CHAPTER V

BIOCHEMICAL CHANGES IN MANNITOL-PEPTONE-YEAST EXTRACT MEDIUM DURING THE GROWTH OF A. CAMPESTRIS S₁₂

The rate of utilization of sugar, nitrogen sources, phosphates etc. by an organism from the growth medium could be calculated from the estimates of these compounds in the medium at different stages of growth. The growth of the microorganism as well as quantitative and qualitative yield of the desired product are dependent on the nutrient utilized and physicochemical environment in the medium. So the studies on the biochemical changes occurring in the medium during the fermentation process is essential before the large scale production methods are standardized.

Reusser, Spencer and Sallans (41) showed that high medium nitrogen concentration favoured protein production and reduced nitrogen concentration induced fat production in T. nudum. Humfeld and Sugihara (50) showed that growth of A. campestris occurs even at small concentration of nitrogen in the medium, but the nitrogen deficiency of the medium is reflected in protein content of the mycelium produced. Litchfield, Overbeck and Davidson (39) showed that C:N ratio influences the yield of morel mushroom. Litchfield and Overbeck (38) also showed that the addition of 0.05% KH₂PO₄ to the medium gave improved yield.

It has been observed during studies on nutritional
requirements of A. campestris S₁₂, that the yield and protein content of the mycelium is dependent on medium carbon and nitrogen sources. Mannitol and peptone have been found to be best carbon and nitrogen sources respectively. In the present chapter, the results of the study on the utilization of mannitol, phosphorus and different forms of nitrogen in relation to cellular growth of A. campestris S₁₂ are reported.

**Experimental**

**Medium**

For the study of biochemical changes, fermentation was carried out in the medium having following composition:

- Mannitol - 20 gms
- Peptone - 10 gms
- KCl - 0.5 gm
- MgSO₄, 7H₂O - 0.5 gm
- KH₂PO₄ - 1.0 gm
- FeSO₄, 7H₂O - Trace
- Yeast extract - 2 gms
- Water - 1000 ml

The 50 ml portions of the medium were distributed in 250 ml Erlenmeyer flasks and heat sterilized.
Cultivation of the cells

The medium was inoculated with approximately equal amount of mycelia of 7 days old culture. The preparation of inoculum is described in Chapter II. The flasks were incubated at room temperature on a rotary shaker.

Analytical methods

The growth of the fungus and nitrogen was estimated as described in Chapter I. The chemical estimation of mannitol is described in Chapter III. Inorganic phosphorus was measured by conventional method of Fiske and Subba Row (104) and pH, by a Beckman glass electrode pH meter. The acid production was measured by titration with 0.01 N NaOH using phenolphthalein as internal indicator. Amino and ammonia-nitrogen were determined by Sorensen's formol titration (105) procedure and the aeration method of VanSlyke (106) respectively.

Results

Fig. 7 represents the growth pattern of A. camnestris S12 in mannitol-peptone-yeast extract medium. The mycelial growth starts with one day lag after inoculation and reaches a maximum on 7th day. Exponential rate of growth is maintained for about 4 days i.e. from 2nd day to 6th day of inoculation. The pH of the broth rises during the first two days of fermentation, the rise during 5th day onward is slow. In between 3rd and 4th day,
FIG. 7: GROWTH OF *A. CAMPESTRI*S 12 IX MANNITOL-PEPTONE-YEAST EXTRACT MEDIUM
there is appreciable drop in medium pH values. Similar fluctuation is recorded for titratable acidity, when pH rises titratable acidity decreases and vice versa (Fig. 8). The change in mannitol concentration during the entire period is marked by a steady fall from 20 gm/l to 0.867 gm/l. The rate of mannitol utilization is greatly accelerated from 3rd day. Medium inorganic phosphorus concentration steadily falls till maximum mycelial yield is attained. A slight rise in the concentration of inorganic phosphate after the exponential growth phase may be due to autolysis of the mycelia.

Amino nitrogen concentration rises during the initial lag period (Fig. 9). This may be due to hydrolysis of the peptides present in the medium. But during entire exponential phase there is slow but steady disappearance of amino nitrogen from the medium. Total nitrogen concentration of the broth falls till maximum mycelial growth attains. Lysis of the cell may contribute to the rise in amino and total nitrogen after 7th day of inoculation. The cell nitrogen during the entire period is marked by a gradual fall from 8.32 mg/100 mg dry wt. to 6.4 mg/100 mg dry wt. Ammonia nitrogen of the broth increases steadily for the first two days, then decreases till the organism attains maximum growth after that it increases sharply.
FIG. 8: CARBON, PHOSPHORUS AND ORGANIC ACID UTILIZATION DURING A. CAMPESTRE S12 FERMENTATION

(SYMBOL: A, MYCELIAL GROWTH; P, MANNITOL;
C, TITRATABLE ACIDS; D, pH;
E, INORGANIC PHOSPHORUS.)
FIG. 9: NITROGEN UTILIZATION DURING A. CAMPESTRIS S12 FERMENTATION

(SYMBOL: A, MYCELIAL GROWTH; B, MYCELIAL NITROGEN;
C, AMINO NITROGEN; D, AMMONIA NITROGEN;
E, TOTAL NITROGEN).