**Applied Biology and Bioengineering (BT520)**

**EXPERIMENT- 2**

**BACTERIAL GROWTH CURVE and SPECIFIC GROWTH RATE**

**PURPOSE**
1. To plot a growth curve and determine a) Generation time and
   b) Specific growth rate of bacterial culture

**PRINCIPLE**
Bacterial population growth studies require inoculation of viable cells into a sterile broth medium and incubation of the culture under optimum temperature, pH and gaseous conditions. Under these conditions, the cells will reproduce rapidly and the dynamics of the microbial growth can be charted by means of a population growth curve, which is constructed by plotting the increase in cell numbers versus time of incubation. The curve can be used to delineate stages of the growth cycles. It also facilitates measurement of cell number and the rate of growth of a particular organism under standardized conditions as expressed by its generation time, the time required for a microbial population to double. The stages of a typical growth curve are:
1. **Lag phase:** During this stage the cells are adjusting to their new environment. Cellular metabolism is accelerated, resulting in rapid biosynthesis of cellular macromolecules, primarily enzymes, in preparation for the next phase of cycle. Although the cells are increasing in size, there is no cell division and therefore no increase in numbers.
2. **Logarithmic (log) phase:** Under optimum nutritional and physical conditions, the physiologically robust cells reproduce at a uniform and rapid rate by binary fission. Thus there is a rapid exponential increase in population, which doubles regularly until a maximum number of cells is reached. The time required for the population to double is the generation time. The length of the log phase varies, depending on the organism and the composition of the medium. The average may be estimated to last from 6 to 12 hours.
3. **Stationary phase:** During this phase the number of cells undergoing division is equal to the number of cells that are dying. Therefore there is no further increase in cell number and the population is maintained at its maximum level for a period of time. The primary factors responsible for this phase are the depletion of some essential metabolites and the accumulation of toxic acidic or alkaline end products in the medium.
4. **Decline or death phase:** The decrease in population due to death closely parallels its increase during the log phase. Theoretically the entire population should die during a time interval equal to that of log phase. This does not occur, however, since a small number of highly resistant organisms persist for an indeterminate length of time.

Construction of a complete bacterial growth curve requires that aliquots of a 24-h shake flask culture be measured for population size at intervals during the incubation period. Spectrophotometric measurement of developing turbidity at regular intervals can be used as an index of increasing cellular mass. The generation time can be determined by simple extrapolation from the log 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:

$$dX/dt = \mu X$$

where $\mu$ is the specific growth rate of the cells.

## MATERIALS

**Culture:** 12-18 h nutrient broth culture of *E. coli* DH5α

**Medium:** Nutrient Broth Ingredients      $gl^{-1}$

Peptic digest of animal tissue      5.00

Beef extract      3.00

Final pH (at 25°C)      $6.9 \pm 0.2$

The above constituents were dissolved in requisite amount of distilled water. The media was sterilized in an autoclave.

## Equipment & Accessories

Laminar hood, Orbital incubator shaker, 250 ml conical flasks, 15 ml test tubes, Glassware marker, 1.0 and 0.2 ml sterile disposable tips, Micropipettes

## PROCEDURE

1. An over-night culture of *E. coli* DH5α is used to inoculate 100 ml of nutrient broth in a 250 ml conical flask at 1% level.
2. The flask containing culture was incubated in an orbital shaker at 37°C, 180 rpm.
3. Aliquots of the culture were taken aseptically at regular intervals and the turbidity was measured in a spectrophotometer at 600 nm using nutrient broth as blank.
4. Optical density of the samples at 600 nm was recorded till 24 h of growth.
5. The $O.D_{600}$ values as a function of time were plotted in a semi-log paper to generate the growth curve.
6. The generation time of the bacteria can be determined by extrapolation from the growth curve.
7. Plot the growth curve and calculate the generation time from the curve.
8. The biomass concentration in different samples is obtained by use of calibration curve obtained earlier.
9. A graph is plotted between biomass concentrations vs. time.
10. Linear part of the graph, which is exponential phase of growth, is taken for specific growth calculation.

## OBSERVATIONS AND RESULTS

| Incubation time (h) | Optical Density (600 nm) |
|---|---|
| 0 | |
| 1.0 | |
| 2.0 | |
| 3.0 | |
| 4.0 | |
| 5.0 | |
| 6.0 | |
| 8.0 | |
| 12.0 | |
| 18.0 | |
| 24.0 | |

**REVIEW QUESTIONS**

1. Why do variations in the generation time exist: (a) among different species of microorganisms? (b) within a single microbial species
2. What is the rationale of choosing a wavelength of 600 nm to measure turbidity of bacterial cells growing in a culture medium?
3. While taking samples regularly for measuring O.D, you did not take proper measures in aseptic transfer during the sampling of the 2h sample. What changes do you predict while measuring optical density of subsequent samples?