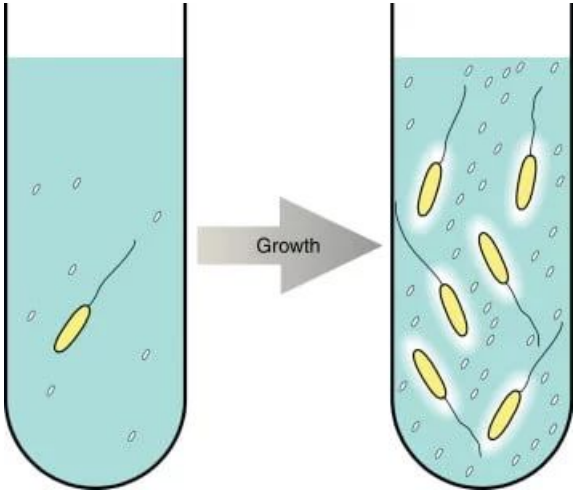
Al- Farabi Kazakh National University

Microbial Growth Kinetics. Comparison of Batch and Continuous Culture

Lecture 2

Studying growth of a microorganism is the basis of biotechnological exploitation of microflora for production of desired product.

Optimization of growth of microorganism in a particular media is desirable due to economical and availability of particular growth constituent.



https://biokimicroki.com/methods-for-measuring-bacterial-growth/

Microbial Kinetics

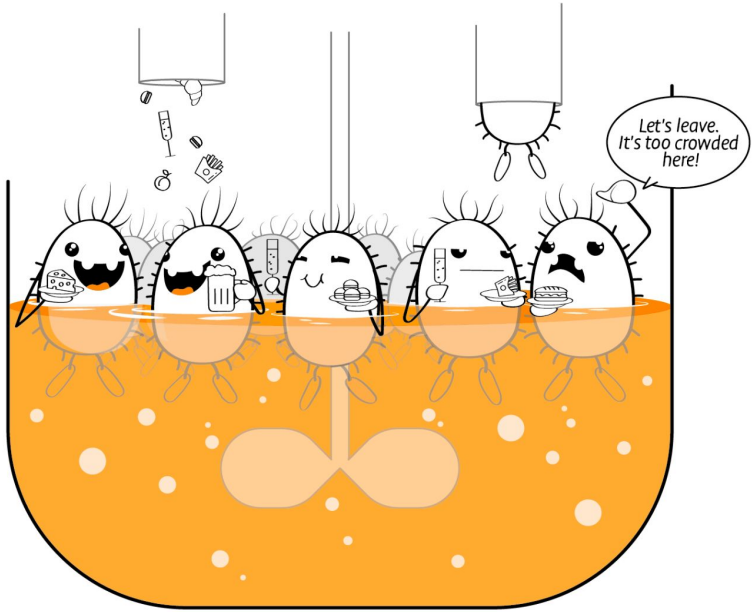
Kinetic studies in microbiology cover all dynamic manifestations of microbial life: growth itself, survival and death, product formation, adaptations, mutations, cell cycles, environmental effects, and biological interactions.

Kinetics provides a theoretical framework for optimal design in biotechnologies based on fermentation and enzyme catalysis, as well as on employment of outdoor activity of natural microbial populations (wastewater treatment, soil bioremediation, etc.)

Microbial Growth

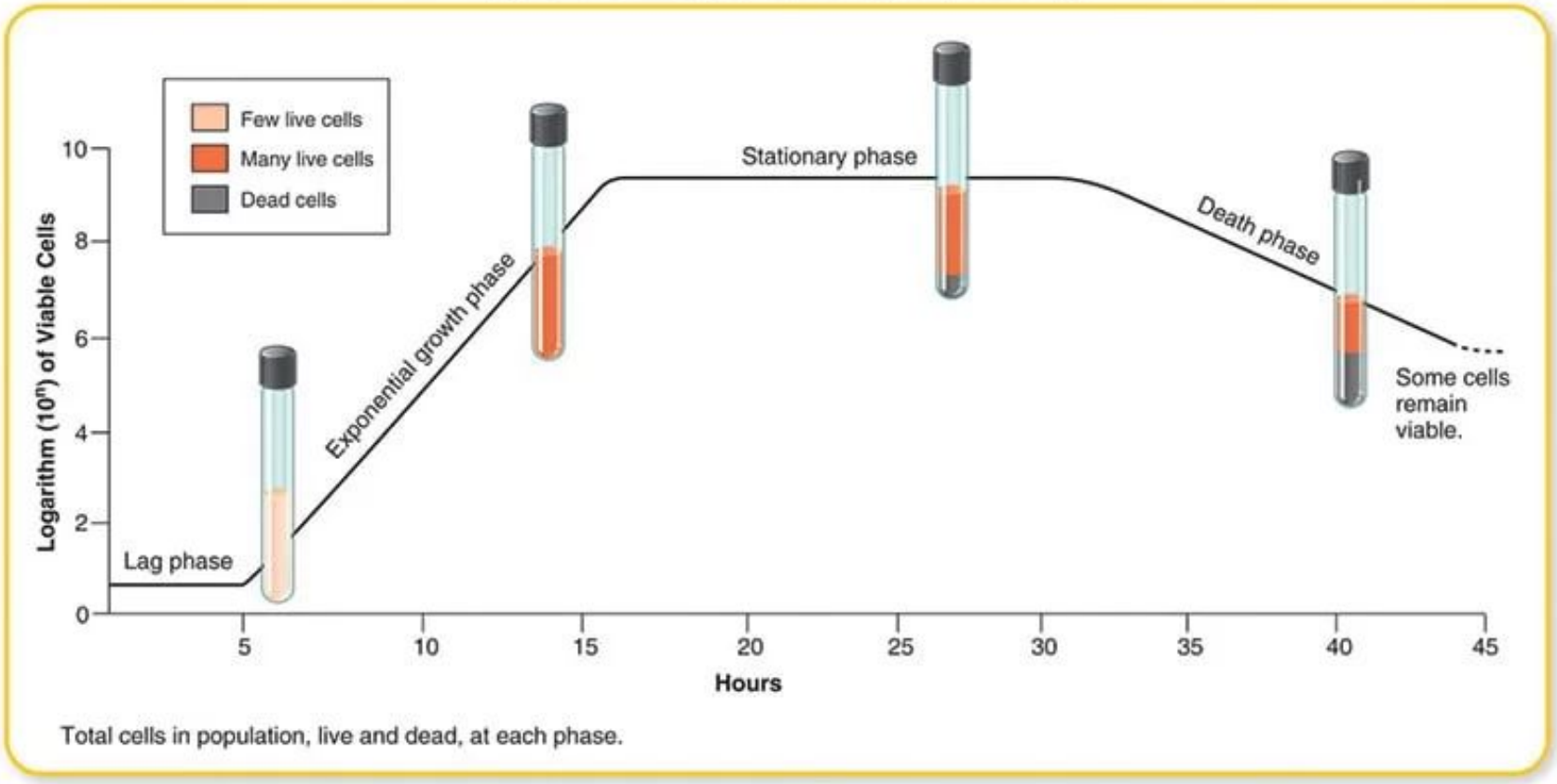
Bacterial Growth is defined as increase in cell size and number.

The bacteria increase its number by reproducing asexual methods such binary fission, budding, multiple fission or by producing spores.



https://www.infors-ht.com/en/blog/the-difference-between-batch-fed-batch-and-continuous-processes/

Four phases of bacterial growth



Total cells in population, live and dead, at each phase.

Lag phase

The length of the lag phase is apparently dependent on a wide variety of factors including the size of the inoculum; time necessary to recover from physical damage or shock in the transfer; time required for synthesis of essential coenzymes or division factors; and time required for synthesis of new (inducible) enzymes that are necessary to metabolize the substrates present in the medium.

This is the period of adjustment to new conditions. Little or no cell division occurs, population size doesn’t increase. This is the phase of intense metabolic activity, in which individual organisms grow in size. It may last from one hour to several days.

Exponential Phase

The exponential phase of growth is a pattern of balanced growth wherein all the cells are dividing regularly by binary fission, and are growing by geometric progression.

The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation. The rate of exponential growth of a bacterial culture is expressed as generation time, also the doubling time of the bacterial population.

$$G=t/n,$$

Generation time (G) is defined as the time (t) per generation (n = number of generations)

Stationary Phase

Number of cells produced is equal to number of cells dying. Factors that slow down microbial growth:

- Exhaustion of available nutrients;
- Accumulation of toxic waste materials
- Exhaustion of biological space
- Acidic pH of media
- Insufficient oxygen supply
- Cell functions necessary for growth will cease, but different functions necessary for survival are turned on.

Bacteria produces secondary metabolites, spore-forming bacteria have to induce or unmask the activity of dozens of genes that may be involved in sporulation process.

Death or decline phase

Population size begins to decrease. This is the stage where number of cells dying starts exceeding number of cells produced.

During the death phase, the number of viable cells decreases geometrically (exponentially), essentially the reverse of growth during the log phase.

Batch Culture

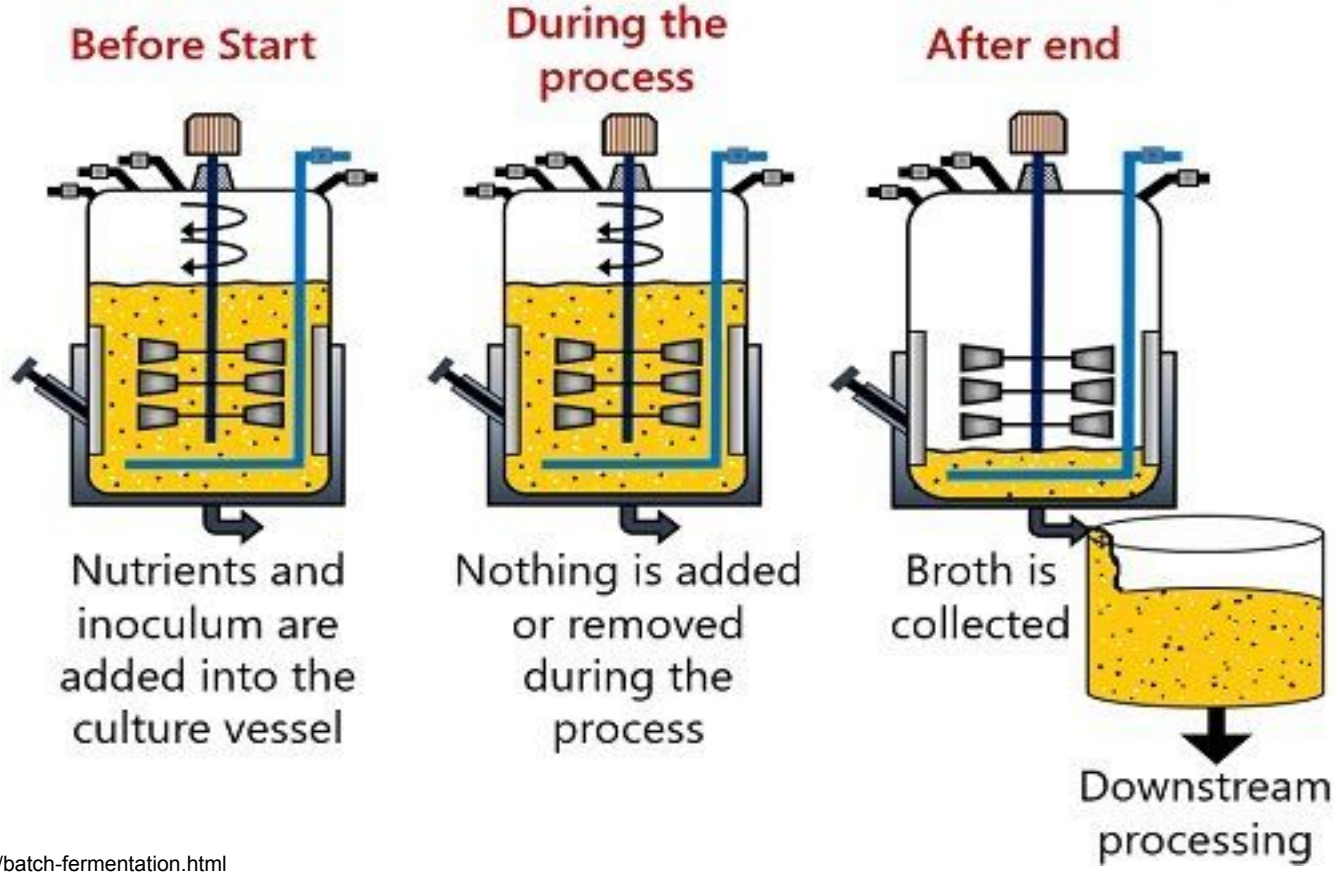
Batch culture is a closed culture system that contains an initial, limited amount of nutrient.

In batch culture cells grow in a finite volume of liquid medium and are usually maintained in conical flasks on orbital shakers at a speed of 80–120 rpm.

There are many types of batch culture: slowly rotating culture, shake culture, spinning culture, and stirred culture.

Batch culture is more suitable for the production of **secondary metabolites** such as antibiotics.

Batch Culture Fermentation Process



https://biologyreader.com/batch-fermentation.html

Advantages

Disadvantages

Its setup is **easy** to make and run.

It has wide applicability in laboratories and industries.

Due to a closed fermentation system, there is **less chance of contamination**.

It is easier in comparison to the continuous culture. Here, you can **reinstall** the setup, if contamination occurs.

Complete conversion of a substrate into a product is possible.

Product isolation is difficult. This is because, fermentation broth contains nutrients, products, reagents, cell debris and toxins.

It involves **high downtime** between two consecutive batches.

It requires high labour cost and involves batch to **batch variability**.

Continuous Cultures

Cultures that require a continuous supply of the cell suspension or the product in the medium are known as continuous cultures.

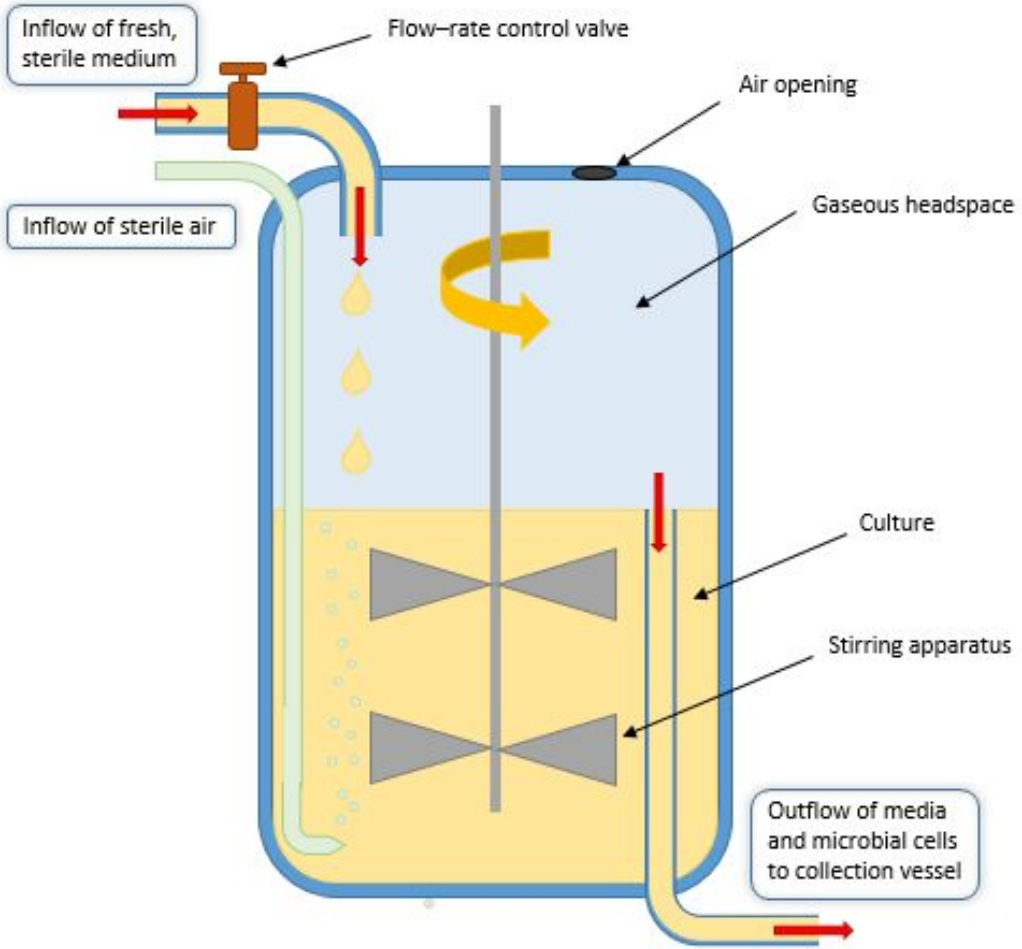
This system is maintained in a steady state for prolonged periods by draining out the used liquid medium and adding fresh medium to stabilize the physiological state of growing cells.

Types of Continuous Culture

There are two types of continuous cultures:

(i) *closed continuous culture* – in this system cells are separated from the drained medium and added back to suspension culture

(ii) *open continuous culture* – in this system addition of the medium is accompanied by harvest of an equal volume of suspension culture.



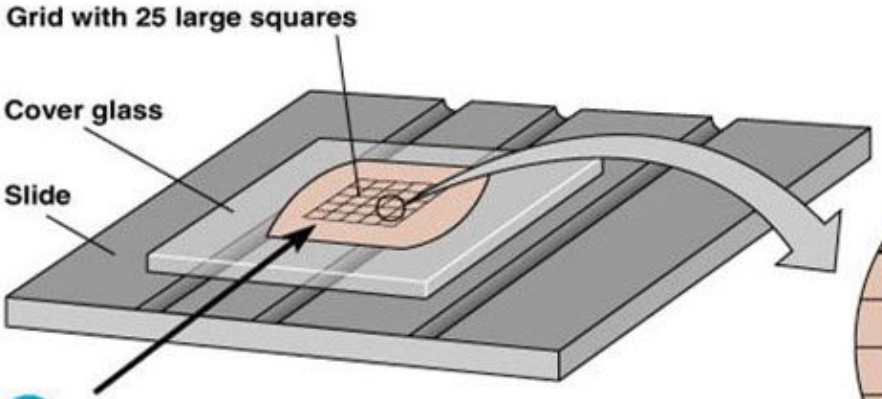
- Continuous culture is used in industries when it is required to extract useful **primary metabolites** such as amino acids, organic acids, etc. from the microorganisms.
- Primary metabolites are produced at the highest rate when the microorganisms are at their exponential phase.
- Fresh medium is added continuously from one end while metabolic products are continually extracted from the other end of the chemostat to keep the culture volume at a constant level.

Quantitative Measurement of Growth

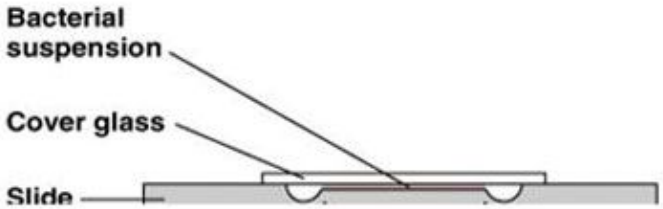
Methods for measurement of the cell mass involve both direct and indirect techniques:

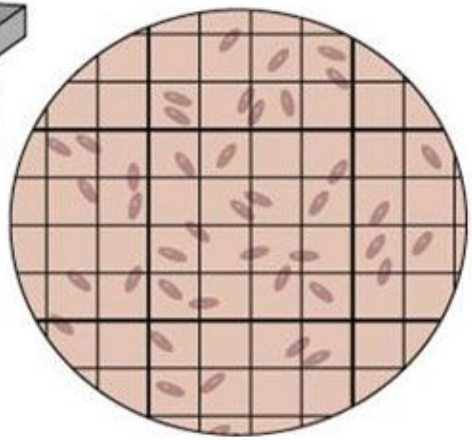
- Direct physical measurement of dry weight, wet weight, or volume of cells after centrifugation.
- Direct chemical measurement of some chemical component of the cells such as total N, total protein, or total DNA content.
- Indirect measurement of chemical activity such as rate of O_2 production or consumption, CO_2 production or consumption, etc.
- Turbidity measurements employ a variety of instruments to determine the amount of light scattered by a suspension of cells.

Direct Microscopic Counts



1 Bacterial suspension is added here and fills the shallow volume over the squares by capillary action.





3 Microscopic count: All cells in several large squares are counted, and the numbers are averaged. The large square shown here has 14 bacterial cells.

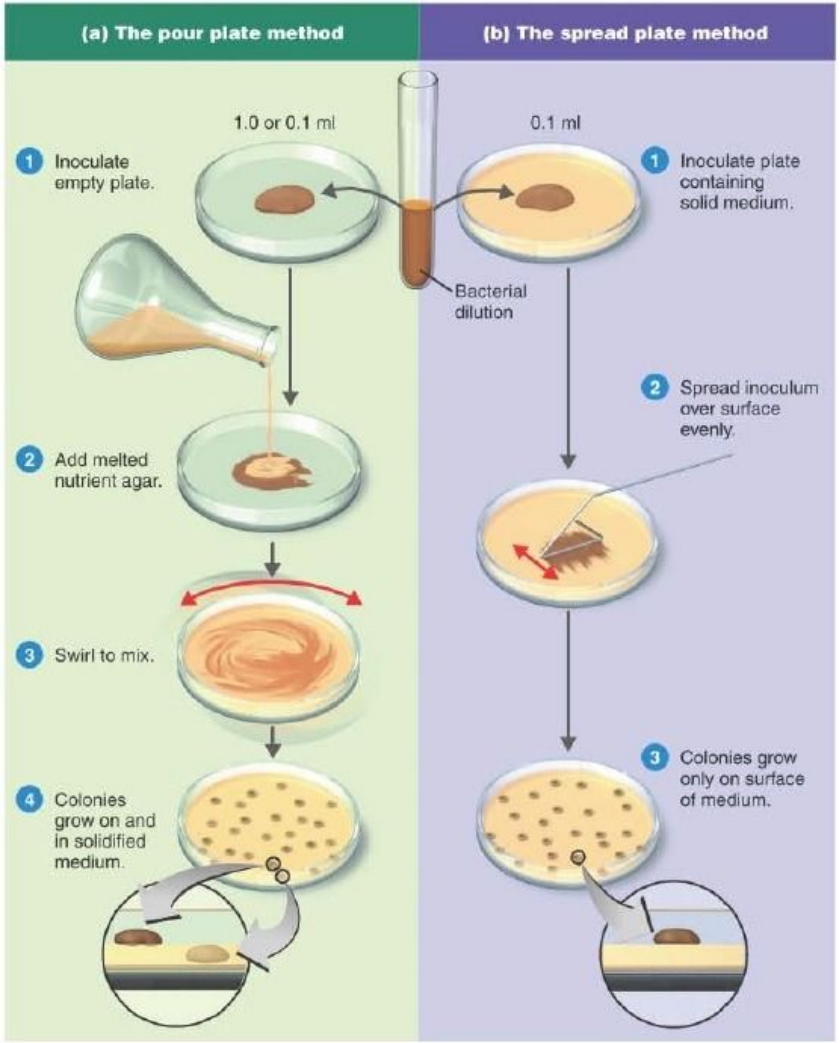
Electronic Enumeration of Cell Numbers

- In this method of microbial growth measurement, bacterial suspension is kept inside an electronic particle counter, within which the bacteria are passed through tiny orifice 10 to 30 **µm in diameter.**
- This orifice is then connected to the two compartments of the counter which contains an electrically conductive solution.
- The electrical resistance between two compartments will increases momentarily, when bacterium passes through the orifice. This generates an electrical signal which is automatically counted.
- The main disadvantage of this method is that there is no way to determine whether the cell count is viable or not.

The Plate Count Method

This method of bacterial counting is most commonly used with satisfactory results for the estimation of bacterial populations in **milk**, water, foods and many other materials.

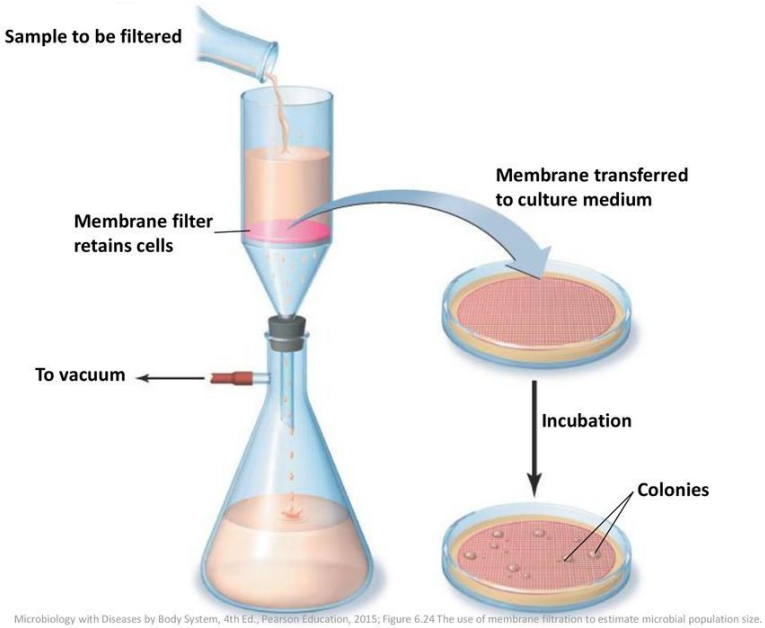
This technique has some drawbacks because some relatively heat-sensitive microorganisms may be damaged by the melted agar and will therefore be unable to form colonies



Membrane filter count method

This method is suitable for liquid or semi-liquid samples (e.g. water) and commonly used for enumeration of Coliform and Staphylococcus spp.

Membrane filtration method is used with relatively low numbers. A known volume of liquid passed through membrane filter. Filter pore size retains organism. It filters microorganism of size more than 0.45 micrometer



Microbiology with Diseases by Body System, 4th Ed., Pearson Education, 2015; Figure 6.24 The use of membrane filtration to estimate microbial population size.

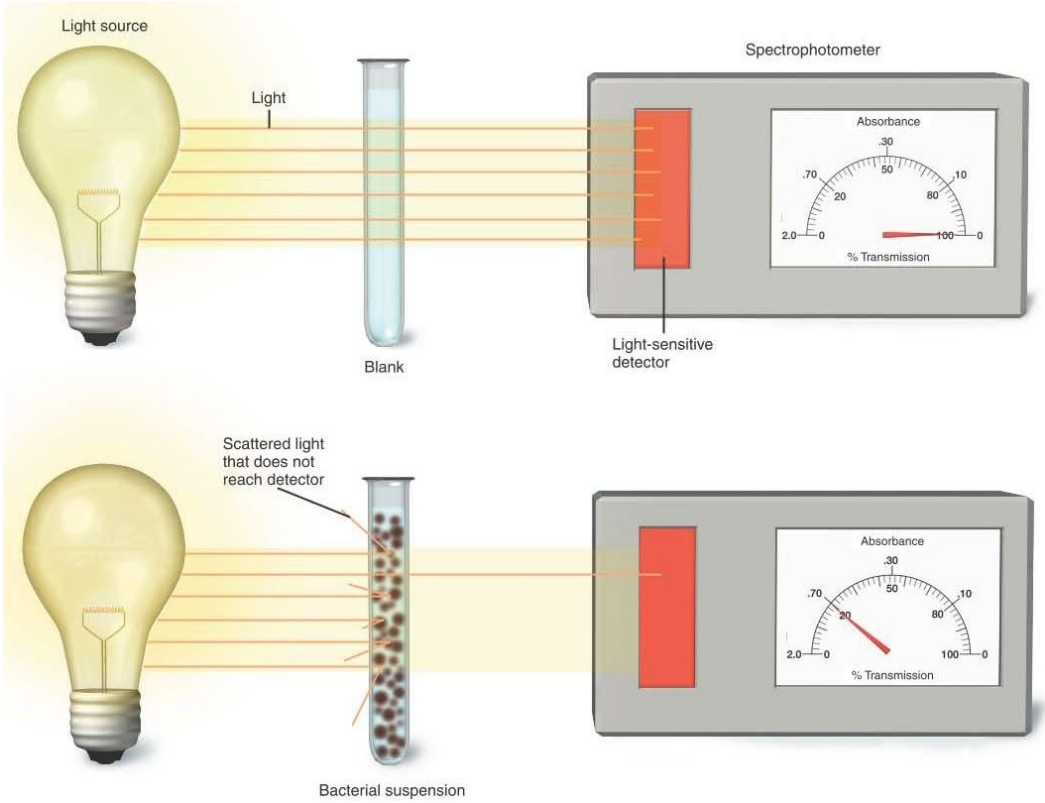
Turbidity measurement methods

When bacteria growing in a liquid medium are mixed, the culture appears turbid. This is because a bacterial culture acts as a colloidal suspension that blocks and reflects light passing through the culture.

Within limits, the light absorbed by the bacterial suspension will be directly proportional to the concentration of cells in the culture.

By measuring the amount of light absorbed by a bacterial suspension, one can estimate and compare the number of bacteria present.

The instrument used to measure turbidity is a spectrophotometer.



https://microbiologynotes.org/measurements-of-microbial-growth/#more-1406

Determination of Nitrogen Content

Bacteria average approximately 14 percent nitrogen on a dry weight basis, although this figure is subject to some variation introduced by changes in culture conditions or differences between species.

To measure growth by this technique, you must first harvest the cells and wash them free of medium and then perform a quantitative chemical analysis for nitrogen.

Determination of Dry Weight

The simplest technique of this sort is to measure the weight of cells in a sample.

Portions of a culture can be taken at particular intervals and centrifuged at high speed to sediment bacterial cells to the bottom of a vessel.

The sedimented cells (a cell pellet) are then washed to remove contaminating salt, and dried in an oven at 100-105°C to remove all water, leaving only the mass of components that make up the population of cells.

An increase in the dry weight of the cells correlates closely with cell growth.

Measurement of Specific Chemical Changes

The bacterial growth can be indirectly estimated by detecting specific changes caused in growth medium as a result of activity and multiplication of bacterial cells. It includes detecting activity cell products such as acid and gas production.

Dye reduction tests such as methylene blue and resazurin reduction tests are used to detect gas production of the bacteria.