Nutritional Requirements

All forms of life, from microorganisms to human beings, share certain nutritional requirements for growth and normal functioning.

1. All organisms require a source of energy. Some rely on chemical compounds for their energy and designated as chemotrophs. Other can utilize radiant energy (light) and are called phototrophs. Both chemotrophs and phototrophs exist among bacteria.
2. All organisms require a source of electrons for their metabolism. Some organisms can use reduced inorganic compounds as electron donors and are termed lithotrophs (some may be chemolithotrophs, others photolithotrophs). Other organisms use organic compounds as electron donors and are called organotrophs (some are chemoorganotrophs others photoorganotrophs).
3. All organisms require nitrogen in some form for cell components. Bacteria are extremely versatile in this respect. Unlike eucaryotes, some bacteria can use atmospheric nitrogen. Others thrive on inorganic nitrogen compounds such as nitrates, nitrites, or ammonium salts, and still other derive nitrogen from organic compounds such as amino acids.
4. All organisms require oxygen, sulfur and phosphorus for cell components. Oxygen is provided in various forms such as water. Sulfur is needed for synthesis of certain amino acids (cysteine, cystine and methionine). Some bacteria require organic sulfur compounds, some are capable of utilizing inorganic sulfur compounds, and some can even use elemental sulfur. Phosphorus, usually supplied in the form of phosphate, is an essential component of nucleotides, nucleic acids, phospholipids, teichoic acids, and other compounds.
5. All living organisms require metal ions, such as K^{+}, Ca^{+}, Mg^{2+}, and Fe^{2+} for normal growth. Other metal ions are also needs but usually only at very low concentrations, such as Zn^{2+}, Cu^{2+}, Mn^{2+}, and Mo^{6+} Ni^{2+}, B^{3+} and Co^{2+}; these are often termed trace elements.
6. All living organisms contain vitamins and vitamin like compounds. These function either as coenzymes for several enzymes.
7. All living organism require water and in the case of bacteria all nutrients must be in aqueous solution before they can enter the cells.

Bacteriological Media

Chemically defined media are needed for the cultivation of autotrophs and are also useful for defining the nutritional requirements of heterotrophs. However, for the routine cultivation of heterotrophs, chemically defined media are not generally used. Instead, certain complex raw materials such as peptones, meat extract, and yeast extract are used, and the resulting media support the growth of a wide variety of heterotrophic bacteria. Agar is included as a nonnutritive solidifying agent when a solid medium is desired. A description of these raw materials is given

in Table-1. Examples of relatively simple liquid and solid media that support the growth of many common heterotrophs are nutrient broth and nutrient agar (Table-2). The addition of yeast extract to each of these formulas improves the nutrient quality, since yeast extract contains several of the B vitamis and other growth-promoting substances. Other complex supplements such as bovine rumen fluid, animal blood, blood serum, or extracts of plant and animal tissues may be required for the cultivation of certain fastidious heterotrophs.

Table-1

Raw Material	**Characteristic**	**Nutritional Value**
Beef extract	An aqueous extract of lean beef tissue concentrated a paste	Contains the water-soluble substances of animal tissue, which include carbohydrates, organic nitrogen compounds, water-soluble vitamins and salts
Peptone	The product resulting from the digestion f proteinaceous materials, e.g., meat, casein and gelatin; digestion of the protein material is accomplished with acids or enzymes; many different peptones (depending upon the protein used and the method digestion) are available for use in bacteriological media; peptones differ in their ability to support growth of bacteria.	Principal source of organic nitrogen; may also contain some vitamins and sometimes carbohydrate, depending upon the kind of proteinaceous material digested.
Agar	A complex carbohydrate obtained from certain marine algae; processed to remove extraneous substances	Used as a solidification agent for media; agar, dissolved in aqueous solution, gels when the temperature is reduced below 45^0C; agar not considered a source of nutrient to the bacteria
Yeast extract	An aqueous extract of yeast cells, commercially available as a powder	A very rich source of the B vitamins; also contains organic nitrogen and carbon compounds.

Table-2

Nutrient Broth:	
Beef Extract	: 3g
Pepton	: 5g
Water	: 1000ml
Nutrient Agar:	
Beef Extract	: 3g
Pepton	: 5g
Agar	: 15g
Water	: 1000ml

Types of Media

Many special-purpose media are needed to facilitate recognition, enumeration and isolation of certain types of bacteria.

Selective Media

These media provide nutrients that enhance the growth and predominance of a particular type of bacterium and do not enhance (and may even inhibit) other types of organisms that may be present.

Differential Media

Certain reagents or supplements, when incorporated into culture media, may allow differentiation of various kinds of bacteria.

Assay Media

Media of prescribed compositions and used for the assay of vitamins, amino acids, and antibiotics. Media of special composition are also available for testing disinfectants.

Media for Enumeration of Bacteria

Specific kinds of media are used for determining the bacterial content of such materials as milk and water. Their composition must adhere to prescribed specifications.

Maintenance Media

Satisfactory maintenance of the viability and physiological characteristics of a culture over time may require a medium different from that which is optimum for growth.

Solid and Semisolid Media

In addition to liquid media, solid and semisolid media are widely used for cultivation of bacteria. Solid media are useful for isolating bacteria or for determining the characteristics of colonies. The solidifying agent is usually agar, which at concentrations of 1.5 to 2.0 percent forms firm, transparent gels that are not degraded by most bacteria. Silica gel is sometimes used as an inorganic solidifying agent for autotrophic bacteria.

Semisolid media, prepared with agar at concentrations of 0.5 percent or less, have a soft, custard like consistency and are useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility.

Preparation of Media

The preparation of bacteriological media usually involves the following steps:

1. Each ingredient, or the complete dehydrated medium is dissolved in the appropriate volume of distilled water.
2. The pH of the fluid medium is determined with a pH meter and adjusted if necessary.
3. If a solid medium is desired, agar is added and the medium is boiled to dissolve the agar.
4. The medium is dispensed into tubes or flasks.
5. The medium is sterilized, generally by autoclaving. Some media (or specific ingredients) that are heat-labile are sterilized by filtration.

Temperature

The temperature that allows for most rapid growth during a short period of time (18 to 24 h) is known as the optimum growth temperature).

Psychrophiles are able to grow at 0^{o}C or lower, though they grow best at higher temperatures. May microbiologists restrict the term *psychrophile* to organisms that can grow at 0^{o}C but have an optimum temperature of 15^{o}C or lower and a maximum temperature of about 20^{o}C.

Psychrotroph or facultative psychrophile term is used for those organisms able to grow at 0^{o}C but which grow best at temperatures in the range of about 20 to 30^{o}C.

Mesophiles grow best within a temperature range of approximately 25 to 40^{o}C. For example, all bacteria that are pathogenic for humans and warm-blooded animals are mesophiles, most growing best at about body temperature (37^{o}C).

Themophiles grow best at temperatures about 45^{o}C. The growth range of many thermophiles extends into the mesophilic region; these species are designated facultative thermophiles. Other theromophiles cannot grow in the mesophilic range; these are termed true thermophiles, obligate thermophiles, or stenothermophiles.

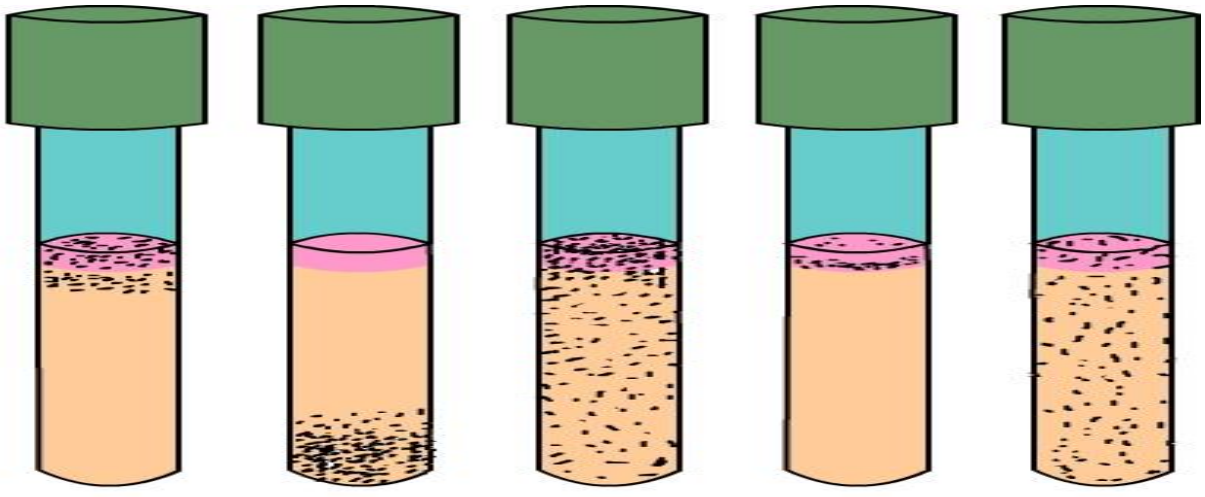
Table-3

Name of Species	Minimum	Optimum	Maximum
Vibrio marinus strain MP-1	-1	15	20
Vibrio psychroerythus	0	15	19
Pseudomonas fluorescens	4	25-30	40
Staphylococcus aureus	6.5	30-37	46
Corynebacterium diphtheria	15	37	40
Nissseria gonorrheae	30	35-36	38.5
Streptococcus thermophilus	20	40-45	50
Thermoactinomyces vulgaris	27-30	60	65-70

Thermus aquticus	40	70-72	79

Gaseous Requirements

1. **Aerobic bacteria** require oxygen for growth and can grow when incubated in an air atmosphere (i.e., 21 percent oxygen).
2. **Anaerobic bacteria** do not use oxygen to obtain energy; moreover, oxygen is toxic for them and they cannot grow when incubated in the air atmosphere. Some can tolerate low levels of oxygen (non-stringent or tolerant anaerobes), but others (stringent or strict anaerobes) cannot tolerate even low levels and may die upon brief exposure to air.
3. **Facultative anaerobic** bacteria do not require oxygen for growth, although they may use it for energy production if it is available. They are not inhibited by oxygen and usually grow as well under an air atmosphere as they do in the absence of oxygen.
4. **Microaerophilic bacteria** require low levels of oxygen for growth but cannot tolerate the level of oxygen present in an air atmosphere.
5.



Position of growth of microbial cells in culture medium depending on oxygen requirement and tolerance. a. Obligate aerobes, b. Obligate anaerobes, c. Facultative anerobes, d. Microaerophiles, e. Aerotolerant anaerobes.

Reproduction

The most common, and no doubt the most important, mode of cell division in the usual growth cycle of bacterial populations is transverse binary fission, in which a single cell divides after developing a transverse septum (crosswall) (Fig.1 A, B, C). Transverse binary fission is an asexual reproductive process. (Infrequently, in some species, binary fission may be preceded by a mating or conjugation of cells a type of sexual process).

Budding

(Some bacteria, such as *Rhodopseudomonas acidophila*, reproduce by budding, a process in which a small protuberance (bud) develops at one end of the cell; this enlarges and eventually develops into a new cell which separates from the parent (Fig.1D). In some budding bacteria, such as *Hyphomicrobium* species, the bud may develop at the end of a prostheca.

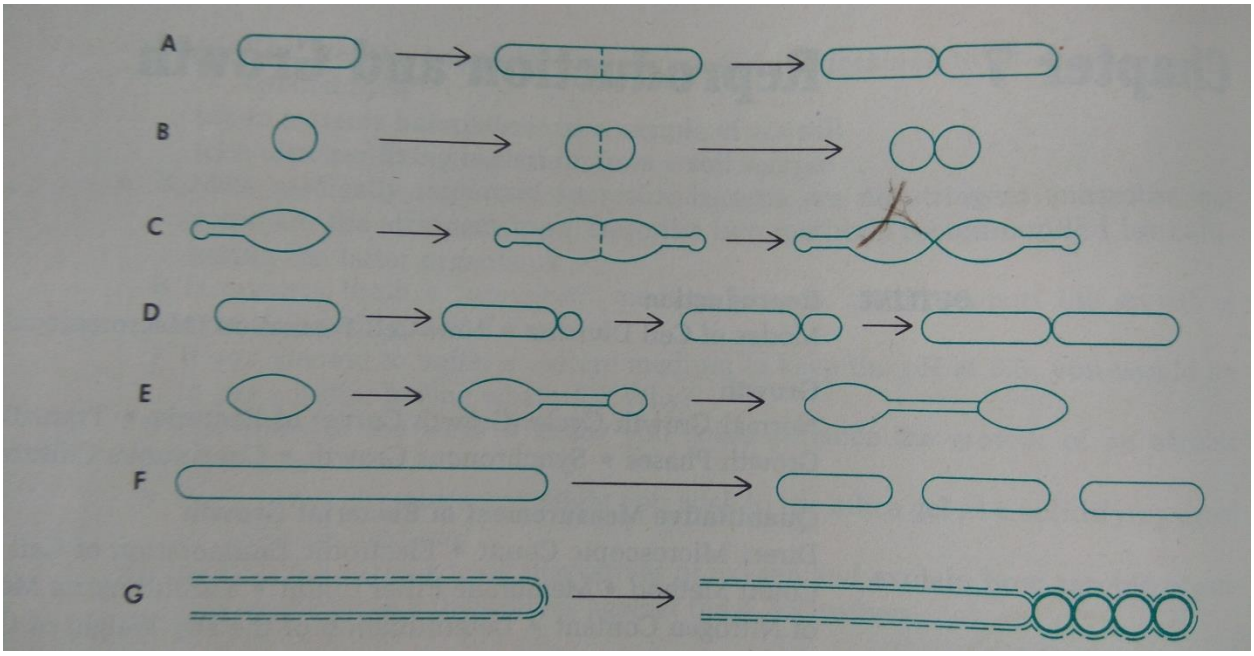


Fig-1

Fragmentations

Bacteria that produce extensive filamentous growth, such as *Nocardia* species, reproduce by fragmentation of the filaments into small bacillary or coccoid cells, each of which gives rise to new growth (Fig.1E).

Formation of Conidiospores or Sporangiospores

Species of the genus *Streptomyces* and related bacteria produce many spores per organism by developing cross walls (septation) at the hyphall tips; each spore gives rise to a new organism (Fig. 1G).

New Cell Formation (Macromolecular Synthesis)

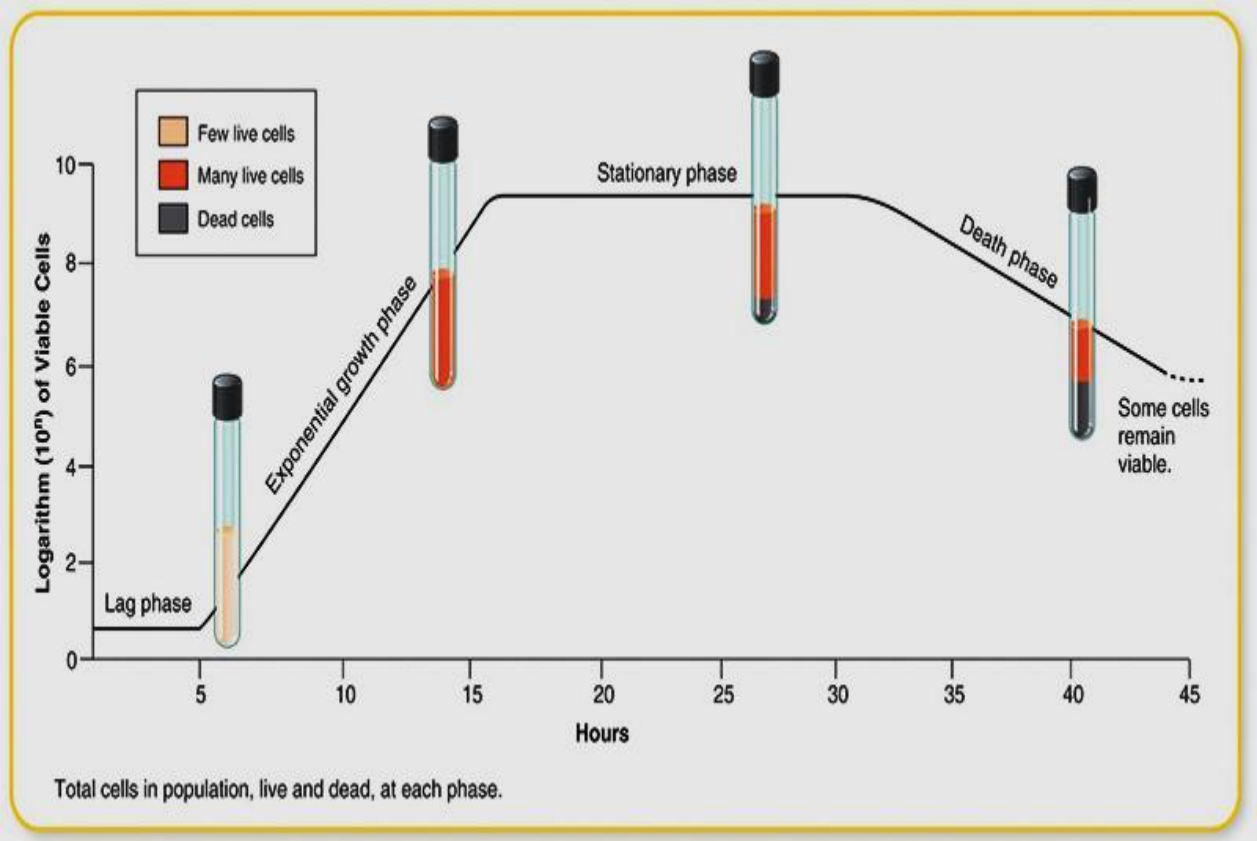
A bacterial cell inoculated into a fresh medium selectively takes up nutrients from its environment. Many biochemical syntheses then take place. The nutrients are converted into cell substance –RNA, DNA, protein, enzymes and other macromolecules. Cell mass and cell size increase and new cell wall building blocks are synthesized. Subsequently, the process of binary fission is initiated, ultimately resulting in the formation of two new cells.

Bacterial/ Microbial Growth

The most common means of bacterial reproduction is binary fission; one cell divides, producing two cells.

Bacterial Normal Growth Cycle (Growth Curve) Protocol

- When fresh liquid medium is inoculated with a given number of bacteria and incubated for sufficient period of time, it gives a characteristic growth pattern of bacteria.
- If the bacterial population is measured periodically and log of number of viable bacteria is plotted in a graph against time, it gives a characteristic growth curve which is known as **growth curve.**
- Measuring the growth rate of bacteria is a fundamental microbiological technique, and has widespread use in basic research as well as in agricultural and industrial applications.



Principle of Bacterial Growth Curve

When bacteria are inoculated into a liquid medium and the cell population is counted at intervals, it is possible to plot a typical bacterial growth curve that shows the growth of cells over time. It shows **four distinct phases** of growth.

1. **Lag phase:** Slow growth or lack of growth due to physiological adaptation of cells to culture conditions or dilution of exoenzymes due to initial low cell densities.
2. **Log or exponential phase:** Optimal growth rates, during which cell numbers double at discrete time intervals known as the mean generation time.
3. **Stationary phase:** Growth (cell division) and death of cells counterbalance each other resulting in no net increase in cell numbers. The reduced growth rate is usually due to a lack of nutrients and/or a buildup of toxic waste constituents.
4. **Decline or death phase:** Death rate exceeds growth rate resulting in a net loss of viable cells.

Turbidimetric determination is useful for plotting growth curves of bacteria in broth or liquid media. It is one of the simplest methods used to analyze trends in growth because it uses a spectrophotometer to track changes in the optical density (OD) over time. In other words, as the number of cells in a sample increase, the transmission of light through the sample will decrease.

Materials Required for Bacterial Growth Curve

1. Bacterial culture (E. coli), Broth (Luria Bertani (LB) Broth, Nutrient Broth)
2. Glass wares: Conical flasks, Measuring cylinder, Sterile test tubes, Sterile Petriplates
3. Reagents: Distilled water
4. Other requirements: Incubator, Shaker, Spectrophotometer, Micropipettes, Tips, Sterile Loops

Procedure of Bacterial Growth Curve

Day 1:

1. Using sterile loop, streak a loopful of bacterial culture onto the agar plate.
2. Incubate at 37^{o}C for 18-24 hours.

Day 2:

1. Pick up a single colony of each strain from the agar plate and inoculate it into a test tube containing 10 ml of autoclaved broth.
2. Incubate the test tube overnight at 37^{o}C.

Day 3:

1. Take 250 ml of autoclaved broth in a sterile 500 ml conical flask.
2. Inoculate 5 ml of the overnight grown culture in above flask.
3. Take OD at zero hour. Incubate the flask at 37^{o}C.
4. Aliquot 1 ml of the culture suspension at an interval of every 30 minutes and take the optical density (OD) at a wavelength of 600 nm using spectrophotometer, till the reading becomes static.

Alternatively, 50-100 µl of formaldehyde can be added to all the 1 ml aliquots of culture suspension taken after every 30 minutes. Optical density of all the aliquots can be taken at the end of the experiment.

5. At the end of experiment, plot a graph of time in minutes on X axis versus optical density at 600nm on Y axis to obtain a growth curve of bacteria.

Expected Result of Bacterial Growth Curve

A logarithmic growth curve is obtained showing the changes in size of a bacterial population over time in the culture. The growth curve is hyperbolic due to exponential bacterial growth pattern.

Quantitative Measurement of Bacterial Growth

We have seen that the term growth as commonly applied in microbiology refers to the magnitude of the total population. Growth in this sense can be determined by numerous techniques based on one or more of the following types of measurement:

Electronic Enumeration of Cell Numbers

In this method, the bacterial suspension is placed inside an electronic particle counter, within which the bacteria are passed through a tiny orifice 10 to 30 µm in diameter. This orifice connects the two compartments of the counter which contain an electrically conductive solution. As each bacterium passes through the orifice, the electrical resistance between the two compartments increase momentarily. This generates an electrical signal which is automatically counted. Although this method is rapid it requires sophisticated electronics equipments; moreover the orifice tends to become clogged.

The Plate-Count Method

This method, allows determination of the number of cells that will multiply under certain defined conditions. A measured amount of the bacterial suspension is introduced into a Petri dish, after which the agar medium (maintained in liquid form at 45^0C) is added and the two thoroughly mixed by rotating the plate. When the medium solidifies, the organisms are trapped in the gel. Each organism grows, reproducing itself until a visible mass of organisms –a colony - develops; i.e., one organism gives rise to one colony hence, a colony count performed on the plate reveals the viable microbial population of the inoculums. The original sample is usually diluted so that the number of colonies developing on the plate will fall in the range of 30 to 300. Within this range the count can be accurate, and the possibility of interference of the growth of one organism with that of another is minimized.

Culture of bacteria or any other sample containing bacteria in suspension. 1 ml transferred to 99 ml dilution blank; 1 ml transferred to 2d 99 ml dilution blank; 1 ml transferred to 3d 99 ml dilution blank. After addition of inoculums to plate, 15 to 20 ml of agar medium is poured into each plate. The plate is gently rotated for thorough distribution of inoculums throughout the medium. Plates are placed, inverted, in an incubator for 24 hr or longer. A plate is selected which contains from 30 to 300 colonies. Number of Colonies counted on plate X dilution of sample = number of bacteria per ml.

The development of the colony from one cell can occur when the bacterial suspension is homogeneous and no aggregates of cells are present; however, if the cells have a tendency to aggregate, e.g., cocci in clusters (staphylococci), chains (streptococci), or pairs (diplococcic), the resulting counts will be lower than the number of individual cells. For this reason the "counts" are often reported as colony-forming units per milliliter rather than number of bacteria per milliliter.

Membrane-Filter Count

A very useful variation on the plate-count technique is based on the use of molecular or membrane filters. These filters have a known uniform porosity of predetermined size sufficiently small to trap microorganisms. This technique is particularly valuable in determining the number of bacteria in a large sample that has a very small number of viable cells: e.g., the bacteria in a large volume of air or water can be collected simply by filtering them through an assembly. The membrane, with its trapped bacteria, is then placed in a special plate containing a pad saturated with the appropriate medium. Special media and dyes can be used to make it easier to detect certain types of organisms than with the conventional plate count.

Turbidmetric Methods

Anyone who has tried to see through a fog realizes that visibility is reduced in proportion to the density of the fog and the distance between the observer and the object that he or she is looking at. This is because each droplet of water in fog absorbs and scatters the light passing through it and the more droplets in the light path, the less one can see. Similarly, bacteria in a suspension absorb and scatters the light passing through them, so that a culture of more than 10^7 to 10^8 cells per milliliter appears turbid to the naked eye. A spectrophotometer or colorimeter can be used for turbidimetric measurements of cell mass. Turbidimetry is a simple, rapid method for following growth; however, the culture to be measured must be dense enough to register some turbidity on the instrument. Moreover, it may not be possible or measure cultures grown in deeply colored media or cultures that contain suspended material other than bacteria. It must also be recognized that dead as well as living cells contribute to turbidity.

Determination of Nitrogen Content

The major constituent of cell material is protein, and since nitrogen is a characteristic part of proteins, one can measure a bacterial population or cell crop in terms of bacterial nitrogen. Bacteria average approximately 14 percent nitrogen on a dry-weight basis, although this figure is subject to some variation introduced by changed in cultural 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. Bacterial nitrogen determination are somewhat laborious and can be performed only on specimens free of all other sources of nitrogen. Furthermore, the method is applicable only for concentrated populations. For these and other reasons, this procedure is used primarily in research.

Determination of Dry Weight of Cells

This is the most direct approach for quantitative measurement of a mass of cells. However, it can be used only with very dense suspensions, and the cells must be washed free of all extraneous matter. Moreover, dry weight may not always be indicative of the amount of living material in cells. Yet, for may organisms the determination of dry weight is an accurate and reliable may to measure growth is widely used in research.

Measurement of a Specific Chemical change Produced on a Constituent of the Medium

As an example of this method of estimating cell mass, we may take a species that produces an organic acid from glucose fermentation. The assumption is that the amount of acid produced, under specified condition and during a fixed period of time, is proportional to the magnitude of the 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.

Table-3. Summary of Methods for Measuring Bacterial Growth

Method	Some Applications	Manner in which Growth Is Expressed
Microscopic count	Enumeration of bacteria in vaccines and cultures	Number of cells per ml
Electronic enumeration	Same as for microscopic count	Same as for microscopic count
Plate count	Enumeration of bacteria in milk, water foods, soil, cultures, etc.	Colony-forming units per ml
Membrane filter	Same as plate count	Same as plate count
Turbidmetric measurement	Microbiological assay, estimation of cell crop in broth, cultures, or aqueous suspensions	Optical density (absorbance)

Method	Some Applications	Manner in which Growth Is Expressed
Nitrogen determination	Measurement of cell crop from heavy culture suspensions to be used for research in metabolism	Mg nitrogen per ml
Dry weight determination	Same as for nitrogen determination	Mg dry weight of cells per ml
Measurements of biochemical activity, e.g. acid production by cultures	Microbiological assays	Milliequivalents and acid per ml or per culture