

# Chapter 6

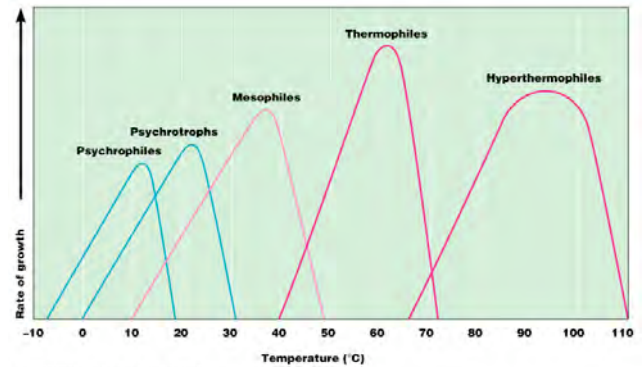
## Microbial Growth

Microbial growth = increase in number of cells, not cell size

Two categories of requirements for growth:

1. Physical
  - Temperature, pH, Osmotic Pressure
2. Chemical
  - Sources of: carbon, nitrogen, sulfur, phosphorus, trace elements, oxygen, and organic factors

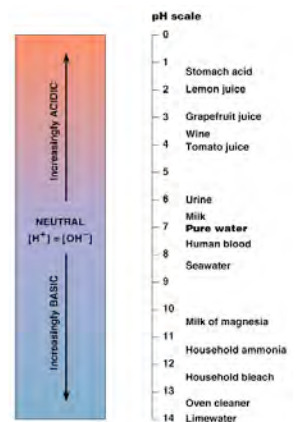
- Temperature
    - Minimum growth temperature
    - Optimum growth temperature
    - Maximum growth temperature
- Usually within a 30-40 degree range



Four general groups of bacteria based on preferred temperature:

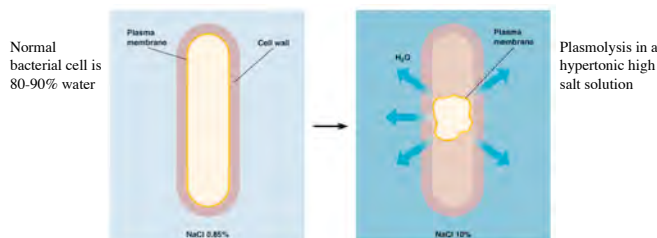
1. Psychrophiles
  - min -10°C
  - max 30°C
  - optimal 15°C
2. Mesophiles
  - min 10°C
  - max 50°C
  - optimal 37°C
3. Thermophiles
  - min 40°C
  - max 70°C
  - optimal 60°C
4. Hyperthermophiles
  - min 70°C
  - max 110°C
  - optimal 95°C

- pH
  - Most bacteria grow between pH 6.5 and 7.5
  - Molds and yeasts grow between pH 5 and 6
  - Acidophiles grow in acidic environments



### • Osmotic Pressure


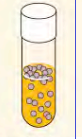



- Hypertonic environments, (increased salt or sugar), cause plasmolysis
- Extreme or obligate halophiles require high osmotic pressure
- Facultative halophiles tolerate high osmotic pressure



The Requirements for Growth: Chemical Requirements

- Carbon
  - Structural organic molecules, energy source
  - Chemoheterotrophs use organic carbon sources
  - Autotrophs use CO<sub>2</sub>
- Nitrogen
  - In amino acids, proteins
  - Most bacteria decompose proteins
  - Some bacteria use NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup>
  - A few bacteria use N<sub>2</sub> in nitrogen fixation
- Sulfur
  - In amino acids, thiamine, biotin
  - Most bacteria decompose proteins
  - Some bacteria use SO<sub>4</sub><sup>2-</sup> or H<sub>2</sub>S
- Phosphorus
  - In DNA, RNA, ATP, and membranes
  - PO<sub>4</sub><sup>3-</sup> is a source of phosphorus

- Trace Elements
  - Inorganic elements required in small amounts (Potassium, Magnesium, Calcium, Iron, Copper, Zinc)
  - Usually as enzyme cofactors
- Organic Growth Factors
  - Organic compounds obtained from the environment
  - Vitamins, amino acids, purines, pyrimidines
- Oxygen (O<sub>2</sub>)

Obligate aerobes	Facultative anaerobes	Obligate anaerobes	Aerotolerant anaerobes	Microaerophiles
				

## Culture Media

- Culture Medium: Nutrients prepared for microbial growth in the lab
- Sterile: No living microbes
- Inoculum: Introduction of microbes into medium
- Culture: Microbes growing in/on culture medium

Culture Medium:  
liquid form = broth  
solid gel form using agar = plates, slants, deeps

### Agar

- Complex polysaccharide
- Used as solidifying agent for culture media in Petri plates, slants, and deeps
- Generally not metabolized by microbes
- Liquefies at 100°C
- Solidifies ~40°C

## Culture Media

- Chemically Defined Media: Exact chemical composition is known
- Complex Media: Extracts and digests of yeasts, meat, or plants
  - Nutrient broth (liquid)
  - Nutrient agar (solid gel)

Fastidious organisms require many growth factors

## Selective Media

- Suppress unwanted microbes and encourage desired microbes.

## Differential Media

- Make it easy to distinguish colonies of different microbes.

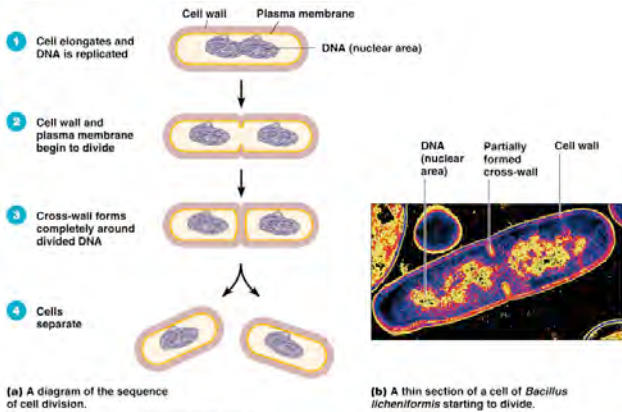
- A pure culture contains only one species or strain
- A colony is a population of cells arising from a single cell or spore or from a group of attached cells
- A colony is often called a colony-forming unit (CFU)

## Streak Plate

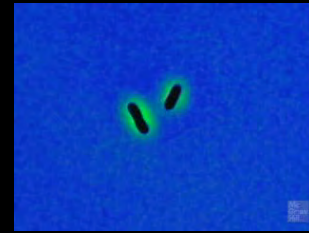


# Reproduction in Prokaryotes

## Binary Fission



## Binary Fission



[Play ExponentialGrowth.mpg.](#)

Generation time - the time required for a cell to divide, to undergo one round of binary fission

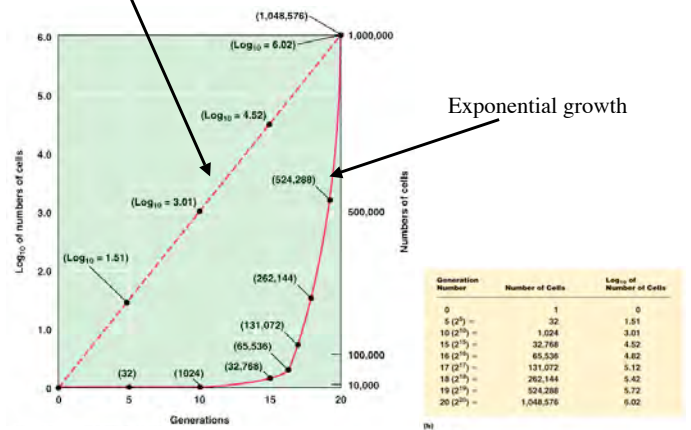
Common bacterial generation times range 1-3hrs

*E. coli* has a generation time of 20 min: one cell in 20 generations will become ~1 million cells (~7 hrs)

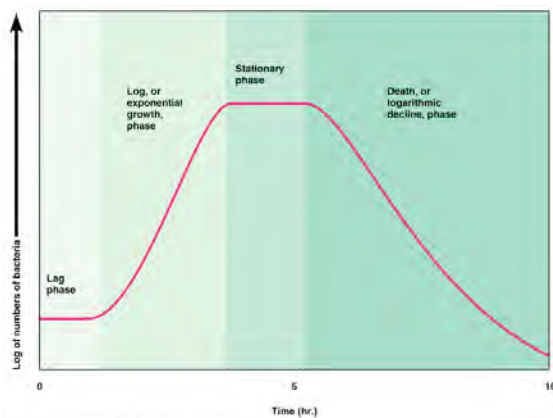
Exponential growth is graphed on a logarithmic scale:

A logarithm of a number X to base 10 is the power/exponent to which 10 must be raised to give that number X

## Log scale of exponential growth

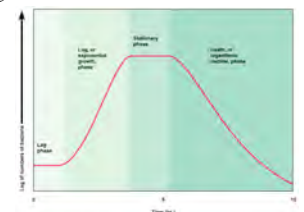


## Bacterial Growth Curve



## Phases of Bacterial Growth in a New Culture

- Lag phase: initial period of little to no cell division as bacteria acclimate to new media
- Log phase: period of exponential growth with a constant generation time
- Stationary phase: cell growth is equal to cell death
- Death phase: cell death exceeds cell growth



# Quantifying Microbial Growth

## Direct Measurements

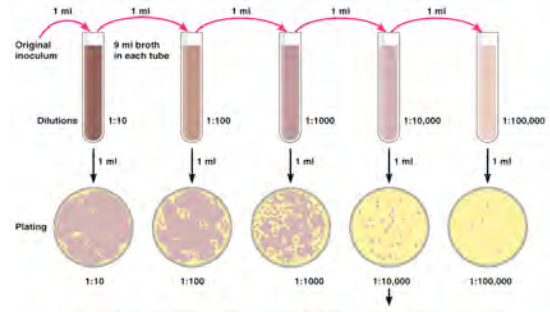
- Plate Counts
- Filtration
- Most Probable Number (MPN)
- Direct Microscopic Count

## Indirect Estimations

- Turbidity
- Metabolic Activity
- Dry Weight

# Direct Measurements of Microbial Growth

- Plate Counts: Perform serial dilutions of a sample, plate, and count resulting colonies



Calculation: Number of colonies on plate  $\times$  reciprocal of dilution of sample = number of bacteria/ml  
 (For example, if 32 colonies are on a plate of  $1/10,000$  dilution, then the count is  $32 \times 10,000 = 320,000$  in sample.)

- Filtration



- Multiple tube MPN (most probable number) test

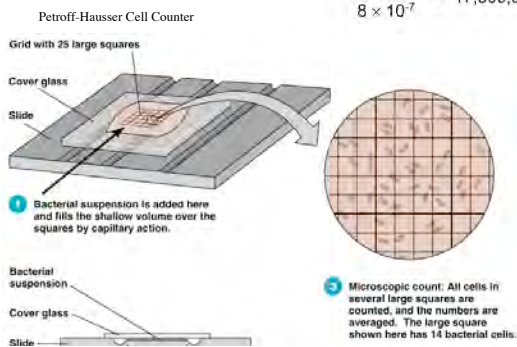
Combination of Positives	MPN Index/ 100 ml	95% Confidence Limits	
		Lower	Upper
4-2-0	22	9	56
4-2-1	26	12	65
4-3-0	27	12	67
4-3-1	33	15	77
4-4-0	34	16	80
5-0-0	23	9	86
5-0-1	30	10	110
5-0-2	40	20	140
5-1-0	30	10	120
5-1-1	50	20	150
5-1-2	60	30	180
5-2-0	50	20	170
5-2-1	70	30	210
5-2-2	90	40	250
5-3-0	80	30	250
5-3-1	110	40	300
5-3-2	140	60	360

Count positive tubes and compare to statistical MPN table.

- Direct Microscopic Count

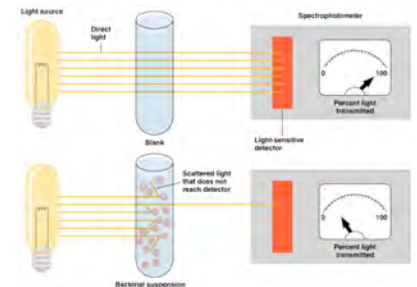
$$\text{Number of bacteria/ml} = \frac{\text{number of cells counted}}{\text{volume of area counted}}$$

$$\frac{14}{8 \times 10^{-7}} = 17,500,000$$



# Estimating Bacterial Numbers by Indirect Methods

- Turbidity
- Metabolic activity
- Dry weight



**Lab 2 Goals and Objectives:**

Lecture: Chapter 6 (Microbial Growth)

Exercise 9: Aseptic Technique

Each person make 3 inoculations:

1. Broth to broth - *E. coli* - 37°C
2. Slant to slant - *E. coli* - 30°C
3. Plate to slant - *S. marcescens* - 30°C (changed)

Each pair will need:

- 1 broth culture *Escherichia coli*, 1 slant culture *Escherichia coli*
- 1 plate culture *Serratia marcescens*

Each person will need:

- 1 Nutrient Broth/BHI tubes, 2 Nutrient Agar/BHIA slants

Exercise 10: Pure Culture Technique

Each person make 2 streak plates: Quadrant Streak Method B, page 85.

Each pair will need:

- 1 mixed culture (which contains: *Escherichia coli*, *Serratia marcescens* and *Micrococcus luteus*)

Each person will need:

- 2 Nutrient Agar/BHIA plates

Finish microscope worksheet if necessary

Turn in cultures from home for incubation

Get help **now**, today, if you are having any difficulty with oil immersion lens use or specimen measurements using the ocular micrometer!!!