CHAPTER 6

METABOLIC CHANGES DURING THE PRODUCTION OF PECTINASES BY A. niger JU

It has been observed during studies on the effect of growth factors and metabolic inhibitors on the production of pectinases by A. niger JU that the yield of the enzyme is greatly enhanced by the presence of yeast extract and methylene blue. It seems to be of interest, therefore, to study the nitrogen and carbon metabolism of the organism in relation to other biochemical changes during the production of pectinases. In the present experiment, a study has been made on the utilization of galacturonic acid (free and combined) and different forms of nitrogen viz., amino nitrogen and total nitragen in relation to cellular growth and production of pectinases.

EXPERIMENTAL AND RESULTS

(i) Rate of Elaboration of Pectinases in Relation to pH, Galacturonic Acid (Free and Combined) Concentration and Cellular Growth

The medium employed for this investigation consisted of Pectin - 4%, Peptone - 1.5%, Yeast extract - 0.75%, K_2HPO_4 - 0.2%, $MgSO_4,7H_2O$ - 0.025%, KCl - 0.05%, Na_2SO_4 - 0.05%, $CaCl_2.2H_2O$ - 0.022%, $FeSO_4,7H_2O$ - 0.0005%,

$ZnSO_4,7H_2O$ - 0.0004%, $Na_2MoO_4,2H_2O$ - 0.001% and $CuSO_4.5H_2O$ - 0.0004%. The biochemistry of fermentation was studied in the above medium both in the absence and presence of 0.025 m M methylene blue, the most potent activator for the synthesis of pectinases. The medium was dispensed in 30 ml volumes in 100 ml Erlenmeyer flasks and inoculated with 0.5 ml of spore suspension containing 5×10^3 spores. After inoculation the flasks were incubated at 30^oC on a rotary shaker (150 r.p.m.) for different periods of time. The contents of the flasks were analyzed at regular intervals to indicate the change in medium composition in relation to pH, galacturonic acid (free and combined) concentration, cellular growth and concentration of pectinases. The galacturonic acid content of the medium was determined by the cysteine reaction method of Dische (106) which was as follows.

The fermentation broth was filtered to separate the cells. The filtered broth was then diluted 100 times with distilled water. To 0.5 ml of this diluted broth was added with shaking 4 c.c. of conc. H_2SO_4 and the solution was cooled in tap water. 0.1 c.c. of a 2.5% solution of cysteine hydrochloride was then added and the reaction mixture left at room temperature for 24 hrs. The intensity of the colour was then read at 540 mμ in a Zeiss Spectrophotometer against a reagent blank. The concentration of galacturonic acid was obtained from the standard curve prepared

with the spectrophotometer reading as the ordinate and concentrations of galacturonic acid as the abscissa.

The pH of the broth was determined by a Beckman glass electrode pH meter, the titrable acidity by simple titration with 0.1 N NaOH, concentration of pectinases and cellular growth by the usual methods as described before. The results are indicated in Tables 33 and 34.

TABLE 33

Rate of Elaboration of Pectinases by A. niger JU in Relation to pH, Acidity, Galacturonic Acid Utilization and Cellular Growth

Time (hours)	pH	Acidity (ml 0.1 N acid per 10 ml)	Cellular growth (gm/ litre)	Galacturonic acid concentration (free & combined) (mg/ml)	Concentration of pectinas (units/m
0	4.5	3.28	0.0	38.0	0.0
24	4.4	3.45	1.02	33.0	1.5
48	4.38	3.72	3.18	28.0	6.0
72	4.30	4.00	6.42	24.0	23.0
96	4.1	4.65	8.59	14.5	70.0
120	3.9	6.10	9.18	6.0	126.0
144	3.8	7.02	9.44	3.0	158.5
168	4.0	5.02	9.18	1.5	139.0

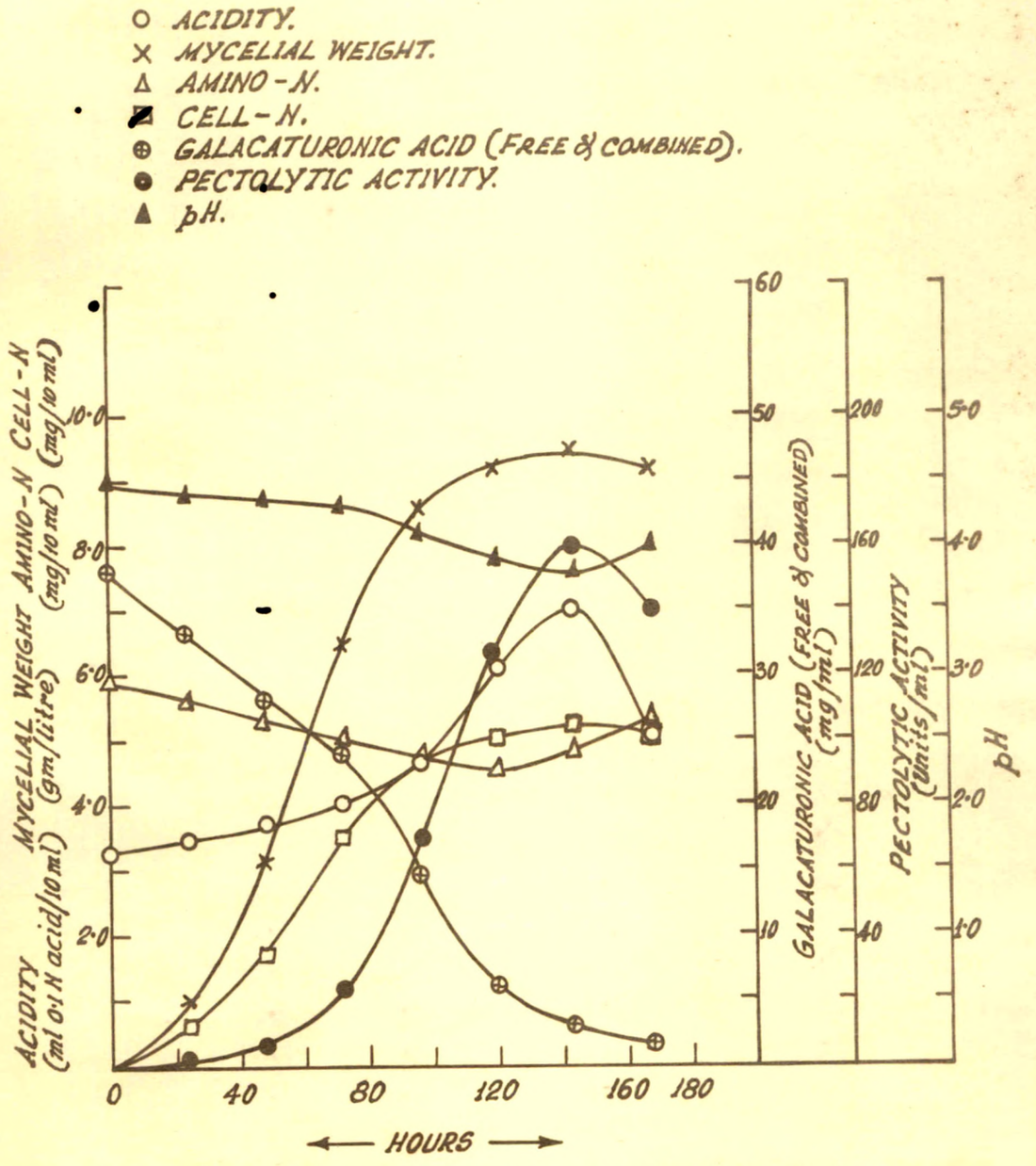

FIG. 1. METABOLIC CHANGES DURING PRODUCTION OF PECTINASES BY *A. niger* Ju.

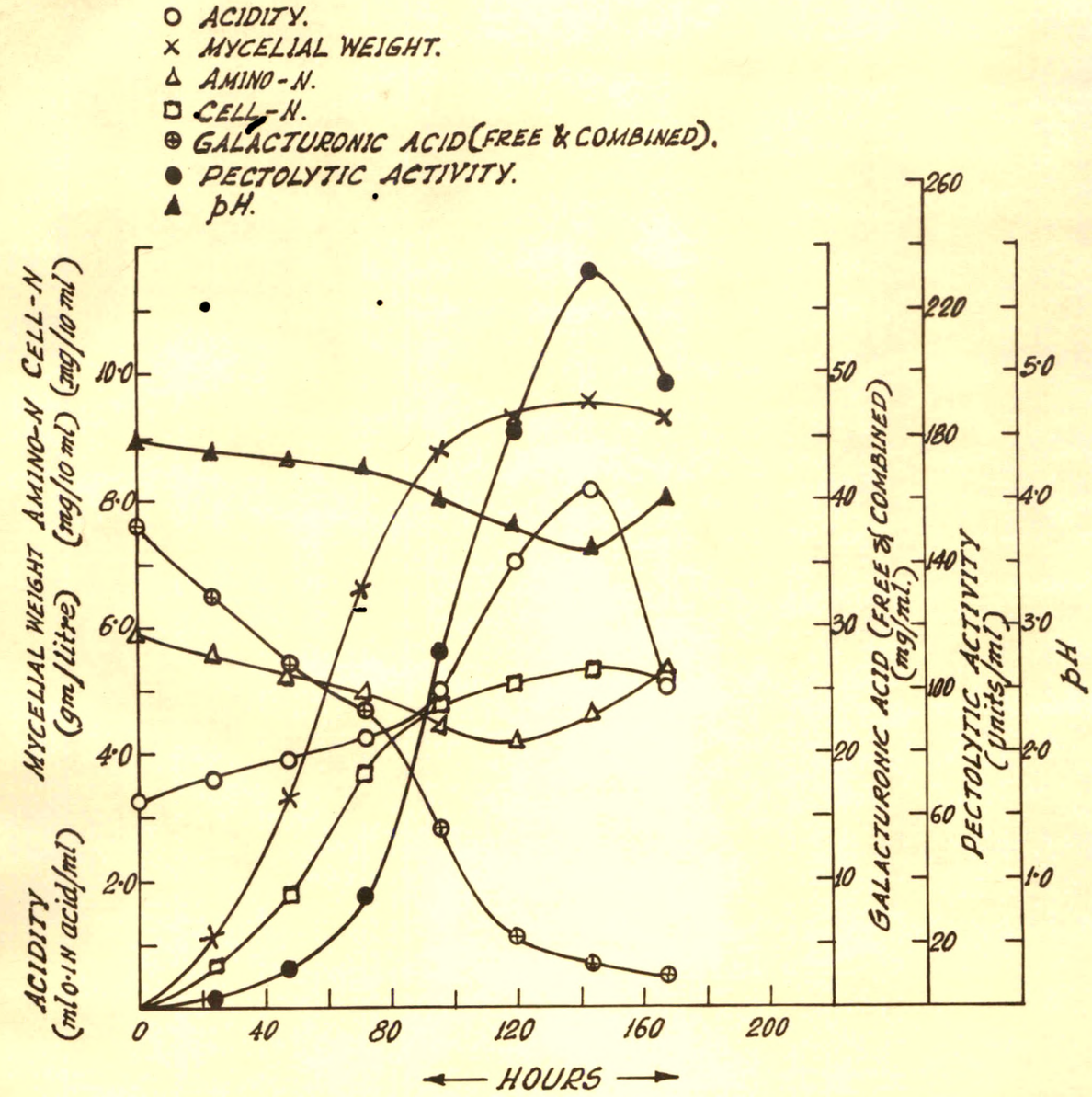

FIG. 2. EFFECT OF METHYLENE BLUE ON THE METABOLIC CHANGES DURING PRODUCTION OF PECTINASES BY *A. niger* Ju.

TABLE 34

Effect of Methylene Blue on the Rate of Elaboration of Pectinases by A. niger Ju in Relation to pH, Acidity, Galacturonic Acid Utilization and Cellular Growth

Time (hours)	pH	Acidity (ml 0.1 N acid per 10 ml)	Cellular growth (gm/ litre)	Galacturonic acid (free and combined) concentration (mg/ml)	Concentration of pectinases (units/ml)
0	4.50	3.28	0.0	38.0	0.0
24	4.38	3.60	1.13	32.5	3.0
48	4.32	3.92	3.33	27.0	12.0
72	4.25	4.23	6.60	23.5	35.0
96	4.00	5.00	8.80	14.0	112.0
120	3.80	7.02	9.31	5.5	182.0
144	3.60	8.13	9.57	3.5	231.0
168	4.00	5.00	9.30	2.5	196.0

It appears from Tables 33 and 34 (Figs. 1 and 2) that the rate of production of pectinases is extremely slow upto 48 hours of growth. The rates of production of pectinases, galacturonic acid utilization, acid production and cellular growth are, however, greatly accelerated between the period 48-144 hrs. As shown in Figs. 1 and 2, the utilization of galacturonic acid (free and combined) takes place at the maximum rate between the period 72 to 120 hrs when there is a rapid rise in the acid content and pectinase level of the

broth. The peak values of cellular growth and production of pectinases are attained on the 6th day of fermentation. However, the presence of the methylene blue (Table 34) greatly stimulates the formation of pectinases and organic acid in the broth.

(ii) Nitrogen Balance during the Production of Pectinases

The rate of change in the composition of the fermentation broth was determined in relation to its amino and total nitrogen content. Amino nitrogen of the broth was determined by Sorenson formol titration method (107) and total nitrogen by micro-Kjeldahl method (108). The cell nitrogen at a particular instant was calculated from the difference between the initial nitrogen of the broth and that left in the broth at that instant after removal of cells. The results are shown in Tables 35 and 36.

TABLE 35

Nitrogen Balance during Production of Pectinases by A. niger JU

Time (hours)	Amino nitrogen in broth (mg/10 ml)	Total nitrogen in broth (mg/10 ml)	Cell nitrogen (mg/10 ml)
0	5.90	27.2	-
24	5.60	26.6	0.6
48	5.30	25.5	1.7
72	5.05	23.7	3.5
96	4.74	22.5	4.7
120	4.51	22.2	5.0
144	4.83	22.0	5.2
168	5.33	22.2	5.2

TABLE 36

Effect of Methylene Blue on the Nitrogen Balance during Production of Pectinases by A. niger JU

Time (hours)	Amino nitrogen in broth (mg/10 ml)	Total nitrogen in broth (mg/10 ml)	Cell nitrogen (mg/10 ml)
0	5.90	27.20	-
24	5.55	26.5	0.7
48	5.24	25.4	1.8
72	4.98	23.5	3.7
96	4.47	22.4	4.8
120	4.19	22.1	5.1
144	4.63	21.9	5.3
168	5.23	22.1	5.1

It appears from the Tables 35 and 36 (Figs. 1 and 2) that during the phase of rapid cell synthesis and enzyme production (48-144 hrs), there is a steady fall in the amino and total nitrogen concentration in the broth upto the 5th day of fermentation. Methylene blue has got practically no effect on the rate of utilization of amino and total nitrogen from the broth.