Experimental No. 3

Determination of Specific Growth Rate

Objective: To determine the specific growth rate of a given bacterial culture

Introduction:

When microbial cells are inoculated into a fresh culture medium under batch conditions and their increase in concentration is monitored, several distinct phases of growth can be observed. There is an initial lag phase, which is of variable duration. This is then followed by the exponential growth phase, where cell number (and dry weight) increases exponentially. This is also referred to as the logarithmic phase, the name arising from the common method of plotting the logarithm of cell number against time. Following this is a short period of declining growth, and then the stationary phase. Here the cell numbers are highest. Finally the cells numbers decline during the death phase.

Instead of cell number, it is often more convenient to use dry cell weight per volume X as a measure of cell biomass concentration. During the exponential phase in batch we can write:

$$\frac{dX}{dt} = \mu X$$

where μ is the specific growth rate of the cells.

List of Reagents and Instruments

A. Equipment: Flasks, Spectrophotometer, Sample tubes, Micropipette

B. Reagents: Flask of culture, Growth media, sterile water

C. Organism

- *Bacillus licheniformis* NRRL B-642

D. Media composition

- **For culture maintenance (Slant and/or Plate)**
  - Nutrient agar medium, 28 g/l

- **For Growth media in flask (Minimal Salt medium)**

Chemical name	Composition (g/l)
Glucose	2.0
Potassium Dihydrogen Phosphate	0.2
Di –Potassium hydrogen phosphate	0.8
Magnesium Sulphate Hepta hydrate	0.5
Ammonium Sulphate	1.0
Calcium Chloride	0.05

Procedure:

1. 150 ml. of fresh growth media taken in a 500 ml Erlenmeyer flask is inoculated with 5 % of seed bacterial culture under aseptic conditions.
2. The inoculated flask is kept under agitation (150 rpm) at 30^{o}C temperature.
3. 2ml of sample is withdrawn from the flask at following time intervals: 0hr, 2hrs, 4hrs, 6hrs, 8hrs, 10 hrs, 12hrs, 14hrs, 16hrs, 18hrs 20 hrs, 22hrs and 24 hrs. Samples collected are immediately subjected to optical density measurement at its λ_{max}
4. The biomass concentration in different samples is obtained by use of calibration curve obtained earlier.
5. A graph is plotted between biomass concentrations vs. time.
6. Linear part of the graph, which is exponential phase of growth, is taken for specific growth calculation.

Task Required

Calculation of specific growth rate of the microorganism.