Measurement of bacterial growth (indirect count)by increase in cell mass.

Indirect measurement of bacterial growth by increase in cell mass primarily involves assessing turbidity through **spectrophotometry** or determining **dry weight**. Turbidity methods, like using a **spectrophotometer**, measure how much light is absorbed or scattered by the bacterial culture, correlating increased turbidity with greater cell mass. Dry weight determination involves centrifuging, washing, and drying cells to directly measure the mass of the bacterial population, though this method doesn't differentiate between live and dead cells.

How do we measure microbial growth?

- Direct measurement
  - Standard Plate counts
    - most common, need to DILUTE to get individual, countable colonies
  - Microscopic Count
    - count with microscope
  - Filtration
    - when # microbes small,
    - water run thru filter and filter applied to TSA plate and incubated
  - Coulter Counter
    - Automated cell counter
- Indirect (Estimation)
  - Turbidity
    - more bacteria, more cloudiness
    - can measure w/ spectrophotometer or eye
  - Metabolic Activity
    - assumes amount of metabolic product is proportional to #
  - Dry Weight
    - used for filamentous organisms, like molds
  - Genetic Probing
    - Real-time PCR

Methods:

1. Turbidimetry:

- **Principle:**

Bacteria scatter light, and the amount of light scattered is proportional to the cell concentration in the culture.

- **Spectrophotometer:**

A spectrophotometer measures the absorbance or transmittance of light through a liquid culture.

- **Procedure:**

A spectrophotometer is used to measure the absorbance or transmittance of light through a bacterial culture at a specific wavelength (often 600 nm).

- **Correlation:**

Higher absorbance or lower transmittance values indicate increased turbidity, which correlates with greater bacterial cell mass.

2. Dry Weight Determination:

- **Principle:** The total mass of bacterial cells in a sample is measured.
- **Procedure:**
  - Bacteria are centrifuged to form a pellet at the bottom of a tube.
  - The pellet is washed to remove any media components or salts.
  - The pellet is dried in an oven at a controlled temperature (e.g., 100-105°C) until all water is removed.
  - The remaining dry mass represents the total bacterial cell mass.
- **Advantage:** Provides a direct measure of the bacterial biomass.
- **Disadvantage:** Counts both living and dead cells, and can be more time-consuming than turbidimetry

Notes: Methods for measurement of the cell mass involve both direct and indirect techniques.

1. **Direct physical measurement** of dry weight, wet weight, or volume of cells after centrifugation. . Wet and Dry Weights: A known volume of a microbial sample is centrifuged so that the cells form a pellet and are separated from the medium. The supernatant medium is discarded and the cell pellet can be weighed and the mg cells/ml of culture can be determined (wet weight).

The cell pellet can be dried before weighing to get mg cell/ml (dry weight). Filtration (preparation after staining with acridine orange SEM)

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

3. **Indirect measurement of chemical** activity such as rate of **O2** production or consumption, **CO2** production or consumption.

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.

A spectrophotometer measures the absorbance or transmission of light through a sample at specific wavelengths. The basic procedure involves warming up the instrument, selecting the appropriate wavelength, calibrating with a blank solution, measuring the sample, and recording the data. This allows for the determination of the concentration or properties of the substance in the sample.

The procedure:

1. Warm-up and Initialization:

- Turn on the spectrophotometer and allow it to warm up for the manufacturer's recommended time (typically 15-20 minutes).
- This ensures stable readings and accurate measurements.

2. Wavelength Selection:

- Choose the appropriate wavelength for your sample. This is usually determined by the substance you are testing and its absorption spectrum.

- **Some spectrophotometers have automatic wavelength selection or allow for manual adjustment.**

3. **Blank Calibration:**

- **Fill a cuvette (a small, transparent container) with a blank solution (a solution without the substance you are testing, often the solvent).**
- **Place the blank cuvette into the spectrophotometer's sample holder.**
- **Calibrate the instrument to zero absorbance (or 100% transmittance) using the blank solution. This sets a baseline for your measurements.**

4. **Sample Measurement:**

- **Prepare your sample solution by diluting it if necessary and ensuring it is free of any contaminants.**
- **Fill a clean cuvette with the prepared sample solution, ensuring no air bubbles are present.**
- **Carefully wipe the cuvette to remove any fingerprints or smudges.**

- **Insert the sample cuvette into the spectrophotometer and close the lid.**
- **Wait for the reading to stabilize (usually a few seconds).**
- **Record the absorbance or transmittance value displayed by the instrument.**

5. **Analysis and Repetition:**

- **Multiple readings should be taken and averaged for accuracy.**
- **The absorbance values can be used to determine the concentration of the analyte using Beer-Lambert's Law or compared to reference data for qualitative analysis.**
- **Clean the cuvettes thoroughly after each measurement to prevent cross-contamination**

Spectrophotometer

Principle, Instrumentation, Applications