Lab 4

The Growth of bacterial populations

For unicellular organisms such as the bacteria, growth can be measured in terms of two different parameters: changes in cell mass and changes in cell numbers.

Measurement of Bacterial Growth

Individual cells of many species double in size between division. Cell mass thus increase at the same rate as cell number.

Methods for Measurement of cell Mass

Methods for Measurement of cell Mass involve both direct and indirect techniques.

1- Direct physical measurement of dry weight or wet weight and Measure volume of cells after centrifugation.

Dry weight is the most direct approach for quantitative measurement of a mass of cell.

The method of work:

1- The sample is centrifuged or filtered.

2-Take the residue or the pellet and washed a number of times to remove all extraneous matter.

3-The residue is then dried by oven and then weighed.

It is commonly used for measuring growth of moulds in certain phases of industrial work.

2- Direct chemical measurement of some chemical component of the cells such as total N, total protein, or total DNA content.

Measurement of cell nitrogen

The major constituent of cell material is protein, and nitrogen is a characteristic constituent of protein. A bacterial population can be measured in terms of cell nitrogen. The sample is taken and the same steps are taken for the previous experiment, and then the cell nitrogen is estimated by chemical analysis. This is also a tedious method, and can be used only with dense cell suspension.

3- Indirect measurement of chemical activity

Include rate of O^2 production or consumption, CO^2 production or consumption, etc.

4-Turbidity measurements: employ a variety of instruments to determine the amount of light scattered by a suspension of cells. particulate objects such as bacteria scatter light in proportion to their numbers. The turbidity or optical density of a suspension of cells is directly related to cell mass or cell number, after construction and calibration of a standard curve. The method is simple and nondestructive, but the sensitivity is limited to about 10^7 cells per ml for most bacteria. This method has some limitations. Turbidity is most effective with suspensions of moderate density. Suspensions with very high or very low density gives erroneous results. Secondly, it is not possible to measure cultures that are deeply coloured or contain suspended material other than cells. It must be recognized that turbidity measures both living as well as dead cells.

Spectrophotometer: It is an instrument that measures the amount of light absorbed by a sample. It is techniques are used to measure the concentration of solutes in solution by measuring the amount of the light that is absorbed by the solution in a cuvette placed in the spectrophotometer

Spectrophotometer

- measures absorbance or transmittance of light, as a function of wavelength

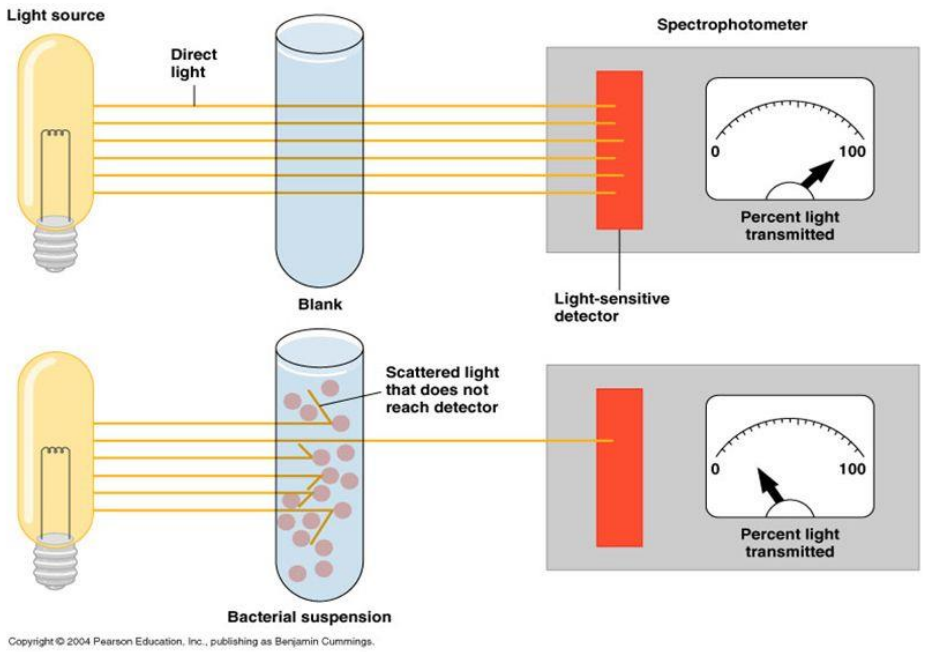
a cuvette

General procedure:

- sample is placed into cuvette
- light of selected wavelength (λ_{max}) is passed through sample
- instrument measures the amount of light absorbed by the sample

spectrophotometer

Bacterial Count Spectrophotometer



Types of Spectrophotometer

2-Measuring of increase methods in the numbers of bacteria

A-Total count (Direct microscopic count of bacteria).

B-Viable count (Indirect microscopic count of bacteria).

A-Total count (Direct microscopic count of bacteria)

Can directly estimate the number of cells in the sample of liquid media by the microscope and this method is characterized such 1-ease of take place 2- speed to obtain its results, but they do not distinguish between viable and dead cells and to conduct that there are several methods.

A-Breedś Counting Method

This method does not distinguish between live and dead cells.

Work method

1-Prepare a clean glass slide and draw them square area of 1 cm.

2- Serial dilution are prepared.

3- Spread 0.1 ml over the square by loop and let to dry on the air.

4- Fixed by heat and dye by simple stain.

5- Exam by the oil immersion and calculate the number of cells in a number of random microscopic fields.

TBC=Total Bacteria Count

MF=Microscopic Factor (number of microscopic field in

1 cm^2e)

MF= 5000

TBC= Avg. number of cells × MF × 100 × dilution inverted

B- Haemocytometer (counting chamber)

The most common method of enumerating the total microbial cells is the direct counting of cell suspension in a counting chamber of known volume using a microscope. Originally designed for performing blood cell counts.

B-Viable count (Indirect microscopic count of bacteria).

1- Viable count/ Plate Count(Pour Plate Technique)

The Pour Plate Technique can be used on any type of liquefied sample for the enumeration of bacteria . Conditions vary depending upon the type of bacteria being enumerated . Only live bacteria are counted in this method.

Procedure :-

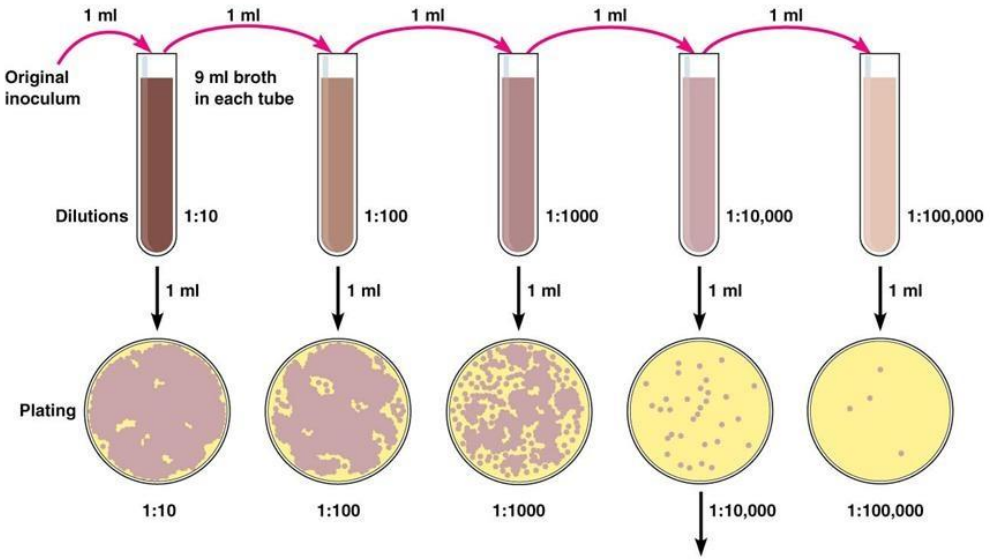
1- Take 10 fold serial dilution of sample
2- Take 10 test tubes. 9 ml of any diluents is taken in each test tube e.g. normal saline .
3- 1 ml of sample is poured first in first test tube by a pipette and it is then mixed thoroughly . (dilution 1/10)
4- 1 ml is transferred from first test tube to second test tube by pipette. It is mixed again . (dilution 1/100)
5- In the same pattern dilution is done up to the last test tube (in this fashion as dilution is increasing, bacterial number is decreasing).
6- 10 Petri dishes are prepared now with general purpose nutrient agar in each, then held at 44-46°C in a water bath .
7- 1.0 mL of the sample or dilution is transferred to a sterile, empty petri dish from each test tube is done in each respective plate . Agar is melted by heating in boiling water, and then allowed to cool in a water bath to 44-46°C
8- Approximately 15 mL of agar medium is poured into the petri dish containing the sample . The sample and agar are mixed thoroughly by rotating the plate several times .
9- When the media has solidified , the plates are inverted and incubated
10- Incubate these plates at 37°C for 24 hours Dense colonies are formed in first two plates and then gradual decrease in intensity is seen .
11- Select only one plate having 30-300 colonies and count the number of colonies in it e.g. plate number 4 is giving 240 colonies . (As a colony is formed by a single living cell, so a colony represents a living cell).

Formula :-

The number of cells /ml = average number of colonies apparent × dilution inverse

Law of the dilution is uses :

Additive / Additive + present x the previous dilution



Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000 bacteria/ml in sample.)

b)Spread Plate Technique

Standard Methods Agar (SMA) is used routinely for the spread plate technique to enumerate aerobic bacteria .

1- Plates are allowed to warm to room temperature and dry before inoculating .
2- Serial dilutions are prepared (using 0.1 ml) so that following incubation on nutrient agar or any media , and spreader is uses to bacteria spreading on the plate , one of the dilutions will yield growth of 30-300 colonies (the ideal range for counting) on the agar plate .
3- The plate is inoculated from the dilution (which has been thoroughly mixed), or directly from the sample using a 0.1 ml inoculum , if low counts are expected.
4- The inoculum is transferred onto the agar surface near the center if the plate is spread , or at a designated mark on the plate if it is being spread by an automatic spreading device .

The inoculum is spread over the surface (by spreader) and allowed to be absorbed by the medium . Plates are inverted and incubated as follows :-

Marked dishes and placed inverted and incubated at 37°C and after incubation law is used :

The number of cells /ml = number of colonies apparent * inverse dilution * 10