Indian Institute of Technology Delhi
Department of Biochemical Engineering and Biotechnology

I SEMESTER
BBL132 – GENERAL MICROBIOLOGY LABORATORY

EXPERIMENT # 6

AIM

To determine bacterial growth curve and to perform dilution plating experiments for enumeration.

BACKGROUND

Bacterial growth is the division of one bacterium into two identical daughter cells during a process called binary fission. Hence, doubling of the bacterial population occurs. Bacterial growth in a typical batch culture is divided into 4 phases: lag, log, stationary and death phases. Determination of the growth curve requires bacterial enumeration (cell counting) by direct and individual (microscopic), direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods.

The simplest method to determine the growth curve is spectrophotometeric. At 600 nm, the optical density (OD) is the measure of solution turbidity. Thus, the samples taken at different time intervals are analyzed by taking OD at 600 nm and plotted to give the growth curve. Death phase is not observed in such case because dead cells also contribute to absorbance.

Therefore, dilution plating is performed. In this method the culture is first serially diluted in normal saline and then plated on the appropriate medium (nutrient agar). Serial dilution involves adding 1 ml culture to the 9 ml normal saline to give 1/10 diluted culture. Now, 1 ml of 1/10 diluted culture is added to another 9 ml normal saline to give 1/100 diluted culture and so on.

After dilution, appropriate dilution is plated on the surface of nutrient agar. After the requisite incubation period, visible colonies appear on the plates which are counted to determine the number of viable cells originally present in the culture. Bacterial count here is described not in cells/ ml, but as colony forming units (CFU)/ml, because it may be a group of bacterium which has given rise to a colony.

CFU/ ml= no. of colonies/volume plated * Dilution factor

Dilution factor is the reciprocal of the dilution plated.

The colony count should be between 30-300. If it < 30, it is called ‘too less to count’ (TLTC) and if >300 it is called TNTC or ‘too numerous to count.

APPARATUS AND MATERIALS REQUIRED

a) For turbidity measurements.

Bacterial culture (*Eshcherichia coli*)
Sterile nutrient broth.
Shaker incubator
Sterile epperndorfs
Laminar air flow
Cuvette
Spectrophometer.

b) For dilution plating

Sterile 0.85 % NaCl solution (9 ml) in tubes.
Nutrient agar plates
Pipettes
Tips and spreader
Incubator (at 37°C)
Colony counter

PROCEDURE

1) Take 4 ml of given microbial culture and inoculate 100 ml of the sterile nutrient broth.
2) Harvest a 0 hour sample in sterile epperndorf and in a cuvette after proper shaking.
3) Sample taken in cuvette is measured for O.D at 600 nm.
4) Incubate the culture at 37°C with shaking at 250 rpm.
5) Harvest the culture in a manner similar to described in step 2 and perform step 3.
6) Plot the value of the O.D with time to get growth curve.
7) The samples harvested in sterile microfuge tubes are to be serially diluted and plate 100 µl of diluted culture on nutrient agar.
8) Incubate the plates at 37°C incubator.
9) After overnight incubation, count the colonies and calculate CFU/ ml and obtain CFU/ml v/s time plot to get the growth curve.

Precautions

1. Ensure adequate shaking before harvesting each sample and before taking O.D.
2. While doing dilution plating, vortex before taking samples for further dilution and for plating.
3. Perform serial dilution and plating under sterile conditions.