# CHAPTER 2

## DETERMINATION OF OPTIMUM CULTURAL CONDITIONS FOR THE PRODUCTION OF PECTINASES BY *ASPERGILLUS niger* JU

During an initial survey on the production of pectinases by fungi, it was observed that the isolate $A_{45}$ (identified as a strain of *Aspergillus niger*) gives moderate yield of pectolytic enzymes in a synthetic medium containing a mixture of sucrose (2%) and pectin (2%) as the carbon source and a mixture of $NaNO_3$ (0.2%), $(NH_4)_2NO_3$ (0.2%) as the nitrogen source. Since the yield of a fermentation product is dependent among other factors on the physiological conditions of fermentation and these again on the nature of the strain under investigation, it was considered desirable to standardize the conditions of fermentation for the production of pectolytic enzymes by the selected strain of *Aspergillus niger* JU. The present work, therefore, seeks to study in detail the various cultural conditions affecting the production of pectinases by the strain of *A. niger* JU.

## EXPERIMENTAL AND RESULTS

### Effects of Various Factors Influencing the Production of Pectinases

The optimum conditions for the production of pectolytic enzymes by *A. niger* JU in sucrose-pectin medium were

worked out by keeping all the factors constant except the one which was varied within reasonable limits. The factors studied were (1) pH of the medium, (2) time period of incubation and method of cultivation, (3) temperature of fermentation, (4) aeration and (5) inoculum volume. The sucrose-pectin medium used for the production of pectinases consisted of : Sucrose - 2%, Pectin - 2%, $NH_4NO_3$ - 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 - 5.0. The stock culture of A. niger JU was maintained on slants of same sucrose-pectin medium containing 3% agar (pH 5.0).

In a typical experiment, 30 ml of the fermentation medium were taken in 100 ml Erlenmeyer flasks and inoculated with 0.2 ml of spore suspension containing 2 x $10^3$ spores (preparation of inoculum as described in page 22 ) and the whole thing incubated on a rotary shaker (150 r.p.m.) at a given temperature. At the end of the fermentation, the pectolytic activity of the filtered broth and the growth of the organism were determined by the same methods as described in Chapter I of this thesis.

(i) Effect of Initial pH of the Medium on the Production of Pectinases

The optimum pH for the production of pectolytic enzymes was determined by carrying out the fermentation experiments at different pH values (initial). For this, the initial pH of the medium was adjusted to 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5 with dilute hydrochloric acid or sodium hydroxide. After 7 days of incubation on a rotary shaker (150 r.p.m.) at 30°C the pectolytic activity of the filtered broth and cellular growth were determined and the results are shown in Table 3.

TABLE 3

Effect of Initial pH of the Medium on the Production of Pectinases by A. niger JU and Cellular Growth

| pH | Enzyme activity (units/ml) | Mycelial weight (gm/litre) |
|---|---|---|
| 3.0 | 16 | 7.18 |
| 3.5 | 20 | 7.62 |
| 4.0 | 22 | 7.68 |
| 4.5 | 30 | 8.19 |
| 5.0 | 29 | 8.02 |
| 5.5 | 21 | 7.59 |

Table 3 indicates that the optimal pH (initial) of the medium for cellular growth and for the production of pectolytic enzymes is 4.5, while lower yields of the pectolytic enzymes are obtained at lower or higher pH values.

(ii) Effect of Incubation Period and Method of Cultivation on the Production of Pectolytic Enzymes

The optimum period of incubation was next determined by carrying out the fermentation under stationary and shake flask methods in 100 ml Erlenmeyer flasks each containing 30 ml of the medium (pH 4.5). In the shake flask method, the flasks were placed on a rotary shaker working at a speed of 150 rev./min. Incubation temperature was kept at 30°C in all cases. The activity of the enzymes in the broth and the cellular growth were determined at different periods of fermentation. The results are indicated in Table 4.

TABLE 4

Effect of Period of Incubation on the Production of Pectinases by A. niger JU and Cellular Growth in Shake Flask and Stationary Cultures

| Period of incubation (days) | Shake Flask Cultures | | Stationary Cultures | |
|---|---|---|---|---|
| | Activity (units/ml) | Mycelial wt (gm/litre) | Activity (units/ml) | Mycelial wt (gm/litre) |
| 3 | 9 | 6.62 | 4 | 5.10 |
| 5 | 22 | 7.66 | 12 | 7.00 |
| 6 | 27 | 7.95 | 17 | 7.81 |
| 7 | 30 | 8.16 | 20 | 8.50 |
| 8 | 28 | 8.02 | 24 | 8.60 |
| 9 | 26 | 7.90 | 23 | 8.55 |

It will be evident from the Table 4 that shake flask process is superior to the stationary process as the former gives higher yield of the enzymes in a shorter period of time (7 days) although the stationary process allows maximum development of the mycelium on the 8th day of fermentation.

(iii) Effect of Temperature of Fermentation on the Production of Pectolytic Enzymes

Fermentation was carried out under shake culture process at temperatures of 25°C, 30°C and 37°C. The pectolytic activity of the broth and cellular growth were determined at different periods of fermentation. The results are shown in Table 5.

TABLE 5

Effect of Temperature of Fermentation on the Production of Pectolytic Enzymes by A. niger JU and Cellular Growth

| Temperature of incubation (°C) | Period of incubation (days) | Activity (units/ml) | Mycelial weight (gm/litre) |
|---|---|---|---|
| 25 | 5 | 17 | 7.40 |
| | 7 | 23 | 7.74 |
| | 8 | 26 | 8.25 |
| | 9 | 24.5 | 8.15 |
| 30 | 5 | 22 | 7.64 |
| | 7 | 30 | 8.16 |
| | 8 | 28 | 8.00 |
| | 9 | 26 | 7.89 |
| 37 | 5 | 24 | 7.88 |
| | 6 | 27 | 8.05 |
| | 7 | 25 | 7.84 |
| | 8 | 21 | 7.60 |

It appears from Table 5 that a temperature of 30°C favours the optimum production of the enzymes.

(iv) Effect of Aeration on the Production of Pectolytic Enzymes

The effect of aeration was studied by taking different volumes of medium in 100 ml Erlenmeyer flasks which were incubated for 7 days at 30°C on a rotary shaker (150 r.p.m.), as the degree of aeration of the fermentation broth in shaker flasks is inversely proportional to the volume of liquid. The results are shown in Table 6.

TABLE 6

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

| Volume of medium (ml) | Activity (units/ml) | Mycelial Weight (gm/litre) |
|---|---|---|
| 15 | 18 | 6.85 |
| 20 | 22 | 7.51 |
| 25 | 28 | 7.98 |
| 30 | 30.5 | 8.28 |
| 35 | 28 | 8.19 |
| 40 | 25 | 8.02 |
| 45 | 21 | 7.91 |

It will be evident from the results in Table 6 that 100 ml flask containing 30 ml of medium gives maximum yield of the pectolytic enzymes indicating that adequate aeration is essential for optimum production.

(v) Effect of Inoculum Volume on the Production of the Pectolytic Enzymes

In all the previous experiments for determination of optimum conditions, 0.2 ml of spore suspension containing $2 \times 10^3$ 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 r.p.m.). After 7 days of fermentation at 30°C, the enzyme yield was determined and the results are shown in Table 7.

TABLE 7

Effect of Inoculum Volume on the Production of Pectolytic Enzymes by *A. niger* JU and Cellular Growth

| Inoculum volume (ml) | Enzyme activity (units/ml) | Mycelial weight (gm/litre) |
|---|---|---|
| 0.1 | 27.0 | 7.99 |
| 0.2 | 30.4 | 8.20 |
| 0.5 | 32.0 | 8.29 |
| 1.0 | 32.0 | 8.34 |
| 1.5 | 30.0 | 8.45 |
| 2.0 | 30.0 | 8.49 |

It appears from Table 7 that the optimal volume of inoculum is 0.5 ml (containing 5 x $10^3$ spores) for 30 ml medium. Thereafter an increase in the volume of inoculum added lowers the yield of pectolytic enzymes.