Experiment No. 2

Determination of Specific Substrate Utilization Rate

Objective: To determine the specific substrate utilization 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 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 phase of declining growth, and then the stationary phase. Here the cell numbers are highest. Finally the cell numbers decline during the death phase. During the growth in batch we can write:

$$\frac{dS}{dt} = q_s X$$

where '*S*' is the substrate concentration, '*t*' is time, '*X'* is the biomass concentration, $\frac{dS}{dt}$ is substrate utilization rate and 'q_s' is the specific substrate utilization rate.

Reagents and equipments required:

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

B. Reagents

- Antrone reagent: Dissolve 2 g Antrone in 1000 ml of concentrated sulphuric acid

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. 100 ml. of fresh growth media taken in a 500 ml Erlenmeyer flask is inoculated with 4 % 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3. 2ml of sample is withdrawn from the flask at following time intervals: 0hr, 1hrs, 2hrs, 3hrs, 8hrs, 12hrs, and 24 hrs. Samples collected are subjected to glucose estimation by Anthrone method. After collecting each sample culture optical density (OD) has to be measured using spectrophotometer to get biomass density (X) using previously developed X vs. OD relationship.
4. The glucose concentration of the supernatant in different samples is obtained by use of Antrone reagent.

Task Required

Plot a graph between glucose concentrations (S) vs. time and biomass concentration (X) vs. time.

Calculation of specific substrate utilization rate of the microorganism.