CHAPTER 3

EFFECT OF CARBON AND NITROGEN SOURCES ON THE PRODUCTION OF PECTINASES BY ASPERGILLUS niger JU

The medium employed in the previous experiment for determining the optimum cultural conditions for the production of pectinases by A. niger JU, contained a mixture of sucrose and pectin as the carbon source and a mixture of NH_4NO_3 and $NaNO_3$ as the nitrogen source. Since the composition of the medium is a very important factor in determining the yield of a fermentation product, it was considered necessary to study the requirements of carbon and nitrogen compounds by the fungal strain for the production of pectolytic enzymes. In the present investigation, therefore, a study has been made to assess the effect of different carbon and nitrogen sources on the production of pectolytic enzymes by A. niger JU.

EXPERIMENTAL AND RESULTS

(i) Effect of Different Carbon Sources on the Production of Pectinases by A. niger JU

The culture of A. niger JU used for studying the production of pectinases was maintained on sucrose-pectin agar slants (64).

The effect of different carbon sources was studied in the basal medium consisting of NH_4NO_3 - 0.2%, $NaNO_3$ - 0.2% Na_2SO_4 - 0.05%, $MgSO_4,7H_2O$ - 0.05%, KCl - 0.05%, K_2HPO_4 - 0.1%, $FeSO_4,7H_2O$ - trace (pH 4.5). To this basal medium was added a given carbon source in 4 percent concentration or a combination of 2 carbon sources each in 2% concentration. The carbon compounds were sterilized separately and added to the medium just prior to inoculation. In a typical experiment, 30 ml of the medium taken in 100 ml Erlenmeyer flasks were inoculated with 0.5 ml of spore suspension containing 5×10^3 spores (preparation of inoculum are described in page 22 of this thesis). The flasks were then placed on a rotary shaker (150 r.p.m.), the temperature of incubation being kept at $30^{\circ}C$. The pectolytic activity of the broth and cellular growth were determined at different periods of fermentation by methods as described in Chapter 1 of this thesis. The results are shown in Table 8.

TABLE 8

Effect of Different Carbon Sources on the Production of Pectolytic Enzymes by *A. niger* JU and Cellular Growth

Carbon sources (4%)	Enzyme concentration (units/ml) on days			Mycelial weight (gm/litre) on days		
	6	7	8	6	7	8
Glucose	35	42	39	10.2	11.5	10.4
Fructose	28	36	33	8.7	8.81	8.73
Maltose	37	45	41	10.3	11.6	10.6
Sucrose	19	25	21	8.25	8.35	8.29
Starch	32.5	40	36	9.07	9.19	9.09
Dextrin	32	39	35	8.99	9.12	9.02
Sorbitol	12	18	16	7.90	8.01	7.93
Raffinose	10	15	13	7.85	7.95	7.88
Ribose	11.5	17	14	7.94	8.08	7.99
Xylose	10	16	13.5	7.94	8.06	7.98
Lactic acid	7	11	9.1	7.80	7.91	7.86
Glycerol	11.5	18	15.5	8.01	8.14	8.09
Pectin	49	60	56	6.97	7.07	7.00

TABLE 9

Effect of Different Carbon Sources in Combination on the Production of Pectolytic Enzymes by A. niger JU and Cellular Growth

Carbon sources (each in 2% concentration)	Enzyme concentration (units/ml) on days			Mycelial weight (gm/litre) on days		
	6	7	8	6	7	8
Glucose + Pectin	41	50	46	9.85	9.95	9.83
Fructose + Pectin	35	43	40	8.44	8.65	8.52
Maltose + Pectin	44	54	50	9.99	10.9	9.96
Sucrose + Pectin	26	38	35	8.08	8.20	8.08
Starch + Pectin	39.5	48	43	9.16	9.27	9.15
Dextrin + Pectin	36.3	45	40.5	9.12	9.24	9.13
Sorbitol + Pectin	18	24	21.2	7.95	8.06	7.95
Raffinose + Pectin	20	26	22.4	7.85	7.98	7.88
Ribose + Pectin	19	25	22	8.01	8.15	8.04
Xylose + Pectin	15	20	17.3	8.02	8.14	8.04
Lactic acid + Pectin	16.8	24.5	22.2	7.88	7.99	7.88
Glycerol + Pectin	18.6	25.5	22.8	8.10	8.21	8.12

Tables 8 and 9 show that out of the different carbon sources tested either singly or in combination, pectin favours maximum production of the pectolytic enzymes in the fermentation broth, although sugars like glucose and maltose allow

maximum development of mycelium. There is no correlation between the growth of the organism and the production of the enzymes. The carbon sources like maltose, glucose and starch also give considerable yields of the enzymes.

As a mixture of pectin and maltose was found to give excellent yield of the enzymes a study was next made to find out the effect of different combinations of the carbon sources on the production of pectinases. The enzyme activity of the broth and cellular growth were determined on the 7th day of fermentation and the results are shown in Table 10.

TABLE 10

Effect of Different Combinations of Maltose and Pectin on the Production of Pectolytic Enzymes by A. niger JU and Cellular growth

Carbon source		Enzyme activity (units/ml)	Mycelial weight (gm/litre)
Maltose (%)	Pectin (%)		
4	nil	45	10.50
3	1	50	10.90
2	2	54	10.80
1	3	56	8.60
nil	4	60	7.05

It will be evident from Table 10 that the concentration of the enzyme in the broth increases and cellular growth decreases as the level of pectin in the medium increases.

Studies were next made to find out the optimal level of pectin for the production of pectinases. Samples were assayed on the 7th day of fermentation. The results are shown in Table 11.

TABLE 11

Effect of Pectin Concentration on the Production of Pectolytic Enzymes by A. niger JU and Cellular Growth

Pectin (%)	Enzyme activity (units/ml)	Mycelial weight (gm/litre)
3	54.5	8.36
4	60.5	7.07
5	55.0	6.81
6	40.0	6.01

Table 11 indicates that a 4.0% concentration of pectin gives maximal yield of the pectinases.

(ii) Effect of Different Nitrogen Sources on the Production of Pectolytic Enzymes by A. niger JU

Different inorganic and organic nitrogen sources were examined for their effect on the production of pectinases. The medium (page 43 of this Chapter) after omission of $NaNO_3$ and NH_4NO_3 followed by supplementation with 4.0 percent pectin was used as the basal medium to which a given nitrogen source was added at the level of 102.8 mg nitrogen per 100 ml of medium. Pectin solution was autoclaved separately and added to the basal medium prior to inoculation. Oil cake and bran extracts were prepared by heating 100 gm of the material in 1000 ml of distilled water for 1 hour and then concentrating the extracts. The nitrogen level of concentrated extract was determined by micro-Kjeldahl method. Fermentation conditions and assay methods were the same as described before. The activity of the pectolytic enzymes produced and growth of the organism were recorded on the 5th, 6th, 7th and 8th day of fermentation. The results are shown in Table 12.

TABLE 12

Effect of Different Nitrogen Sources on the Production of Pectolytic Enzymes by A. niger and Cellular Growth

Nitrogen sources	Enzyme concentration (units/ml) on days				Mycelial weight (gm/litre) on days			
	5	6	7	8	5	6	7	8
Ammonium nitrate	50.0	65.0	75.0	70.0	6.80	7.67	7.78	7.66
Ammonium sulphate	32.0	44.0	53.0	50.0	6.71	7.58	7.70	7.60
Diammonium hydrogen phosphate	38.5	53.0	62.0	58.0	6.30	7.19	7.25	7.16
Ammonium acetate	12.0	23.0	30.0	27.0	6.17	6.98	7.07	7.00
Ammonium chloride	32.5	45.0	53.0	49.0	6.69	7.58	7.68	7.59
Ammonium tartarate	33.0	46.0	55.0	51.0	6.84	7.65	7.79	7.64
Urea	32.5	44.5	52.0	48.0	6.95	7.58	7.85	7.60
Sodium nitrate	37.0	40.0	55.0	50.0	6.74	7.49	7.65	7.50
Sodium nitrite	10.0	15.0	20.0	18.5	5.86	6.62	6.70	6.61
Gelatin	80.0	100.0	92.0	88.0	7.70	8.65	8.39	8.37
Casein	83.5	104.0	93.5	85.0	8.43	8.78	8.66	8.47
Peptone	85.0	109.0	95.0	89.0	8.63	8.89	8.74	8.45
Casamino acid	80.5	101.0	90.5	83.0	8.47	8.69	8.58	8.34
Aspartic acid	50.0	67.0	82.0	77.0	7.85	7.93	8.05	7.84

Table 12 (Contd.)

Nitrogen sources	Enzyme concentration (units/ml) on days				Mycelial weight (gm/litre) on days			
	5	6	7	8	5	6	7	8
Tryptone	56.0	75.0	64.0	54.0	7.82	8.01	7.91	7.80
Mustard seed cake extract	8.5	15.0	12.0	10.0	5.90	6.02	5.94	5.86
Yeast flour	32.0	45.0	37.0	30.0	7.13	7.31	7.22	7.12
Soy flour	49.0	64.0	55.0	46.0	7.49	7.68	7.51	7.39
Wheat bran extract	89.0	103.0	91.0	75.0	8.46	8.71	8.59	8.44
Rice bran extract	31.0	42.0	36.0	28.0	7.10	7.29	7.20	7.11
Corn steep liquor	30.0	40.0	35.0	27.0	7.03	7.21	7.14	7.08

Table 12 shows that organic nitrogen sources like peptone, casein, wheat bran and gelatin are superior to other nitrogen sources tested in giving higher yield of pectinases. Peptone appears to be the best of the sources of nitrogen tested for their effect on the production of the enzymes. Among inorganic nitrogen sources, ammonium nitrate is superior to others for the production of pectinases.

(iii) Effect of C/N Ratio on the Production of Pectinases by A. niger JU

As the carbon to nitrogen (C/N) ratio of the medium plays a very important role in the production of the enzymes by microorganisms, investigation was next made to find out the optimum C/N ratio of the medium for the enzyme production using pectin as the carbon source and peptone or ammonium nitrate as the nitrogen source. In this study, pectin was used in 4 percent concentration. The amount of nitrogen source was varied to attain the desired C/N ratios. Cellular growth and the enzyme activity of the broth with ammonium nitrate as the nitrogen source was determined on the 7th day of fermentation while those with peptone measured on the 6th day. The results are indicated in Table 13.

TABLE 13

Effect of C/N Ratio on the Production of Pectolytic Enzymes by A. niger JU and Cellular Growth

Nitrogen source	C/N ratio	Enzyme activity (units/ml)	Mycelial weight (gm/litre)	Final pH
Ammonium nitrate	27.0	70	7.85	4.5
	13.5	80	8.40	4.4
	10.0	88	8.65	4.9
	7.0	75	8.69	6.5
	4.0	50	8.70	6.7
	3.6	25	8.71	6.7
	2.7	20	8.69	6.7
	2.2	20	8.65	6.7

Table 13 (Contd.)

Nitrogen source	C/N ratio	Enzyme activity (units/ml)	Mycelial weight (gm/litre)	Final pH
Peptone	27.0	100	7.93	4.1
	13.5	106	8.80	4.1
	10.0	120	9.00	4.2
	7.5	110	8.99	4.3
	4.3	100	9.10	4.4
	3.6	97	9.19	4.4
	2.7	94	9.08	4.4
	2.2	93	9.06	4.4

It is quite evident from the results in Table 13 that higher yields of the pectolytic enzymes are recorded in the medium with the C/N ratio at 10 whether peptone or ammonium nitrate is used as the nitrogen source. Peptone, however, supports maximum production of the enzymes.