6.8C: Measurements of Microbial Mass

LEARNING OBJECTIVES

- Recall ways of measuring microbial mass

Bacterial growth follows three phases: the lag phase, the log phase, and the stationary phase. The measurement of an exponential bacterial growth curve in a batch culture was traditionally a part of the training of all microbiologists; the basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry), direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods. Models reconcile theory with the measurements.

METHODS OF MEASUREMENT

There are several methods for measuring cell mass, including the gravimetric method which uses ordinary balances to weigh a sample (dry weight/ml) after the water has been removed.
Figure: **Spectrophotometer**: This spectrophotometer can measure as little as one microliter of a sample.

An indirect method for calculating cell mass is **turbidimetry**. Cell cultures are turbid: they absorb some of the light and let the rest of it pass through. The higher the cell concentration is, the higher the turbidity. Spectrophotometers are electrical appliances that can measure turbidity very accurately. The culture is placed in a translucent cuvette; the cuvette is placed in the machine and the turbidity measured immediately. Simple mathematical formulae help convert the detected turbidity to cell concentration. Using spectrophotometry for measuring the turbidity of cultures is known as **turbidometry**. Note the difference in spelling: **turbidimetry** and **turbidometry** are not the same word.

In spectrophotometry, cultures usually do not need to be diluted, although above a certain cell density the results lose reliability. Of all the electrical appliances used for counting cells, a spectrophotometer is the cheapest and its operation the fastest and most straightforward. This has made spectrophotometry the methods of choice for quick measurements of bacterial growth and related applications. There are spectrophotometers in which several cuvettes can be inserted at one time, reducing work time even more. Additionally, there are spectrophotometers that require extremely small volumes of culture, as little as 1 microliter. This, combined with the stochastic nature of liquid cultures, enables only an estimation of cell numbers.

An additional method for the measurement of microbial mass is the quantification of cells in a culture by **plating the cells on a petri dish**. If the cells are efficiently distributed on the plate, it can be generally assumed that each cell will give rise to a single colony. The colonies can then be counted, and based on the known volume of culture that was spread on the plate the cell concentration can be calculated.

As is with counting chambers, cultures usually need to be heavily diluted prior to plating; otherwise, instead of obtaining
single colonies that can be counted, a so-called “lawn” will form, resulting in thousands of colonies lying over each other. Additionally, plating is the slowest method of all: most microorganisms need at least 12 hours to form visible colonies.

Key Points

• Calculating the dry weight of a sample enables one to calculate the cell count, but the sensitivity is limited to samples containing more than 10E8 bacteria per milliliter.
• Spectrophotometry is an indirect method for calculating cell concentrations by measuring the changes in turbidity.
• Bacteria can also be counted by using the plating method, which is based on the number of colonies formed in Petri dishes containing specific growth media.

Key Terms

• **spectrophotometry**: A spectrophotometer is commonly used for the measurement of transmittance or reflectance of solutions. However they can also be designed to measure the diffusivity on any of the listed light ranges that usually cover around 200nm – 2500nm using different controls and calibrations. [2] Within these ranges of light, calibrations are needed on the machine using standards that vary in type depending on the wavelength of the photometric determination. [3]
• **flow cytometry**: A technique used to sort and classify cells by using fluorescent markers on their surface.
• **gravimeter**: An instrument used to measure local variations in the gravitational field