MICROBIAL GROWTH

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*INTRODUCTION

REQUIREMENT FOR MICROBIAL GROWTH

> PHYSICAL REQUIREMENT

><u>NUTRITIONAL REQUIREMENT</u>

BACTERIAL GROWTH CURVE

*FACTORS AFFECTING BACTERIAL GROWTH

COUNTING OF BACTERIAL CELL

INTRODUCTION

What is Microbial GROWTH

Living organisms grow and reproduce. The growth indicates that an organism is in active metabolism. In plants & animals, we can see the increase in height or size. Growth common refers to increase in size but with microorganisms particularly bacteria, this term refers to changes in total population rather than increase in size or mass of individual organisms. The change in population in bacteria chiefly involves Binary fission.

A cell dividing by binary fission is immortal unless subjected to stress by nutrient depletion or environmental stress. Therefore, a single bacterium continuously divides. 1 cell divides and providing 2 cells and 2 cells divide providing 4 cells and so on. Therefore, the population increases by geometric progression.



PHYSICAL REQUIREMENT

CHEMICAL REQUIREMENT

REQUIREMENTS FOR MICROBIAL GROWTH

PHYSICAL REQUIREMENTS

- TEMPERATURE
- pH

27.2012

 OSMOTIC PRESSURE CHEMICAL
 REQUIREMENTS

- CARBON
- NITROGEN, SULFUR & PHOSPHORUS
- TRACE ELEMENTS
- OXYGEN
- ORGANIC GROWTH FACTORS



Temperature

- Temperature is the most important factor that determines the rate of growth, multiplication, survival, and death of all living organisms.
- High temperatures damage microbes by denaturing enzymes, transport carriers, and other proteins.
- Microbial membrane are disrupted by temperature extremes.
- At very low temperatures membranes also solidify and enzymes also do not function properly.



PSYCHROPHILE

- The term psychrophile was first used by S. Schmidt-Nelson.
- Extremophilic organisms that are capable of growth and reproduction in cold temperatures
- Temperature range: -20°C to +10°C.
- Examples: Oscillatoria, Chlamydomonas nivalis, Methanogenium, etc.





MESOPHILES

 Grows best in moderate temperature.
 Temperature range: 20°C to 45°C.
 Examples: Escherichia coli, Streptococcus pneumoniae, etc.



THERMOPHILES

- Derived from Greek word thermotita meaning heat and philia meaning love.
- Heat-loving microorganisms.
- Grow at 50°C or higher. Their growth minimum is usually around 45°C and often optima between 50 and 80°C.
- Examples: Thermus aquaticus, Geogemma barossii, etc.

pН

- pH refers to negative logarithm of hydrogen ion concentration.
- Microbial growth is strongly affected by the pH of the medium.
- Drastic variations in cytoplasmic pH disrupt the plasma membrane or inhibit the activity of enzymes and membrane transport protein.



ACIDOPHILES Grow between pH 0 and 5.5. Examples: Ferroplasma, Thiobacillus thioxidans, Sulfolobus acidocaldarius, etc.

<u>ALKALOPHILES</u>

Grow between pH range of 7.5 to 14. Examples: Thermococcus alcaliphilus, etc.

Neutrophiles

Grow between pH 5.5 to 8.0 Examples: Lactobacillus acidophillus, E. coli, Pseudomonas aerunginosa, etc.



 Osmotic pressure is the minimum pressure which needs to be applied to a solution to prevent the inward flow of water across a SPM.

- Types of solution:
 - 1. Hypotonic 2. Isotonic 3. Hypertonic

 Water activity of a solution is 1/100 the relative humidity of the solution. It is equivalent to the ratio of the vapour pressure of solution to that of pure water.
 Wapour pressure of solution

Water activity=

Vapour pressure of pure water

Classification of bacteria according to osmotic pressu

 Osmotolerant are those microorganisms which can grow at relatively high salt concentration. Examples: Aeromonas spp., Staphylococcus spp, etc.

Halophiles - Grow in the presence of salt at conc. Above 0.2 to 0.6.
 Examples: Halobacterium halobium

CHEMICAL REQUIREMENT

Every organism must find in its environment all of the substances required for energy generation and cellular biosynthesis. The chemicals and elements of this environment that are utilized for bacterial growth are referred to as **nutrients** or **nutritional requirements**. In the laboratory, bacteria are grown in **culture media** which are designed to provide all the essential nutrients in solution for bacterial growth.

At an elementary level, the nutritional requirements of a bacterium such as *E. coli* are revealed by the cell's elemental composition, which consists of C, H, O, N, S. P, K, Mg, Fe, Ca, Mn, and traces of Zn, Co, Cu, and Mo. These elements are found in the form of water, inorganic ions, small molecules, and macromolecules which serve either a structural or functional role in the cells. The general physiological functions of the elements are outlined in the Table below.

MAJOR ELEMENTAL COMPOSITION OF MICROBIAL CELL

Ele	% DCW	Chemical form used by the microbe	Physiological functions		
C	50	Organic compounds, CO, CO ₂	major constituents of cell		
0	20	Organic compounds, H_2O , O_2 , CO_2	material		
N	14	Organic compounds, NH4 ⁺ , NO3 ⁻ , N2	proteins, nucleic acids, lipids, carbohydrates and others		
н	8	Organic compounds, H2O, H2	carbony drates and others		
Ρ	3	HPO42-	nucleic acids, phospholipids, teichoic acid, coenzymes		
S	1	organic sulfur compounds, SO4 ²⁻ , HS ⁻ , S ⁰ , S2O3 ²⁻	proteins, coenzymes		
k	1	К*	major inorganic cation, compatible solute, enzyme cofactor		
Na	1	Na ⁺	transport, energy transduction		
Ca	0.5	Ca ²⁺	enzyme cofactor, bound to cell wall		
Mg	0.5	Mg ²⁺	enzyme cofactor, bound to cell wall, membrane & phosphate esters		
CI	0.5	CI-	major inorganic anion		
Fe	0.2	Fe ²⁺ , Fe ³⁺	cytochromes, ferredoxin, cofactor		

Carbon

Structural backbone of living matter, it is needed for all organic compounds to make up a living cell

Chemoheterotrophs get most of their carbon from the source of their energy---organic materials such as proteins, carbohydrates and lipids

Chemoautotrophs and photoautotrophs derive their carbon from carbon dioxide Nitrogen, Sulfur and Phosphorus

For synthesis of cellular material

Nitrogen and sulfur is needed for protein synthesis

Nitrogen and phosphorus is needed for syntheses of DNA, RNA and ATP

Nitrogen- 14% dry weight of a bacterial cell Sulfur and phosphorus- 4%

Trace Elements

 Microbes require very small amounts of other mineral elements, such as Fe, Cu, Mo, Zn

 Essential for certain functions of certain enzymes

 Assumed to be naturally present in tap water and other components of media

TRACES OF NUTRITION'S

Element	Chemical form used by the microbe	Physiological functions
Mn	Mn ²⁺	superoxide dismutase, photosystem II
Со	Co ²⁺	coenzyme B12
Ni	Ni⁺	hydrogenase, urease
Cu	Cu ²⁺	cytochrome oxidase, oxygenase
Zn	Zn ²⁺	alcohol dehydrogenase, aldolase, alkaline phosphatase, RNA and DNA polymerase, arsenate reductase
Se	SeO3 ²⁻	formate dehydrogenase, glycine reductase
Мо	MoO4 ²⁻	nitrogenase, nitrate reductase, formate dehydrogenase, arsenate reductase
W (tungsten)	WO4 ²⁻	formate dehydrogenase, aldehyde oxidoreductase



Obligate Aerobes - only aerobic growth, oxygen required, growth occurs with high concentration of oxygen

Facultative Aerobes - both aerobic and anaerobic growth, greater growth in presence of water, growth is best in presence of water but still grows without presence of oxygen

Obligate Anaerobe - only anaerobic growth, growth ceases in presence of oxygen, growth occurs only when there is no oxygen

Aerotolerate Anaerobe - only anaerobic growth, but continues in presence of oxygen, oxygen has no effect

Microaerophiles - only aerobic growth, oxygen required in low concentration, growth occurs only where a low concentration of oxygen has diffused into medium



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Organic Growth Factors

Essential organic compounds an organism is unable to synthesize, they must be directly obtained from the environment

Growth factors are organized into three categories.

- 1.purines and pyrimidines: required for synthesis of nucleic acids (DNA and RNA)
- **2.amino acids**: required for the synthesis of proteins
- 3.vitamins: needed as coenzymes and functional groups of certain enzymes

Some bacteria lack the enzymes needed for synthesis for certain vitamins, so they must obtain them directly Examples: amino acids, purines, pyrimidines



Bacterial Growth Curve

The growth of bacteria in batch culture can be modeled with 4 different phases: 1.Lag phase 2.Log phase or exponential phase **3.Stationary phase** 4.Death phase





Period of little or no cell division

Can last for 1 hour or several days

Cells are not dormant

Undergoing a period of intense metabolic activity : DNA and enzyme synthesis

LOG PHASE

Period of growth also known as logarithmic increase

Sometimes called as exponential growth phase

Cellular respiration is most active during this period

Metabolic activity is active and is most preferable for industrial purposes

Sensitive to adverse conditions

STATIONARY PHASE

- Period of equilibrium
- Metabolic activity of surviving cells slows down which stabilizes the population
- Cause of discontinuity of exponential growth is not always clear
- May play a role: exhaustion of nutrients, accumulation of waste products and harmful changes in pH
- Chemostat continuous culture used in industrial fermentation

DEATH PHASE

Also known as Logarithmic Decline Phase

Continues until a small fraction of the population is diminished

Some population dies out completely

Others retain surviving cells indefinitely while others only retain for a few days

FACTORS AFFECTING GROWTH



Temperature

Theoretically, bacteria can grow at all temperatures between the freezing point of water and the temperature at which protein or protoplasm coagulates. Somewhere between these maximum and minimum points lies the optimum temperature at which the bacteria grow best.

Temperatures below the minimum stop bacterial growth but do not kill the organism. However, if the temperature is raised above the maximum, bacteria are soon killed. Bacteria can be classified according to temperature preference: *Psycrophilic* bacteria grow at temperatures below 16°C, *mesophilic* bacteria grow best at temperatures between 16 and 40°C, and *thermophilic* bacteria grow best at temperatures above 40°C.



Bacteria cannot grow without water. Many bacteria are quickly killed by dry conditions whereas others can tolerate dry conditions for months; bacterial spores can survive dry conditions for years. Water activity (AW) is used as an indicator of the availability of water for bacterial growth.

NUTRIENT AVAILABILITY

Bacteria need nutrients for their growth and some need more nutrients than others.

Lactobacilli live in milk and have lost their ability to synthesis many compounds, while *Pseudomonas* can synthesis nutrients from very basic ingredients.

Bacteria normally feed on organic matter; as well as material for cell formation organic matter also contains the necessary energy. Such matter must be soluble in water and of low molecular weight to be able to pass through the cell membrane. Bacteria therefore need water to transport nutrients into the cell.

oxygen supply

Animals require oxygen to survive but bacteria differ in their requirements for, and in their ability to utilise, oxygen. Bacteria that need oxygen for growth are called aerobic. Oxygen is toxic to some bacteria and these are called anaerobic. Anaerobic organisms are responsible for both beneficial reactions, such as methane production in biogas plants, and spoilage in canned foods and cheeses. Some bacteria can live either with or without oxygen and are known as *faculative anaerobic* bacteria.

ACIDITY OF THE MEDIUM

The acidity of a nutrient substrate is most simply expressed as its pH value. Sensitivity to pH varies from one species of bacteria to another. The terms pH optimum and pH maximum are used. Most bacteria prefer a growth environment with a pH of about 7, i.e. neutrality.

Bacteria that can tolerate low pH are called *aciduric*. Lactic acid bacteria in milk produce acid and continue to do so until the pH of the milk falls to below 4.6, at which point they gradually die off. In canning citrus fruits, mild heat treatments are sufficient because the low pH of the fruit inhibits the growth of most bacteria.

Bacteria Enumeration

As part of daily routine, the laboratory microbiologist often has to determine the number of bacteria in a given sample as well as having to compare the amount of bacterial growth under various conditions. Enumeration of microorganisms is especially important in dairy microbiology, food microbiology, and water microbiology.

Some of the methods used involve diluting the sample to a point at which the number of bacteria has been reduced to very small numbers. This enables an estimate to be established for quantifying the bacteria. Direct counts of bacteria require a dye to be introduced to the populations of bacteria to allow the observer to view the bacteria.

VIABLE (STANDARD) PLATE COUNT

Viable Plate Count (also called a Standard Plate Count) is one of the most common methods, for enumeration of bacteria. Serial dilutions of bacteria are plated onto an agar plate. Dilution procedure influences overall counting process. The suspension is spread over the surface of growth medium. The plates are incubated so that colonies are formed. Multiplication of a bacterium on solid media results in the formation of a macroscopic colony visible to naked eye. It is assumed that each colony arises from an individual viable cell. Total number of colonies is counted and this number multiplied by the dilution factor to find out concentration of cells in the original sample.

A major limitation in this method is selectivity. The nature of the growth medium and the incubation conditions determine which bacteria can grow and thus be counted. Viable counting measures only those cells that are capable of growth on the given medium under the set of conditions used for incubation. Sometimes cells are viable but non-culturable. The number of bacteria in a given sample is usually too great to be counted directly. However, if the sample is serially diluted and then plated out on an agar surface in such a manner that single isolated bacteria form visible isolated colonies, the number of colonies can be used as a measure of the number of viable (living) cells in that known dilution. The viable plate count method is an indirect measurement of cell density and reveals information related only to live bacteria.

PROCEDURE VIABLE PLATE COUNT

We will be testing four samples of water for the Viable Count. The samples include:

1) water from a drinking fountain

2) boiled water from a drinking fountain

3) water from the local river

4) boiled water from the local river

You will need **DATA TABLE 1** to input your data and calculate the number of CFU per ml.

Data Table 1					
		V 1-	able Flate Coulit		
Sample	Dilution Factor	# of Colonies	Dilution Factor X # of Colonies	# of CFU / ml	
Faucet Water					
River Water					
Boiled Faucet Water					
Boiled River Water					

CFUs per ml of sample = The # of colonies counted X The dilution factor of the plate counted

1) Take 6 dilution tubes, each containing 9 ml of sterile saline.

2) Dilute 1 ml of a sample by withdrawing 1 ml of the sample and dispensing this 1 ml into the first dilution tube.
3) Using the same procedure, withdraw 1 ml from the first dilution tube and dispense into the second dilution tube. Subsequently withdraw 1 ml from the second dilution tube and dispense into the third dilution tube. Continue doing this from tube to tube until the dilution is completed.



4) Transfer 1 ml from each of only the last three dilution tubes onto the surface of the corresponding agar plates.

5) Incubate the agar plates at 37°C for 48 hours.
6) Choose a plate that appears to have between 30 and 300 colonies.



DF =10,000 DF =1,000,000 DF =1,000,000

DIRECT MICROSCOPIC CELL COUNT

In the direct microscopic count, a counting chamber with a ruled slide is employed. It is constructed in such a manner that the ruled lines define a known volume. The number of bacteria in a small known volume is directly counted microscopically and the number of bacteria in the larger original sample is determined by extrapolation.

The Petroff-Hausser counting chamber for example, has small etched squares 1/20 of a millimeter (mm) by 1/20 of a mm and is 1/50 of a mm deep. The volume of one small square therefore is 1/20,000 of a cubic mm or 1/20,000,000 of a cubic centimeter (cc). There are 16 small squares in the large double-lined squares that are actually counted, making the volume of a large double-lined square 1/1,250,000 cc. The normal procedure is to count the number of bacteria in five large double-lined squares and divide by five to get the average number of bacteria per large square. This number is then multiplied by 1,250,000 since the square holds a volume of 1/1,250,000 cc, to find the total number of organisms per ml in the original sample.







Petroff-Hausser counting chamber



PROCEDURE DIRECT MICROSCOPIC COUNT

- 1) Add 1 ml of the sample into a tube containing 1 ml of the dye methylene blue. This gives a 1/2 dilution of the sample.
 - **2)** Fill the chamber of a Petroff-Hausser counting chamber with this 1/2 dilution.

3) Place the chamber on a microscope and focus on the squares using 400X.

Count the number of bacteria in one of the large double-lined squares.
 Count all organisms that are on or within the lines.

Calculate the number of bacteria per cc (ml) as follows: The number of bacteria per cc (ml)

> The number of bacteria per large square X The dilution factor of the large square (1,250,000)

The dilution factor of any dilutions made prior to placing the sample in the counting chamber, such as mixing it with dye (2 in this case)

TURBIDITY COUNT

When you mix the bacteria growing in a liquid medium, 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. Spectrophotometric analysis is based on turbidity and indirectly measures all bacteria (cell biomass), dead and alive.

The instrument used to measure turbidity is a spectrophotometer.



PROCEDURE TURBIDITY COUNT

We will be testing only two samples of water for the turbidity enumeration test. One of the samples has been drawn from a drinking water faucet while the other was taken from the local river. You will need DATA TABLE 3 and a printable version of the STANDARD CURVE CHART to enumerate your samples bacteria.

DATA TABLE 3 TURBIDITY COUNT

SAMPLE NAME: Faucet Water

Dilutions	Absorbance	# of Bacteria	Dilution Factor		Dilution factor X Bacteria #
Original			1	1 X	=
1/2			2	2 X	=
1/4			4	4 X	=
1/8			8	8 X	=
1/16			16	16 X	=
Total =					
Average # of Bacterial Cells per ml (Total / 5) =					



PROCEDURE TURBIDITY COUNT

1) Place the **ORIGINAL** tube of the sample and four tubes of the sterile broth in a test-tube rack. Each tube of broth contains 5 ml of sterile broth.

2) Use four of these tubes (tubes 2 to 5) of broth to make four serial dilutions of the culture.

3) Transfer 5ml of the **ORIGINAL** sample to the first broth tube. Transfer 5ml from that tube to the next tube, and so on until the last of the four tubes has 5ml added to it. These tubes will be 1/2, 1/4, 1/8, and 1/16 dilutions.



4) Set the display mode on the Spectrophotometer to **ABSORBANCE** by pressing the **MODE** control key until the appropriate red LED is lit.

5) Set the wavelength to 520 nm by using the **WAVELENGTH** dial.

6) Standardize the spectrophotometer by using a **BLANK**. The **BLANK** used to standardize the machine is sterile nutrient broth: it is called the **BLANK** because it has a sample concentration equal to zero (# of bacteria = 0).

7) Place the original bacterial specimen into the spectrophotometer.

8) Next insert the 1/2 dilution and read it. Repeat this with the 1/4, 1/8, and 1/16 dilutions. Read to the nearest thousandth (0.001) on the absorbance digital display.



9) Record your values in TABLE 3 for each of the individual samples, along with the dilutions that they came from.
10) Using the standard curve table given below, calculate the number of bacteria per milliliter for each

dilution.

DATA TABLE 3 TURBIDITY COUNT					
SAMPLE NAME: Faucet Water					
Dilutions	Absorbance	# of Bacteria	Dilution Dilution factor X Bacteria #		
Original			1	1 X	=
1/2			2	2 X	=
1/4			4	4 X	=
1/8			8	8 X	=
1/16			16	16 X	=
Total =					
Average # of Bacterial Cells per ml (Total / 5) =					

Review the example of absorbance counts acquired and the determinations of # of bacteria for the dilutions using the **STANDARD CURVE CHART given below. Be sure to keep track of all of the zeros in your calculations of the subsequent calculations for average bacteria per ml.

EXAMPLE DATA TABLE 3 TURBIDITY COUNT

SAMPLE NAME: EXAMPLE

Dilutions	Absorbance	# of Bacteria	Dilution Factor	Dilution factor X Bacteria #
Original	0.130	26,000,000	1	1 X 26,000,000 = 26,000,000
1/2	0.066	12,900,000	2	2 X 12,900,000 = 25,800,000
1/4	0.034	6,500,000	4	4 X 6,500,000 = 26,000,000
1/8	0.018	3,200,000	8	8 X 3,200,000 = 25,600,000
1/16	0.010	1,750,000	16	16 X 1,750,000 = 28,000,000
				131,400,000

Total =

Average # of Bacterial Cells per ml (Total / 5) = 26,306,280 bacteria per ml



References

TEXT BOOK OF MICROBIOLOGY BY TAMIL NADU PU BOARD
PRESCOTT'S MICROBIOLOGY
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MANY INTERNET ARTICLES.

THANKYOU