18.0	14.13
8.	3.5	42.0	136.0	16.0	11.76
9.	4.0	39.5	142.0	15.0	10.56

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