[J. Ferment. Technol., Vol. 49, No. 9. p. 759~770, 1971]

Fermentative Production of Pectinases By Fungi: Screening of Organisms and Production of the Enzymes by *Aspergillus niger*

S. K. Mukherjee and S. K. Majumdar

(Department of Food Technology and Biochemical Engineering, Jadavpur University, Jadavpur, Calcutta-32 India)

Abstract

During screening of fungi-producing pectic enzymes, a strain of *Aspergillus niger* JU has been selected as the most potent organism.

The optimum cultural conditions for the production of the enzymes by the organism were pH 4.5, period of incubation 7 days, temperature 30°C, and volume of inoculum, a 0.5 ml suspension, containing 5×10^3 spores per 30 ml medium.

The shake flask process was superior to the stationary culture process in giving a higher yield of the enzymes in a shorter period of time, indicating that adequate aeration is essential for optimal yield of the enzymes. Studies on the effect of carbon sources on the production of pectolytic enzymes by *A. niger* JU indicate that pectin at a level of 4 percent gave a maximal yield of the enzymes.

The organism, *A. niger* JU, utilized both inorganic and organic nitrogen sources for growth and enzyme production. Among the inorganic nitrogen sources, ammonium nitrate gave an optimal yield on the 7th day, while the maximal yield of the enzymes was obtained with peptone as the nitrogen source on the 6th day of fermentation. Organic nitrogen sources were, however, superior to inorganic nitrogen sources in giving a maximal yield of the enzymes.

The level of pectolytic enzymes in the fermentation broth reached a maximum value when the C/N ratio of the medium was 10.

The medium that allowed maximum development of mycelium did not necessarily give an optimal yield of the enzymes.

Introduction

The practical application and use of pectin-degrading enzymes in the fruit processing industry for the production and clarification of fruit juice dates back about forty years. Advantages of enzymatic treatment of fruit pulps and juices for extraction and clarification respectively, have been detailed by Reid,[1,2] Kertesz,[3] Tressler and Joslyn[4] Charley,[5] Sreekantiah *et al.*,[6] Joseph *et al.*,[7] and Yamasaki *et al.*[8] Deuel and Stutz[9] reported that pectolytic enzymes are produced by various plants and microorganisms, and also by certain animals. A great variety of strains of bacteria, yeasts, and molds are capable of producing pectolytic enzymes. Although considerable

studies have been made with the pectin-degrading enzymes from different sources, the enzyme preparations for use in the food industry are of fungal origin because the optimum pH of mould enzymes lies very near the pH of many fruits, which ranges from 3.0 to 5.5.

Extensive reviews by Demain and Phaff[10] and Endo[11] show that members of the genera Penicillium, and Aspergillus, and other fungi, like *Rhizopus tritici*, *Sclerotinia libertiana*, *Botrytis cenera*, *Fusarium moniliforme*, and *Coniothyrium diplodiella* produced pectolytic enzymes. In addition, many of the plant-pathogenic fungi and air-borne spores of Aspergillus species are capable of producing pectic enzymes as reported by Puvgi *et al.*[12] and Deuel and Stutz.[9] Details of the commercial production of pectolytic enzymes are rather scanty, but there are two examples in the B.I.O.S. report.[13] Limited information is available on the culture methods employed for the commercial production of pectic enzymes. Some of these are apparently produced on solid bran cultures.[14,15] Most laboratory studies have been made with either surface cultures or shake flasks.[16-18] According to Brooks and Reid,[16] *Aspergillus foetidus* produces both endopolygalacturonase and exopolygalacturonase in surface cultures, but only endopolygalacturonase in submerged cultures. The food technologists are concerned with the action of commercial enzyme preparations, which are complex mixtures of enzymes, on fruit juices and pulps, etc., that are mixtures of heterogenous substances.[19,20] The present investigation is, therefore, concentrated primarily with studying the fermentative production of the overall pectin-degrading enzymes by submerged growth in shake flasks.

The production of pectic enzymes by microorganisms is dependent to a large extent on the metabolic characteristics of the cultures. According to Kertesz,[21,22] a variation in the composition of the medium will cause a shift in the metabolic products produced by the organism, which have a profound effect on the rate of the enzymes. The effect of different carbon and nitrogen compounds on the production of pectolytic enzymes by fungi was studied by Fernando,[23] Willaman and Kertesz,[24] Phaff,[25] Puvgi *et al.*,[12] Gupta,[26] Chatterjee and Bose,[27] Tuttobello and Mill,[28] Sreekantiah *et al.*,[29] Yamasaki *et al.*,[30] and Moldabaeva.[31] Saito[17] studied the factors affecting the production of enzymes in shake flask cultures of *A. niger* and has reported that endopolygalacturonase is adaptive to the presence of pectic substances. Kantio[32] has reported that the pectolytic enzymes produced by *A. niger* are adaptive to the presence of pectin. Phaff and Demain[33] have demonstrated that both pectin esterase and polygalacturonase of *Penicillum chrysogenum* are induced by pectic materials, D-galacturonic acid, mucic acid, and L-galactonic acid. Attempts were first made to screen out molds from soils, decaying fruits, etc., for the selection of a potent organism giving a high yield of pectin-degrading enzymes and then to determine the effect of different cultural conditions on the production of the enzymes by *A. niger*. The effect of different carbon and nitrogen sources on the production of pectolytic enzymes by *A. niger* JU is also reported in this paper.

Materials and Methods

1. Selection of the organism Although the ability to produce pectolytic enzymes is widespread amongst microorganisms, it was necessary to screen them in order to obtain highly active strains. Organisms employed in our investigations were obtained from a wide variety of sources such as soils, rotten fruits, and other plant materials, and also from the culture collection of microorganisms maintained in the Department of Food Technology and Biochemical Engineering, Jadavpur University. Special attention was given to infected and rotten fruits, such as oranges, pears, guavas, apples, and bananas, whose high pectin content might be expected to encourage the development of pectolytic organisms. The majority of the organisms studied were molds belonging to the genera Aspergillus, Penicillium, and Rhizopus, all of which are well known as producers of pectolytic enzymes.

Rapid screening of organisms with pectolytic activity was done with modifications of the method of Tuttobello and Mill.[28] In this method, the organism was inoculated separately into test tubes containing 9 ml of 5 percent pectin in 2 percent malt extract at pH 4.5, whereas Tuttobello and Mill[28] employed a medium of 9 ml of 5 percent pectin in a 2 percent aqueous extract of ground nut meal at pH 4.2. In the presence of pectolytic strains, there is a reduction of the initial high viscosity and the turbid material in the medium gradually precipitates, leaving a clear zone at the top of the tube. This zone increases in size with time, and this increase is a function of the pectolytic activity of the organism.

After preliminary screening, two selected isolates were further studied by a shake flask method for the production of pectolytic enzymes in the sucrose-pectin medium (I) of Tuttobello and Mill,[28] consisting of 2% sucrose, 2% pectin, 0.2% NH_4NO_3, 0.2% $NaNO_3$, 0.05% Na_2SO_4, 0.5% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.1% K_2HPO_4, and a trace of $FeSO_4$ (pH 5.0) and in the Medium (II) of Yamasaki *et al.*,[30] consisting of 3% sucrose, 0.75% pectin, 0.58% $NaNO_3$, 0.5% ammonium tartarate, 0.003% yeast extract, 0.05% $MgSO_4 \cdot 7H_2O$, 0.5% KCl, and 0.1% K_2HPO_4 (pH 5.0). Thirty ml of the medium, placed in 100 ml Erlenmeyer flasks, was inoculated with 0.2 ml of a spore suspension containing 2×10^3 spores prepared from a well sporulated culture (5 days old) with distilled water. The flasks were then incubated on a rotary shaker (150 rpm) at 30°C for 7 days.

The culture of *A. niger*, selected as the most potent organism, was maintained on sucrose-pectin Medium (I). Inoculated slants were incubated at 30°C for 5 days and then stored in a refrigerator.

2. Various factors influencing the production of pectolytic enzymes The optimum conditions for the production of pectolytic enzymes by *A. niger* in sucrose-pectin Medium (I) were worked out by keeping all the factors constant except the one which was varied. The factors studied were (a) pH of the medium, (b) time period of incubation and method of cultivation, (c) temperature of fermentation, (d) aeration,

and (e) inoculum volume. Fermentation conditions were the same as described before. Growth was determined as the dry weight of mycelium. The mycelium was separated by filtration through a Buchner funnel, washed with water, and dried at 90°C overnight. The enzyme activity of the culture filtrate was determined by the method described below.

3. Assay of pectolytic enzymes The activity of the pectolytic enzymes was determined by measuring the reduction in the viscosity of a standard pectin solution at a given temperature, enzyme concentration, and reaction period as recommended by Kertesz,[3] Reid,[2] Charley,[5] Endo[34] and Tuttobello and Mill.[35] This method gives an appropriate estimation of the activities of a mixture of esterase, polymethylgalacturonases, and polygalacturonases. Throughout this work, one viscosity diminishing unit has been taken as that amount of enzyme which reduces the viscosity of 1 ml of a 1 percent solution of pectin by 50 percent in 10 minutes at pH 4.8 and 30°C.[35] Percent viscosity change was calculated from the equations of Roboz *et al.*,[36]

$$\text{Percent viscosity change} = \frac{V_0 - V_t}{V_0 - V_s} \times 100$$

where, V_0 = flow time in seconds of pectin and heat-inactivated enzyme,
V_t = flow time in seconds of pectin and activated enzyme at reaction time,
V_s = flow time in seconds of the buffer and inactivated enzyme.

4. Selection of suitable carbon and nitrogen sources The effects of different carbon and nitrogen sources were studied in the basal medium, consisting of 0.05% Na_2SO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.1% K_2HPO_4, and a trace of $FeSO_4 \cdot 7H_2O$ (pH 4.5). For studying the effect of different carbon sources on the production of pectinases, various carbon compounds were added in 4 percent concentrations to the basal medium containing NH_4NO_3 (0.2%) and $NaNO_3$ (0.2%), while the effect of different nitrogen sources was studied by adding a given source (nitrogen level at 102.8 mg N_2 per 100 ml) to the basal medium containing an optimum amount of the superior carbon source. Solutions of carbohydrates were autoclaved separately and added to the basal medium before inoculation. Thirty ml of the medium, placed in 100 ml Erlenmeyer flasks, was inoculated with 0.5 ml of spore suspension containing 5×10^3 spores. Samples were taken at intervals for the assay of enzymes and determination of cellular growth. Oil-cake and bran extracts were prepared by heating 200 mg of the material in 2,000 ml of distilled water at 100°C for 1 hour and then concentrating the extracts. The nitrogen content of the concentrated extracts added was determined by the micro-Kjeldahl method.

Results and Discussion

1. Selection of the potent organism During an initial survey on the production of pectolytic enzymes by fungi, as many as 60 isolates were tested. It was observed

from the preliminary screening that two strains of an *Aspergillus* species gave substantial yields of pectic enzymes. One of the cultures isolated from a soil sample of Jadavpur University campus was identified as a strain of *A. niger* JU. These two strains were therefore further investigated for selection. For these studies, fermentation experiments were conducted in two media: Medium (I) of Tuttobello and Mill[28] and Medium (II) of Yamasaki *et al.*[30] The results are given in Table 1.

Table 1. Production of pectolytic enzymes by the selected isolates.

Name of the culture	Enzyme activity (units/ml)		Mycelial weight (g/100 ml)	
	Medium I	Medium II	Medium I	Medium II
1. *A. niger* JU	28	22	0.812	0.799
2. *Aspergillus* sp.	21	10	0.780	0.771

Of the two isolates tested for the production of pectolytic enzymes, *A. niger* JU was found to give higher yields of the enzymes in Medium (I) of Tuttobello and Mill.[28] The culture was, therefore, studied to standardize the conditions of fermentation for the production of pectic enzymes in Medium (I) of Tuttobello and Mill.[28]

2. Effect of the initial pH of the medium on the production of pectolytic enzymes The initial pH of the medium was adjusted to 3, 3.5, 4.0, 4.5, 5.0, and 5.5 with 0.1 N hydrochloric acid or sodium hydroxide. Fermentation was carried out in 100 ml Erlenmeyer flasks, each containing 30 ml of medium, the flasks being placed on a rotary shaker (150 rpm) at a temperature of 30°C for 7 days. The resutls are shown in Table 2. The maximum yield of pectolytic enzymes was obtained when the initial pH of the medium was 4.5.

Table 2. Effect of the initial pH of the medium on the production of pectinases by *A. niger* and cellular growth.

Initial pH	Enzyme activity (units/ml)	Mycelial weight (g/100 ml)
3.0	16	0.718
3.5	20	0.762
4.0	22	0.768
4.5	30	0.819
5.0	29	0.802
5.5	21	0.759

3. Effect of the incubation period and method of cultivation on the production of pectolytic enzymes Fermentation was carried out with stationary and shake-flask methods in 100 ml flasks, each containing 30 ml of the medium (pH 4.5). The activity of the enzymes in the broth was determined after different periods of fermentation. The results are shown in Table 3.

It is evident from Table 3 that the shake culture process is superior to the

Table 3. Effect of the period of incubation on the production of pectinases by *A. niger* and cellular growth in both shake-flask and stationary cultures.

Period of incubation (days)	Shake-flask cultures		Stationary cultures	
	Activity (unit/ml)	Mycelial wt. (g/100 ml)	Activity (unit/ml)	Mycelial wt. (g/100 ml)
3	9	0.662	4	0.510
5	22	0.766	12	0.700
6	27	0.795	17	0.781
7	30	0.816	20	0.850
8	28	0.802	24	0.860
9	26	0.790	23	0.855

stationary process, as the former gave a higher yield of the enzymes in a shorter period of time (7 days), although the stationary process allows maximum development of the mycelium.

4. Effect of the temperature of fermentation on the production of pectolytic enzymes Fermentation was carried out with shake cultures at temperatures of 25°C, 30°C, and 37°C. The results are shown in Table 4.

Table 4. Effect of the temperature of fermentation on the production of pectolytic enzymes by *A. niger*. and cellular growth.

Temperature (°C)	Period of incubation (days)	Activity (unit/ml)	Mycelial weight (g/100 ml)
25	5	17	0.740
	7	23	0.774
	8	26	0.825
	9	24.5	0.815
30	5	22	0.764
	7	30	0.816
	8	28	0.800
	9	26	0.789
37	5	24	0.788
	6	27	0.805
	7	25	0.784
	8	21	0.760

A temperature of 30°C favoured the optimum production of the enzymes, although a lower or a higher temperature had no significant effect on the growth of the organism.

5. Effect of aeration on the production of pectolytic enzymes The effect of aeration was studied by placing different volumes of medium: 15 ml, 20 ml, 25 ml, 30 ml, 35 ml, 40 ml, and 45 ml, in 100 ml Erlenmeyer flasks which were incubated for 7 days at 30°C on a rotary shaker (150 rpm). The degree of aeration of the

fermentation broth in the shaker flasks was inversely proportional to the volume of liquid. The results are shown in Table 5.

Table 5 shows that a 100 ml flask containing 30 ml of medium gave a maximum yield of the enzymes, indicating that adequate aeration is essential for optimum production.

Table 5. Effect of aeration on the production of pectolytic enzymes by *A. niger* and cellular growth.

Volume of medium (ml)	15	20	25	30	35	40	45
Activity (unit/ml)	18	22	28	30.5	28	25	21
Mycelial weight (g/100 ml)	0.685	0.751	0.798	0.828	0.819	0.802	0.791

6. Effect of the inoculum volume on the production of pectolytic enzymes

In all the previous experiments for the determination of optimum conditions, 0.2 ml of spore suspension, containing 2×10^3 spores, was used as the inoculum. In the present experiment, different volumes of spore suspension were used to inoculate 30 ml of medium in 100 ml Erlenmeyer flasks placed on a rotary shaker (150 rpm). After 7 days of fermentation, the enzyme yield was determined; the results are shown in Table 6.

The optimal volume of inoculum was 0.5 ml, containing 5×10^3 spores for 30 ml medium.

Table 6. Effect of the inoculum volume on the production of pectolytic enzymes by *A. niger* and cellular growth.

Inoculum volume (ml)	Enzyme activity (unit/ml)	Mycelial weight (g/100 ml)
0.1	27	0.799
0.5	32	0.829
1.0	32	0.834
1.5	30	0.845
2.0	30	0.849

7. Effect of different carbon sources on the production of pectolytic enzymes

The effect of different carbon sources, either singly or in combination, on the production of pectolytic enzymes and the growth of the organism was determined after 6, 7, and 8 days of fermentation and the results are shown in Tables 7 and 8.

Tables 7 and 8 show that, out of the different carbon sources tested either singly or in combination, pectin gave the maximum yield of the pectolytic enzymes in the fermentation broth, while maltose, glucose, and starch were suitable carbon sources. A mixture of pectin and maltose was also found to give excellent production of the enzymes. There was, however, no correlation between the growth of the organism and the production of the enzymes.

Table 7. Effect of different carbon sources on the production of pectolytic enzymes by *A. niger* and cellular growth.

Carbon sources (4%)	Enzyme concentration (units/ml)			Mycelium weight (g/100 ml)		
	6 days	7 days	8 days	6 days	7 days	8 days
Glucose	35	42	39	1.02	1.15	1.04
Fructose	28	36	33	0.87	0.881	0.873
Maltose	37	45	41	1.03	1.160	1.106
Sucrose	19	25	21	0.825	0.835	0.829
Starch	32.5	40	36	0.907	0.919	0.909
Dextrin	32	39	35	0.899	0.912	0 902
Sorbitol	12	18	16	0.790	0.801	0.793
Raffinose	10	15	13	0.785	0.795	0.788
Ribose	11.5	17	14	0.794	0.808	0.799
Xylose	10	16	13.5	0.794	0.806	0.798
Lactic acid	7	11	9.1	0.780	0.791	0.786
Glycerol	11.5	18	15.5	0.801	0.814	0.809
Pectin	49	60	56	0.697	0.707	0.700

Table 8. Effect of different carbon sources in combination on the production of pectolytic enzymes by *A. niger* and cellular growth.

Carbon sources (each in 2% concentration)	Enzyme concentration (units/ml)			Mycelium weight (g/100 ml)		
	6 days	7 days	8 days	6 days	7 days	8 days
Glucose+Pectin	41	50	46	0.985	0.995	0.983
Fructose+Pectin	35	43	40	0.844	0.865	0.852
Maltose+Pectin	44	54	50	0.999	1.090	0.996
Sucrose+Pectin	26	38	35	0.808	0.820	0.808
Starch+Pectin	39.5	48	43	0.916	0.927	0.915
Dextrin+Pectin	36.3	45	40.5	0.912	0.924	0.913
Sorbitol+Pectin	18	24	21.2	0.795	0.806	0.795
Raffinose+Pectin	20	26	22.4	0.785	0.798	0.788
Ribose+Pectin	19	25	22	0.801	0.815	0.804
Xylose+Pectin	15	20	17.3	0.802	0.814	0.804
Lactic acid+Pectin	16.8	24.5	22.2	0.788	0.799	0.788
Glycerol+Pectin	18.6	25.5	22.8	0.810	0.821	0.812

The results of the effect of a mixture of pectin and maltose in different combinations on the production of pectinases are shown in Table 9. The enzyme activity of the broth was determined on the 7th day of fermentation.

It will be evident from Table 9 that the concentration of the enzyme in the broth increased as the level of pectin in the medium increased.

Studies were next made to determine 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 10.

Table 10 indicates that the optimal level of pectin in the medium for the

Table 9. Effect of different combinations of maltose and pection on the production of pectolytic enzymes by *A. niger* and cellular growth.

Carbon source		Enzyme activity (units/ml)	Mycelial weight (g/100 ml)
Maltose (%)	Pectin (%)		
4	nil	45	1.05
3	1	50	1.09
2	2	54	1.08
1	3	56	0.860
nil	4	60	0.705

Table 10. Effect of pectin concentration on the production of pectolytic enzymes by *A. niger* and cellular growth.

Pectin (%)	Enzyme activity (units/ml)	Mycelial weight (g/100 ml)
3	54.5	0.836
4	60.5	0.707
5	55.0	0.681
6	40.0	0.601

production of pectolytic enzymes by *A. niger* was 4 percent.

The pectin-degrading enzymes of *A. niger* JU seem to be adaptive, because the production of the enzymes was increaed remarkably in the presence of pectin. This observation confirms the results concerning endopolygalacturonase production with *A. niger* as reported by Tuttobello *et al.*[28] and Saito *et al.*[37] Increased activity of pectolytic enzymes may, however, be partly dependent on the induced synthesis of exopolygalacturonase as reported by Saito *et al.*[37] The culture, *A. niger* JU, shows a difference in its biochemical characteristics from the culture of Tuttobello and Mill,[28] as the former gave a maximal yield of the enzyme in the presence of pectin as the carbon source, while the latter gave an optimal yield in the presence of a mixture of sucrose and pectin.

8. Effect of different nitrogen sources on the production of pectolytic enzymes

Different nitrogen sources, including both inorganic and organic compounds, were examined in these studies. To the basal medium containing 4 percent pectin as the carbon source were added different nitrogen sources (nitrogen level at 102.8 mg N_2 per 100 ml medium). Fermentation conditions and the assay methods were the same as described previously. The activity of the pectolytic enzymes produced and the growth of the organism were recorded on the 5th, 6th, 7th, and 8th days of fermentation. The results are shown in Table 11.

Table 11 shows that organic nitrogen sources like peptone, casein, and wheat bran were superior to inorganic sources in giving higher yields of the enzymes in a shorter period of time. Of the organic nitrogen sources tested, peptone gave the maximal yield of the enzymes on the 6th day of fermentation. Among inorganic

Table 11. Effect of different nitrogen sources on the production of pectolytic enzymes by *A. niger* and cellular growth.

Nitrogen sources	Enzyme concentration (units/ml)				Mycelial weight (g/100 ml)			
	5 days	6 days	7 days	8 days	5 days	6 days	7 days	8 days
Ammonium nitrate	50.0	65.0	75.0	70.0	0.680	0.767	0.778	0.766
Ammonium sulfate	32.0	44.0	53.0	50.0	0.671	0.758	0.770	0.760
Diammonium hydrogen phosphate	38.5	53.0	62.0	58.0	0.630	0.719	0.725	0.716
Ammonium acetate	12.0	23.0	30.0	27.0	0.617	0.698	0.707	0.700
Ammonium chloride	32.5	45.0	53.0	49.0	0.669	0.758	0.768	0.759
Ammonium tartrate	33.0	46.0	55.0	51.0	0.684	0.765	0.779	0.764
Urea	32.5	44.5	52.0	48.0	0.695	0.758	0.785	0.760
Sodium nitrate	37.0	40.0	55.0	50.0	0.674	0.749	0.765	0.750
Sodium nitrite	10.0	15.0	20.0	18.5	0.586	0.662	0.670	0.661
Gelatin	80.0	100.0	88.0	92.0	0.770	0.837	0.865	0.839
Casein	83.5	104.0	93.5	85.0	0.843	0.878	0.866	0.847
Peptone	85.0	109.0	95.0	89.0	0.863	0.889	0.874	0.845
Casamino acid	80.5	101.0	90.5	83.0	0.847	0.869	0.858	0.834
Aspartic acid	50.0	67.0	82.0	77.0	0.785	0.793	0.805	0.784
Tryptone	56.0	75.0	64.0	54.0	0.782	0.801	0.791	0.780
Mustard seed cake extract	8.5	15.0	12.0	10.0	0.590	0.602	0.594	0.586
Yeast flour	32.0	45.0	37.0	30.0	0.713	0.731	0.722	0.712
Soy bean flour	49.0	64.0	55.0	46.0	0.749	0.768	0.751	0.739
Wheat bran extract	89.0	103.0	91.0	75.0	0.846	0.871	0.859	0.844
Rice bran extract	31.0	42.0	36.0	28.0	0.710	0.729	0.720	0.711
Corn steep liquor	30.0	40.0	35.0	27.0	0.703	0.721	0.714	0.708

nitrogen sources, ammonium nitrate was superior to the others tested in giving maximal production on the 7th day of fermentation.

9. Effect of the C/N ratio on the production of the pectolytic enzymes

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 determine the optimum C/N ratio of the medium for enzyme production using pectin as the carbon source and peptone or ammonium nitrate as the nitrogen source. In this study, pectin was used in a 4 percent concentration. The amount of the nitrogen source was varied to attain the desired C/N ratios. The enzyme activity of the broth with ammonium nitrate as the nitrogen source was determined on the 7th day of fermentation, while that with peptone was measured on the 6th day. The results are indicated in Table 12.

It is quite evident from the results that the maximum production of pectolytic enzymes was obtained in the medium with a C/N ratio of 10 whether peptone or ammonium nitrate was used as the nitrogen source. The maximum yield of the pectin-degrading enzymes was, however, obtained in the medium containing peptone. Tuttobello and Mill[28] also reported on the maximum production of endopolygalac-

Table 12. Effect of the C/N ratio on the production of pectolytic enzymes by *A. niger* and cellular growth.

Nitrogen source	C/N ratio	Enzyme activity (units/ml)	Mycelial weight (g/100 ml)	Final pH
Ammonium nitrate	27.0	70	0.785	4.5
	13.5	80	0.840	4.4
	10.0	88	0.865	4.9
	7.0	75	0.869	6.5
	4.0	50	0.870	6.7
	3.6	25	0.871	6.7
	2.7	20	0.869	6.7
	2.2	20	0.865	6.7
Peptone	27.0	100	0.793	4.1
	13.5	106	0.880	4.1
	10.0	120	0.900	4.2
	7.5	110	0.899	4.3
	4.3	100	0.910	4.4
	3.6	97	0.919	4.4
	2.7	94	0.908	4.4
	2.2	93	0.906	4.4

turonase by *A. niger* in a medium containing organic nitrogen sources. On the contrary, organic nitrogenous sources were found to repress the production of endo-polygalacturonase by *Aspergillus saitoi* as reported by Yamasaki *et al.*[30] and by *Aspergillus aureus* as reported by Sreekantiah *et al.*[29] The present investigation has shown that it is possible to increase the yield of pectolytic enzymes appreciably by maintaining a proper carbon-nitrogen balance in the medium.

Acknowledgement

We wish to thank the University Grants Commission for their financial assistance. Thanks are also due to Dr. A. N. Bose, Head of the Department of Food Technology and Biochemical Engineering, Jadavpur University, for his valuable encouragement during the course of these studies.

References

1) Reid, W. W.: *Recent Advances in Fruit Products Industry*, 145, Comm. Bur. Hort. Plantation Corps (1960).
2) Reid, W. W.: *Production and Application of Enzyme Preparations in Food Manufacture*, 35, Soc. Chem. Ind., London, (1961).
3) Kertesz, Z. I.: *The Pectic Substances*, 628, Interscience Publishers, Inc., N. Y. (1951).
4) Tressler, D. K., Joslyn, M. A.: *Fruit and Vegetable Juice Processing Technology*, Avi Publishing Co., Inc. (1960).
5) Charley, V. L. S.: *Production and Application of Enzyme Preparations in Food Manufacture*, 107, Soc. Chem. Ind., London, (1961).
6) Sreekantiah, K. R., Shastry, M. C. S., Johar, D. S., Ramachandra Rao, T. N., Bhatnagar, H. C.: *Food Science*, **12**, 364 (1963).
7) Joseph, R., Sreekantiah, K. R., Johar, D. S.: *Food Sci.*, **12**, 369 (1963).
8) Yamasaki, M., Kato, A., Chu, S. Y., Arima, K.: *Agr. Biol. Chem.*, **31**, 552 (1967).

9) Deuel, H., Stutz, E.: *Advances in Enzymology*, **20**, 341 (1958).
10) Demain, A. L., Phaff, H. J.: *Wallerstein Lab. Commun.*, **20**, 119 (1957).
11) Endo, A.: *Agr. Biol. Chem.*, **25**, 394 (1961).
12) Pavgi, M. S., Pithawala, M. P., Savoor, G. R., Sreenivasan, A.: *Proc. Ind. Acad. Sci.*, **34**, Sec. B, 33 (1951).
13) B.I.O.S. Final Report 275, Item 22 (London: H.M.S.O.).
14) Swangard, W. M.: *The Pectic Substances* (Z. I. Kertesz), 378, N. Y. Interscience Publishers, Inc. (1951).
15) Sreekantiah, K. R., Johar, D. S.: *Food Sci.*, **12**, 358 (1963).
16) Brooks, J., Reid, W. W.: *Chem. and Ind.*, 325 (1955).
17) Saito, H.: *J. Gen. Appl. Microbiol.*, **1**, 38 (1955).
18) Takehana, H., Ogura, N.: *J. Agr. Chem. Soc., Japan*, **29**, 83 (1955).
19) Reid, W. W.: *J. Sci. Food Agr.*, **1**, 234 (1950).
20) Dingle, J., Solomons, G. L.: *J. Appl. Chem.*, **2**, 395 (1952).
21) Kertesz, Z. I.: *Fermeniforsch*, **9**, 300 (1928).
22) Kertesz, Z. I.: *Fermeniforsch*, **10**, 36 (1928).
23) Fernando, M.: *Ann. Bot. (NS)*, **1**, 727 (1937).
24) Willaman, J. J., Kertesz, Z. I.: *New York State Agric. Expt. Stn. Tech. Bull.*, 178 (1931).
25) Phaff, H. J.: *Arch. Biochem.*, **13**, 67 (1947).
26) Gupta, S. C.: *Ann. Bot. (NS)*, **20**, 179 (1956).
27) Chatterjee, B. C., Bose, A. N.: *J. Sci. Ind. Res.*, **190**, 162 (1960).
28) Tuttobello R., Mill, P. J.: *Biochem. J.*: **79**, 51 (1961).
29) Sreekantiah, K. R., Shah, V. K., Johar, D. S.: *Food Sci.*, **12**, 353 (1963).
30) Yamasaki, M., Yasui, T., Arima, K.: *Agr. Biol. Chem.*, **30**, 142 (1966).
31) Moldabaeva, R. K.: *Mikrobiologiya*, **37**, 674 (1968).
32) Kantio, P.: *Chem. Abstr.*, **46**, 1027b ((1950).
33) Phaff, H. J., Demain, A. L.: *Wallerstein Lab. Commun.*, **20**, 119 (1957).
34) Endo, A.: *Agr. Biol. Chem.*, **25**, 389 (1961).
35) Tuttobello, R., Mill, P. J.: *Biochem. J.*, **79**, 57 (1961).
36) Roboz, E. R., Barrat, R. M., Tatum, E.: *J. Biol. Chem.*, **195**, 459 (1952).
37) Saito, H., Minoda, Y., Marumo, H.: *J. Agr. Chem. Soc., Japan*, **28**, 810 (1954).

(Received May 14, 1971)