Experiment No. 6

Batch operation in a bioreactor to study bacterial growth kinetics

Objective: To study growth kinetics of a bacterial culture in a bioreactor operated under batch mode

Introduction:

A bioreactor provides better control in terms of temperature, pH, antifoam addition, DO etc. in successful fermentation processes.

Media and materials:

Mineral salt medium, pH 6.8, is used as the growth medium for *B. licheniformis*.

A. Equipment

- Flasks
- Graduated cylinder
- Centrifuge
- Oven, 100 ºC
- Balance
- Spectrophotometer
- Fermentor
- Orbital Incubator shaker

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. The fermenter vessel is added with 1.0 L of the growth medium and autoclaved.
2. Calibrate the DO probe according to the manufacturers' instruction
3. Set the temperature of the fermenter to 30^{o}C
4. Under aseptic condition inoculate the fermenter with 2-5 % v/v of seed culture obtained earlier under shake flask conditions.
5. Set the fermenter controller to proper settings of pH, agitator speed and aeration.
6. Take samples at regular interval of time (every two hours) and measure biomass concentration and substrate utilization.

Task Required

Calculation of specific growth rate of the culture

Calculation of specific substrate utilization in the bioreactor