Estimation of Microbial Cell Mass

Presented to:

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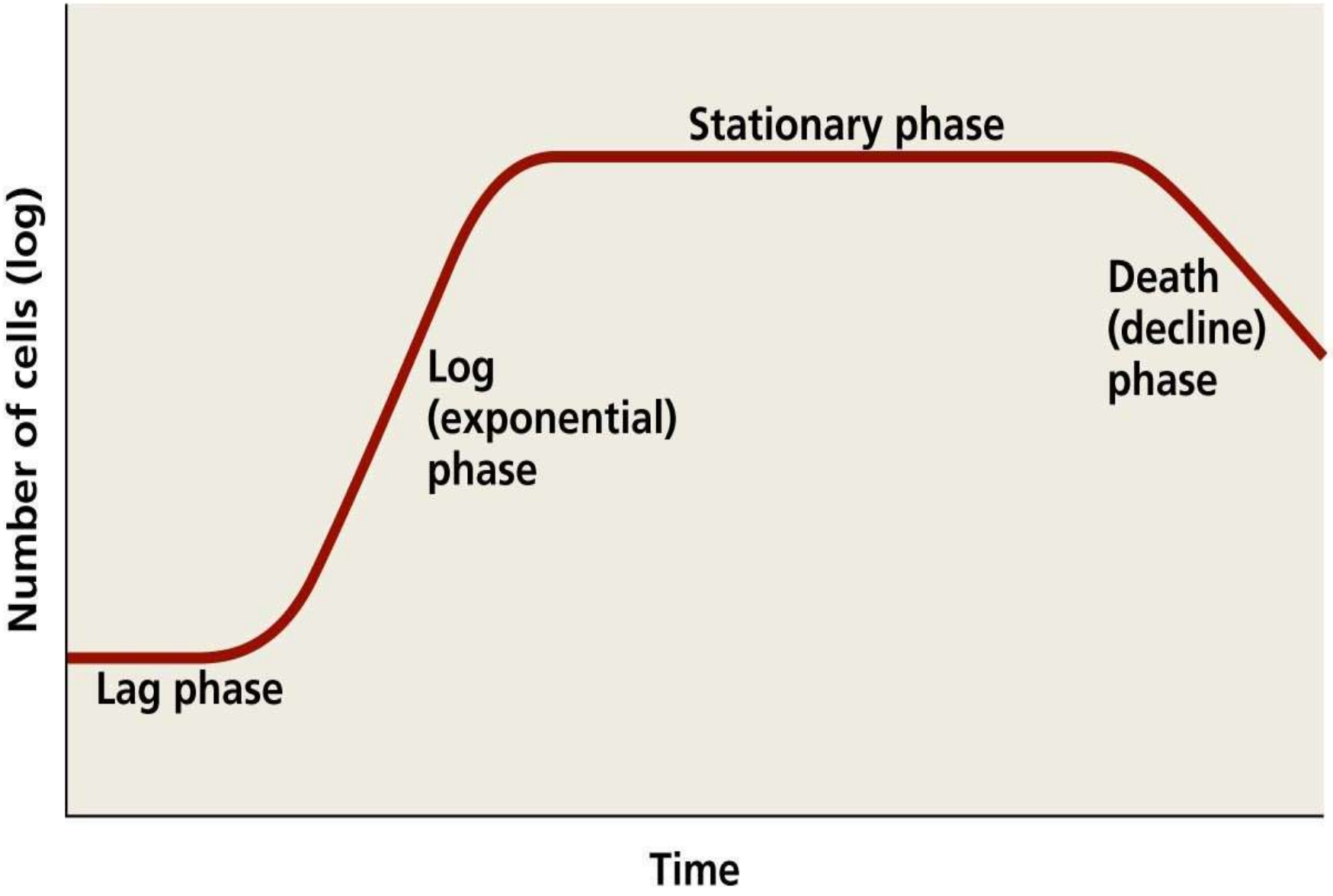
Microbial Growth

- In microbiology growth is defined as a process of increase in the no. of microorganisms by asexual method or
- Increase in cell mass
- Increase in cell activity
- Here growth refers to **population** growth rather than growth of individual cells

Phases of Microbial Growth

- There are four phases of growth which are described as:
  - Lag phase
  - Log phase
  - Stationary phase
  - Death or decline phase


Number of cells (log)
Stationary phase
Death
(decline)
phase
Log
(exponential)
phase
Lag phase
Time

Measurement of Microbial Growth

Direct Counts:

- counting chambers
- electronic counters – flow cytometry
- on membrane filters

Viable Counting Methods:

- Spread and pour plate techniques
- Membrane filter technique
- Most Probable Number (MPN)

Measurement of Cell Mass by Indirect Methods

- Dry Weight Analysis
- Measurement of metabolic activity
- Turbidity

Direct Count

Counting Chambers

- The most direct method of counting microorganism is by the use of a microscope and a slide with special chambers of known volume. These slides allow the counting of a small number of cells in a small volume and extrapolating the result to determine the population.
- A culture is placed on the slide marked with precise grids. The number of cells present in each grid is counted and an average determined.
- Conversion using a formula gives the number of cells per milliliter in the culture.
- e.g. Petroff Hauser Chamber

Petroff Hauser Chamber

New improved Neubauer ruling pattern with 1/400 sq. mm of ruling. Designed for the counting of small cells such as bacteria, sperm, blood platelets.

➢*Procedure*

A clear area at the center of this special slide is divided, by colored lines, into carefully calculated areas.

A coverslip is then placed over the bacterial suspension on the grid, and held a very accurate distance away from the surface.

The microbes are thus contained within a small, but known, volume of liquid, and can easily be counted by looking at the chamber through an appropriate microscope lens.

Most Petroff-Hausser counting chambers consist of 400 small squares each with an area of 0.0025 mm^2, 25 large squares with areas of 0.04 mm^2 and the suspension depth (height of the coverslip) is 0.02 mm.

An investigator places a small sample of the microbe culture between the grid (on the surface of the slide) and the coverslip and makes several counts of the number of cells found within one or more of the larger squares. These numbers are recorded.

The total number of cells in a milliliter of the original growth population can then be calculated.

Direct Count

- Membrane Filter Method

- Cells filtered through special membrane that provides dark background for observing cells
- Cells are stained with fluorescent dyes
- Useful for counting bacteria
- With certain dyes, can distinguish living from dead cell.

Direct Count

Flow Cytometry

- Microbial suspension forced through small orifice with a laser light beam
- Movement of microbe through orifice impacts electric current that flows through orifice
- Instances of disruption of current are counted
- Specific antibodies can be used to determine size and internal complexity

Direct Count

- **Advantage of Direct Microscopic count**
- Rapid, simple and easy method requiring minimum equipment.
- Morphology of the bacteria can be observed as they counted.
- Very dense suspensions can be counted if they are diluted appropriately.

Direct Count

- **Limitations of Direct Microscopic count**

- Dead cells are not distinguished from living cells.
- Small cells are difficult to see under the microscope, and some cells are probably missed.
- Precision is difficult to achieve
- A phase contrast microscope is required when the sample is not stained.
- The method is not usually suitable for cell suspensions of low density i.e. < 10^7 Cells per ml, but samples can be concentrated by centrifugation or filtration to increase sensitivity

Viable Counting Methods

- A **viable cell count** allows one to identify the number of actively growing/dividing cells in a sample.
- A viable cell is defined as a cell which is able to divide and form a population.
- A viable Count is done by diluting the original sample, placing aliquots of dilutions on appropriate culture medium and then incubating then plates under proper conditions so that colonies are formed.

Viable Cell Count

- **Pour plate method** is usually the method of choice for counting the number of colony-forming bacteria present in a liquid specimen.

➢ *Procedure*

- In this method, fixed amount of inoculum (generally 1 ml) from a broth/sample is placed in the center of sterile Petri dish using a sterile pipette.

- Molten cooled agar (approx. 15mL) is then poured into the Petri dish containing the inoculum and mixed well.

- After the solidification of the agar, the plate is inverted and incubated at 37°C for 24-48 hours.

Cont.

- Microorganisms will grow both on the surface and within the medium.

Colonies that grow within the medium generally are small in size and may be confluent; the few that grow on the agar surface are of the same size and appearance as those on a streak plate. Each (both large and small) colony is carefully counted (using magnifying colony counter if needed). Each colony represents a “colony forming unit” (CFU).

- The number of microorganisms present in the particular test sample is determined using the formula:

- **CFU/mL= CFU * dilution factor * 1/aliquot**
- For accurate counts, the optimum count should be within the range of 30-300 colonies/plate. To insure a countable plate a series of dilutions should be plated.

Viable Cell Count

- **Disadvantages of Pour plate method**
- Preparation for pour plate method is time consuming compared with streak plate/and or spread plate technique.
- Loss of viability of heat-sensitive organisms coming into contact with hot agar.
- Reduced growth rate of obligate aerobes in the depth of the agar.

Viable Cell Count

Spread plate technique is the method of isolation and enumeration of microorganisms in a mixed culture and distributing it evenly. The technique makes it easier to quantify bacteria in a solution.

- **Procedure of Spread Plate Technique**
- Make a dilution series from a sample.
- Pipette out 0.1 ml from the appropriate desired dilution series onto the center of the surface of an agar plate.
- Dip the L-shaped glass spreader into alcohol.
- Flame the glass spreader (hockey stick) over a Bunsen burner.

Cont.

- Spread the sample evenly over the surface of agar using the sterile glass spreader, carefully rotating the Petridish underneath at the same time.
- Incubate the plates in inverted position at 37°C for 24 hours.
- Calculate the CFU value of the sample. Once you count the colonies, multiply by the appropriate dilution factor to determine the number of CFU/mL in the original sample.Only plates with 30-300 colonies are statistical.
- **Limitations of Spread Plate Technique**
- Strick aerobes are favored while microaerophilic tends to glow slower.
- Crowding of the colonies makes the enumeration difficult.

Viable Cell count

- **Membrane filter technique** (used in our lab during water testing)
  - bacteria from aquatic samples are trapped on membranes
  - membrane placed on culture media
  - colonies grow on membrane
  - colony count determines number of bacteria in sample

Viable Count Mrthod

- **Most Probable Number (MPN)** is a method used to estimate the concentration of viable microorganisms in a sample by means of replicate liquid broth growth in ten-fold dilutions.
- MPN is most commonly applied for quality testing of water i.e *to ensure whether the water is safe or not in terms of bacteria present in it*.

Cont.

- **MPN test is performed in 3 steps**
- Presumptive test
- Confirmatory test
- Completed test
- **1. Presumptive test**
- It is achieved by performing serial dilutions of a bacterial culture ,dividing the sample into aliquots or replicates followed by incubation and subsequent visual examination of each sample for growth.

Cont.

- **Confirmatory Test**
- Performed when presumptive test is positive.
- In this process, a loopful of sample from each tube showing positive test *(color change with gas)* is streaked onto two selective medium like EMB (Eosine Methylene Blue) or Endo's medium.
- Incubated at 37°C for 24 hrs.
- **Results**
- Dark centred colonies with metallic sheen on **EMB** agar.
- Pinkish colonies on Endo's medium.

Cont.

- **Completed Test**

From each of the solid-medium plates used for the confirmed test, transfer selected colonies

- ✓ 3 ml lactose-broth
- ✓ to an agar slant

Incubate the inoculated lactose-broth fermentation tubes at 37°C and inspect gas formation after 24 ± 2 hours. If no gas production is seen,further incubate up to maximum of 48 ±3 hours to check gas production.

The agar slants should be incubated at 37°C for 24± 2 hours and **Gram Stained Preparations** made from the slants should be examined microscopically.

The formation of gas in lactose broth and the demonstration of Gram negative, non-spore-forming bacilli in the corresponding agar indicates the presence of **a member of the coliform group** n the sample examined.

Indirect Methods

- Dry Weight Analysis
- Sample is centrifuged
- Resulting cell pellet is filtered and then it is weighed.
- **Advantages-** only way to determine growth of filamentous bacteria. It is rapid and easy.
- **Disadvantages-** cumbersome, not very accurate. It does not give you cell numbers or increase in mass. It cannot distinguish between live and dead cells and must work within certain absorbency (more than 107 and less than 108).

Indirect Methods

- **METABOLIC ACTIVITY**
- Another indirect way of estimating bacterial numbers is measuring the metabolic activity of the population (for example, acid production or oxygen consumption, nutrient utilization, waste production, pH, etc).
- The assumption is that the amount of acid produced or oxygen consumed under specific conditions and during a fixed period of time is proportional to the magnitude of bacterial population.
- Admittedly, the measurement of acid or any other end product is a very indirect approach to the measurement of growth and is applicable only in special circumstances.

Indirect Method

- **Turbiditimetric method**

➢ A quick and efficient method of estimating the number of bacteria in a liquid medium is to measure the turbidity or cloudiness of a culture and translate this measurement into cell numbers. This method of enumeration is fast and is usually preferred when a large number of cultures are to be counted.

❑ *Procedure*

- A spectrophotometer or calorimeter can be used for turbidimetric measurements of cell mass.
- A spectrophotometer is used to determine turbidity ("cloudiness") by measuring the amount of light that passed through a suspension of cells.
- More cells = more turbidity; more turbidity = less light passing through the suspension
- However, the culture to be measured must be dense enough to register some turbidity on the instrument.

Thank You!!!

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