FDE 330
FOOD BIOTECHNOLOGY

- Measurement Methods of Microbial Growth
- Effects of Environmental Conditions on Microbial Growth

Measurement Methods of Microbial Growth

Measurement of Mirobial Growth

Monitoring microbial growth in culture

- During a fermentation, methods are required for the routine determination of the microbial population, cell number and/or biomass, in order to monitor its progress.
- Numerous direct and indirect methods are available for this purpose.
- **Direct procedures** involve dry weight determination, cell counting by microscopy and plate counting methods.
- **Indirect methods** include turbidimetry, spectrophotometry, estimation of cell components (protein, DNA, RNA or ATP), and online monitoring of carbon dioxide production or oxygen utilization.
- The method adopted in any given situation depends upon the fermentation and any specific requirements. Several factors must be considered, such as the degree of accuracy and sensitivity needed, and the duration of the analysis. Estimation of unicellular organisms, provided that they are not prone to flocculation, is relatively straightforward, but filamentous organisms, fungi and actinomycetes present additional problems. Also, culture media vary in viscosity, colour and the quantity of particulate solids, all of which may influence the choice of monitoring method. The speed of analysis may be critical, as an instant indication of biomass or cell concentration is often required. However, faster methods are frequently less reliable, and longer procedures are generally more accurate and reproducible.

Measurement of Mirobial Growth

- can measure changes in number of cells in a population
- can measure changes in mass of population

Measuring Growth

- Direct methods – count individual cells
- Indirect Methods – measure effects of bacterial growth

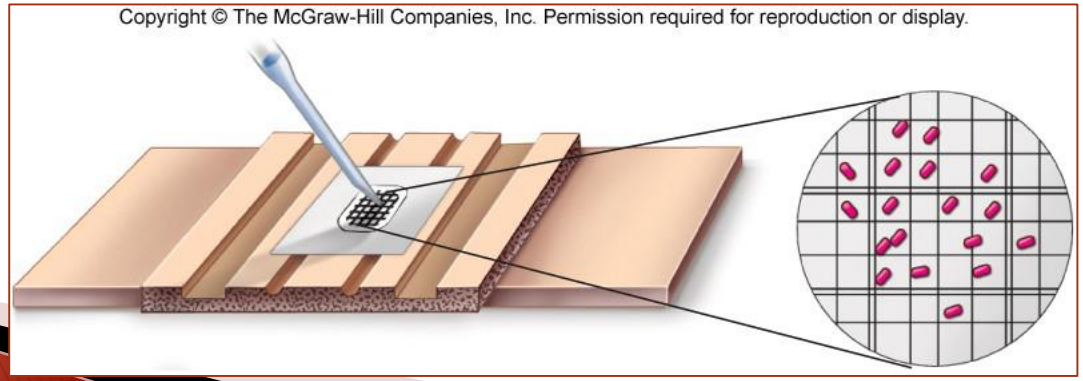
Direct microscopic counting methods

- **Cell numbers** in a suspension can be measured, except for filamentous organisms, by direct microscopic counts, using **Petroff-Hauser** or **Neubauer-type counting chambers**.

- The former is more suitable for counting bacteria. The counting grids, when the chamber is covered with a glass coverslip, hold a known volume of culture. By counting the number of cells within a proportion of the grid, the number of cells per millilitre can be determined.

- Direct microscopic counts are rapid, but limited **by their inability to distinguish living from dead cells**, unless differentiated by use of a vital staining technique.

- Also, samples must contain relatively high cell concentrations, normally a minimum of 5 x 10^6 cells/mL.



Direct microscopic counting methods

- It is the counting technique used to determine the total cell number.
- This is done with the aid of a **Petroff-Housser** or **Thoma slide** or **Hemocytometer**.
- In this method, specially developed slides are used.
- The Petroff Hausser Counting Chamber is used for bacteria counting.
- The Thoma Counting Chamber is used for yeast counting.
- Also, Howard slide method, which is the most commonly used method in mold counting, is used to determine the microbiological quality of raw material used in the production of tomato products such as tomato paste, tomato juice, ketchup and some fruit products.
- These are slides with certain depths into which cell samples are placed.
- There are horizontal and vertical lines on this section and the number of cells in each square is counted with the help of a microscope.
- Dyes such as methylene blue or trypan blue should be used to distinguish between living and dead cells. These dyes only stain dead cells. Thus, both total and viable cell counts can be done.

Electronic cell counters

- Electronic cell counting equipment is also rapid, but most methods are more suitable for larger unicellular microorganisms, such as yeasts, protozoa and some algae, and less useful for estimating bacteria.
- The **Coulter Counter**, for example, is used to count and size particles, and is based on the measurement of changes in electrical resistance produced by non-conductive particles suspended in an electrolyte.
- This method involves drawing a suspension of cells through a small aperture across which an electrical current is maintained.
- As a cell passes through the aperture it displaces its own volume of electrolyte and changes the electrical resistance.
- These changes are detected and converted to a countable pulse.
- However, it essentially counts particles, and is consequently prone to errors due to cells clumping and the presence of particulate debris.

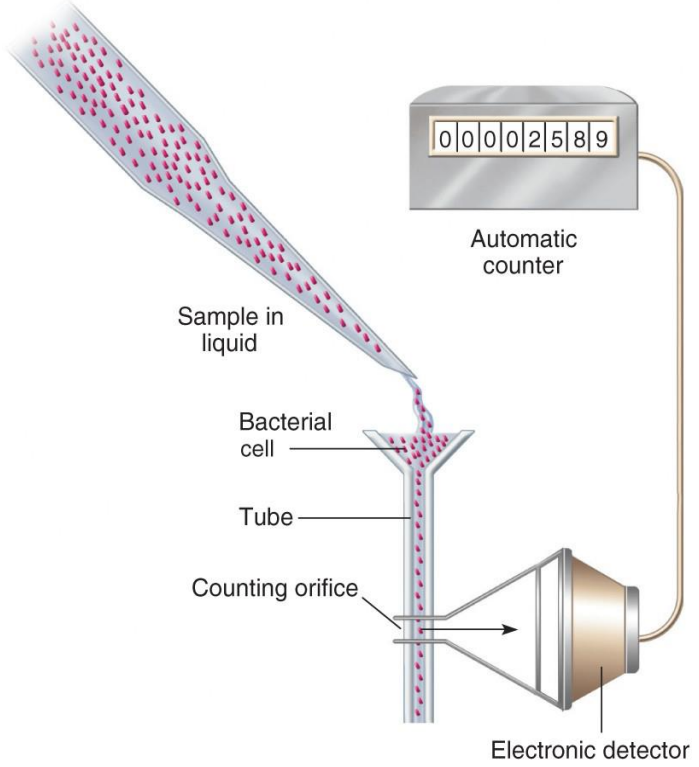


Plate counting techniques

- Plate counting methods detect viable cells, i.e. those able to form colonies on an appropriate solid nutrient medium.
- The two methods routinely used are **spread plating** and **pour plating**.
- Prior to plating, it is usually necessary to prepare a serial dilution series in sterile diluent for concentrated cell suspensions.
- Alternatively, for samples with low cell numbers, as in water analysis, a concentration step is required.

Plate counting techniques

- **In spread plating**, samples, usually 0.1 mL, are spread on the surface of a suitable agar-based nutrient medium using a sterile spreading device, e.g. a bent glass rod. The plates are then inverted and incubated at the optimum growth temperature. All resultant colonies should be well separated and easily counted. This also enables the isolation of pure cultures where required. However, microorganisms with low tolerance to oxygen do not grow and pour plating may be preferred.

- **In pour plating**, a suspension of microorganisms, normally a 1mL sample, is placed in a Petri dish and thoroughly mixed with molten agar media at 48–50°C, and allowed to solidify prior to incubation. Pour plates result in the development of microbial colonies throughout the agar. Those organisms with lower oxygen tolerance grow within the agar. Colonies of aerobic organisms often have variable sizes, as those nearer the surface have a better oxygen supply. Consequently, it is often harder to see similarity in colonial morphology between colonies on the surface and those within the agar. Also, counting may be more difficult than for spread plates. Care has to be taken to ensure that the molten agar is not too hot, otherwise some microbial cells may be injured and slow to form visible colonies or even killed.

Plate counting techniques

- For statistical reliability, results are recorded only for plates containing 30–300 colonies. Calculation of the cell concentration in the original sample is then carried out, taking into account the dilution and volume plated.
- Both methods measure **colony-forming units** (CFU).
- These techniques are accurate, but a minimum of 1–2 days incubation is usually necessary before the colonies are countable.
- However, more rapid microcolony counting techniques are also available.

Turbidimetric and spectrophotometric techniques

- These methods provide a simple, rapid and convenient means of **total biomass estimation**.
- They are usually performed at a specific optimum wavelength for each microorganism.
- Turbidimetric methods measure the light scattered by a suspension of cells, which is proportional to the cell concentration.
- Alternatively, spectroscopy may be employed, using absorbance or transmittance of a cell suspension. Some modern fermentation monitoring systems now employ methods based on near-infrared spectroscopy.
- Turbidimetric and spectrophotometric methods require the construction of appropriate calibration curves, prepared using standard cell suspensions containing known concentrations of cells.
- Also, care must be taken when interpreting the results if the fermentation broth contains particulate matter or is highly coloured.

Dry weight estimation

- This method determines the weight of total cells, both living and dead, in liquid culture samples.

- It involves separating the biomass from a known volume of a homogeneous cell suspension. This is usually achieved by filtration under vacuum, through a preweighed membrane filter with a pore size of 0.2µm or 0.45 µm. The filter with collected cells is 'washed' with water to remove any residual growth medium and dried to a constant weight in an oven at 105°C.

- Results are normally expressed as milligrams of cells per millilitre of culture.

- Obviously, any other suspended non-cellular materials above the size of the filter pores is also collected and can lead to errors. Further limitations are the time needed to obtain the results and the relatively large volume of sample required to obtain sufficient biomass for accurate weighing, as an individual bacterium weighs only about 10^{-12} g.

ATP bioluminometry

- ATP is rapidly lost from dead cells; consequently, it is very useful for determining the concentration of viable microorganisms.
- The amount of ATP in a sample can be quantified using ATP bioluminometry.
- This technique utilizes an enzyme–substrate complex, luciferase–luciferin, obtained from the firefly, *Photinus pyralis*, which generates a photon of light for each molecule of ATP.
- When an aliquot of luciferase–luciferin is added to ATP extracted from a sample of cell suspension, the light generated can be detected in a bioluminometer. The resulting signal is amplified and then expressed as a digital or analogue data output.

ATP bioluminometry

$$ATP + O_2 + \text{luciferin} \xrightarrow{\textit{luciferase}} \text{oxyluciferin} + AMP + CO_2 + PP_i + \text{photon of light (562 nm)}$$

- This procedure is very rapid and sensitive. Under optimum conditions as little as 10 femtomoles (1femtomole = 10^{-15} mol) of ATP can be detected, which is approximately equivalent to 1 yeast cell or about 10 bacteria.
- ATP bioluminometry is most suitable for direct measurement of samples that are not coloured, as quenching of light may be a problem.
- However, as the method is very sensitive, samples may require considerable dilution, which often overcomes any colour quenching problem.

On-line estimation

- On-line monitoring of fermenters can provide real-time estimation of biomass, and minimizes the requirement for repeated sampling and off-line analysis.
- Monitoring systems may involve optical density or capacitance-based probes.
- Also, the microorganisms involved in most fermentation processes will either have a requirement for oxygen and/or will produce carbon dioxide. In such cases it is theoretically possible to establish a mathematical relationship between factors, such as carbon dioxide evolution or oxygen utilization, and the biomass concentration within the bioreactor.
- Therefore, estimation of biomass concentrations and even product formation can be made by measuring these parameters on-line using carbon dioxide or oxygen detectors and biosensors attached to a computer.
- This can give an accurate estimate of the biomass concentration, provided that the mathematical algorithms developed are reliable.

Effects of Environmental Conditions on Microbial Growth

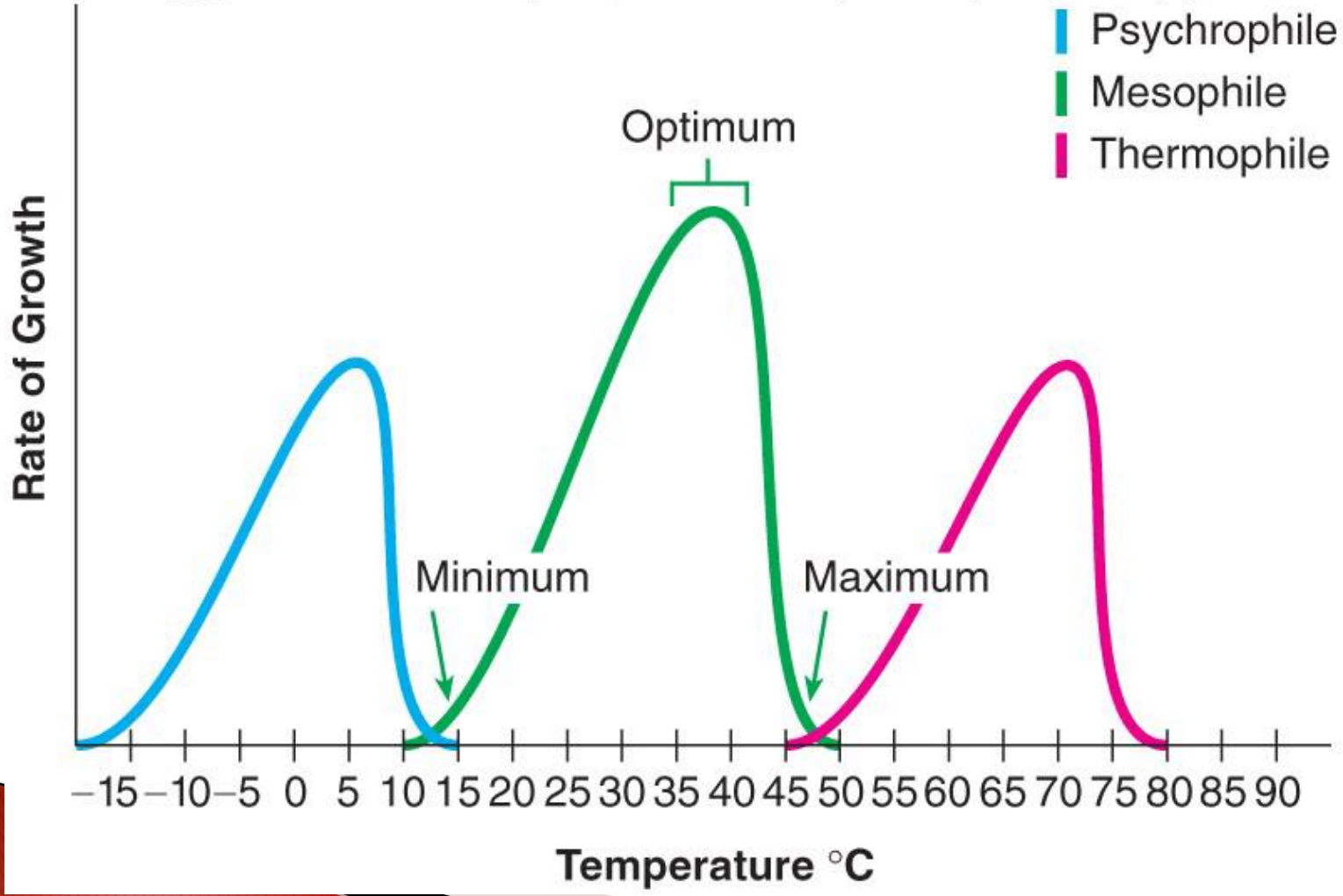
Effects of Environmental Conditions on Microbial Growth

- Growth and development of microorganisms are greatly affected by the chemical and physical conditions of their environment.

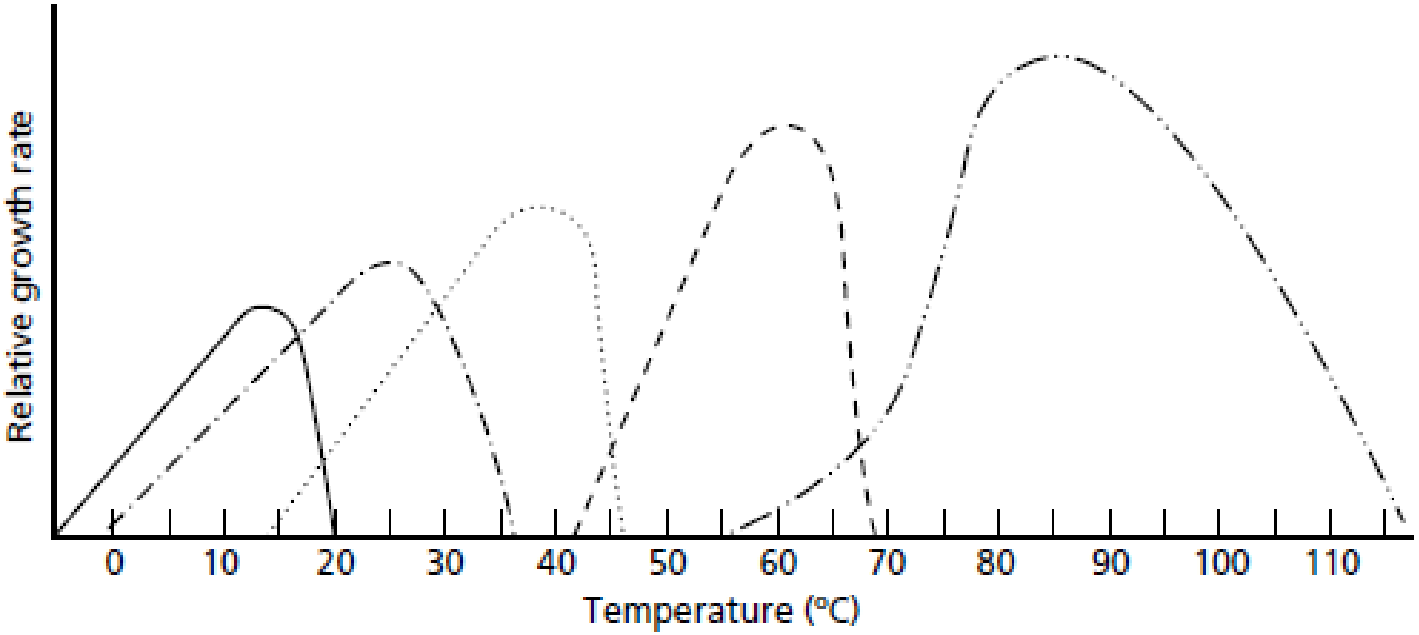
- Important environmental factors affect microbial growth;

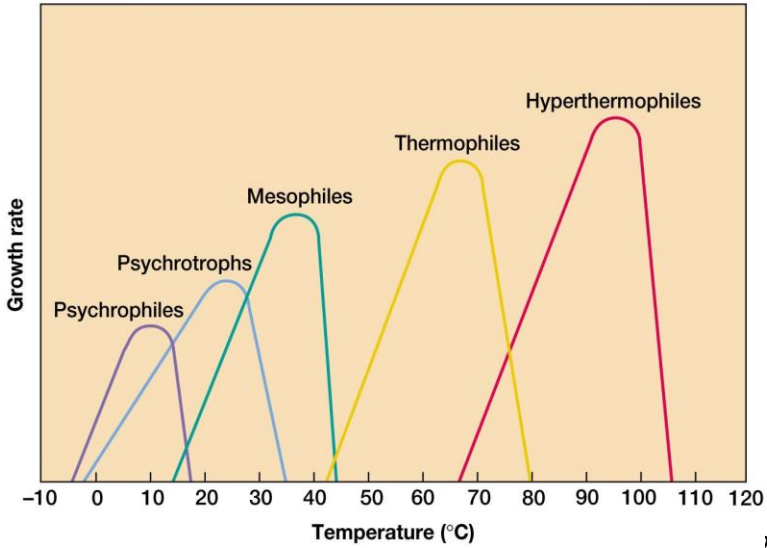
1. Temperature
2. pH
3. Oxygen
4. Solutes, water activity
5. Radiation
6. Hydrostatic pressure

Effects of temperature on growth



Temperature ranges for microbial growth





Effects of oxygen on growth

- Microorganisms are classified into five main groups on the basis of their requirements for oxygen.

1. **Obligate aerobes** grow only in the presence of oxygen, as it is required as the terminal electron acceptor for electron transport in aerobic respiration.
2. **Facultative anaerobes** function with or without oxygen, but grow more efficiently when oxygen is available.
3. **Microaerophiles** require some oxygen for the biosynthesis of certain compounds, but cannot grow at normal atmospheric oxygen concentrations of 21% (v/v). They must have lower oxygen levels of 2–10% (v/v).
4. **Aerotolerant anaerobes** essentially ignore oxygen and grow equally well in its presence or absence.
5. **Obligate anaerobes** cannot tolerate oxygen; exposure to it results in their death.