General microbiology

Lecture-12
Microbial growth (Part-2)

Content

- Bacterial Cell Division
  - Cell Growth and Binary Fission
- Population growth
  - The Concept of Exponential Growth
  - The Microbial Growth Cycle
- Measuring Microbial Growth
- Environmental Factors Affecting Growth
  - Temperature
  - Acidity and Alkalinity
  - Osmotic Effects on Microbial Growth
  - Oxygen and Microorganisms
  - Toxic Forms of Oxygen

Measuring Microbial Growth

Microscopic Counts

Viable Counts

Turbidimetric Methods

Measuring Microbial Growth

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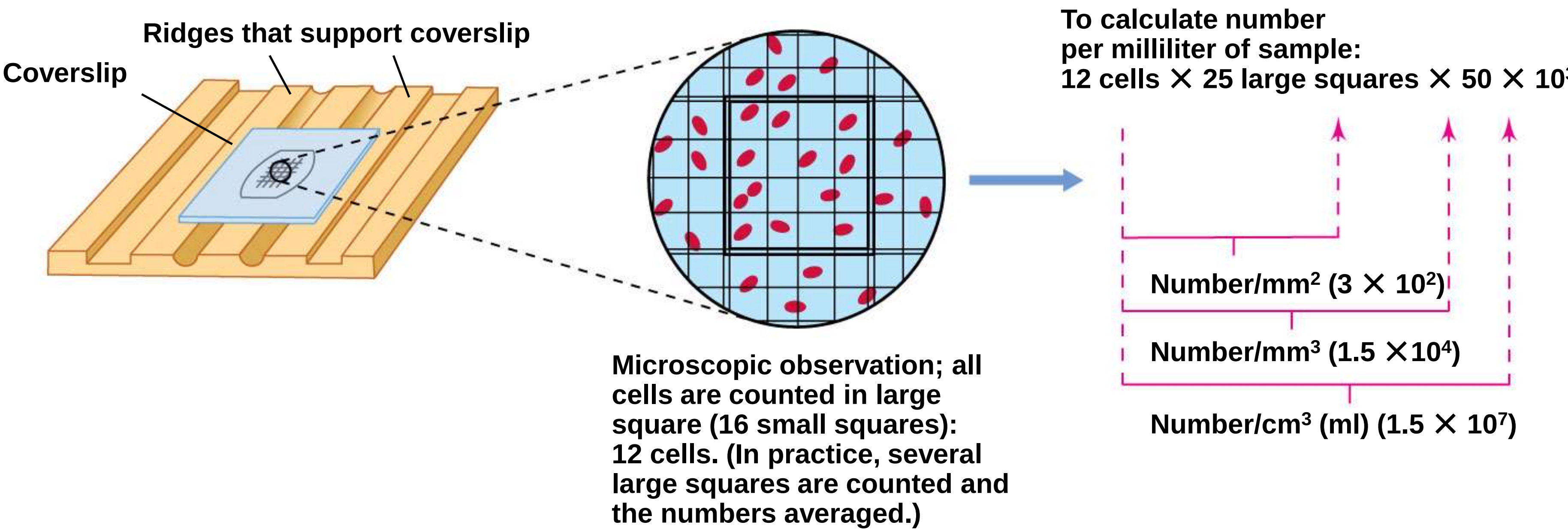
Turbidimetric Methods

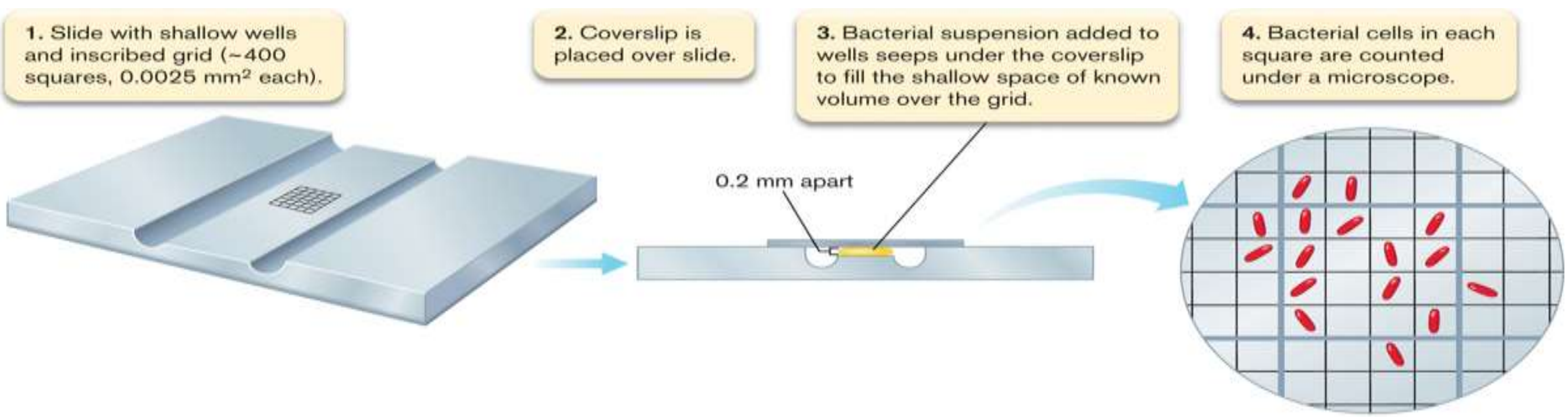
Microbial cells are enumerated by microscopic observations

- Results can be unreliable

Limitations of microscopic counts

- Cannot distinguish between live and dead cells without special stains
- Small cells can be overlooked
- Precision is difficult to achieve
- Phase-contrast microscope required if a stain is not used
- Cell suspensions of low density ($<10^6$ cells/ml) hard to count
- Motile cells need to immobilized
- Debris in sample can be mistaken for cells


Ridges that support coverslip
Coverslip
Microscopic observation; all cells are counted in large square (16 small squares): 12 cells. (In practice, several large squares are counted and the numbers averaged.)
To calculate number per milliliter of sample:
12 cells × 25 large squares × 50 × 10^3
Number/mm^2 (3 × 10^2)
Number/mm^3 (1.5 × 10^4)
Number/cm^3 (ml) (1.5 × 10^7)


1. Slide with shallow wells and inscribed grid (~400 squares, 0.0025 mm^2 each).
2. Coverslip is placed over slide.
3. Bacterial suspension added to wells seeps under the coverslip to fill the shallow space of known volume over the grid.
4. Bacterial cells in each square are counted under a microscope.
0.2 mm apart

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Turbidimetric Methods

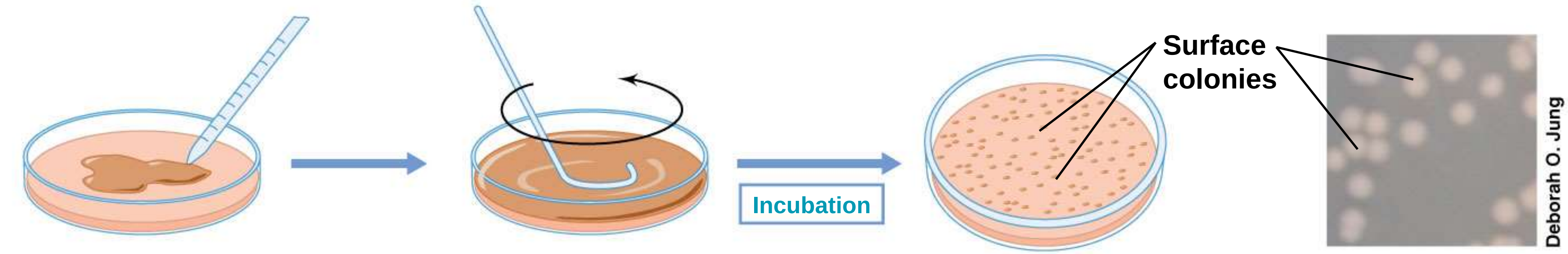
Viable cell counts (plate counts):

measurement of living, reproducing population

- Two main ways to perform plate counts:
  - *Spread-plate method*
  - *Pour-plate method*

To obtain the appropriate colony number, the sample to be counted should always be diluted.

Spread-plate method

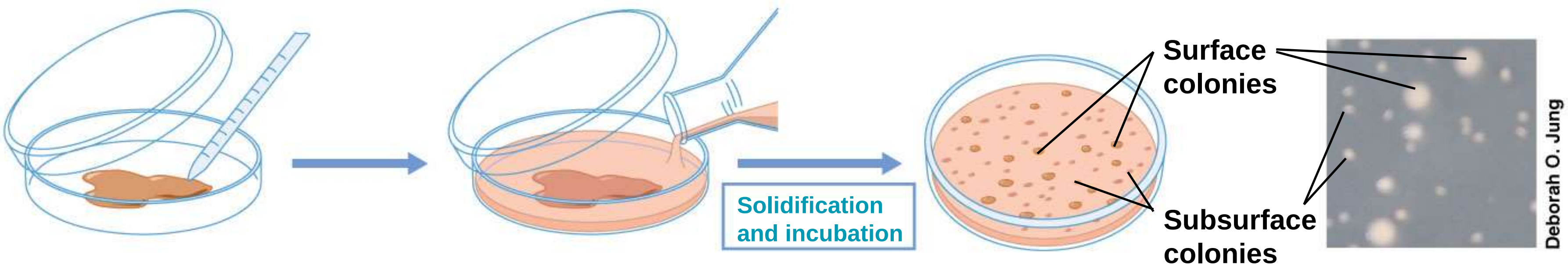


Sample is pipetted onto surface of agar plate (0.1 ml or less)

Sample is spread evenly over surface of agar using sterile glass spreader

Typical spread-plate results

Pour-plate method

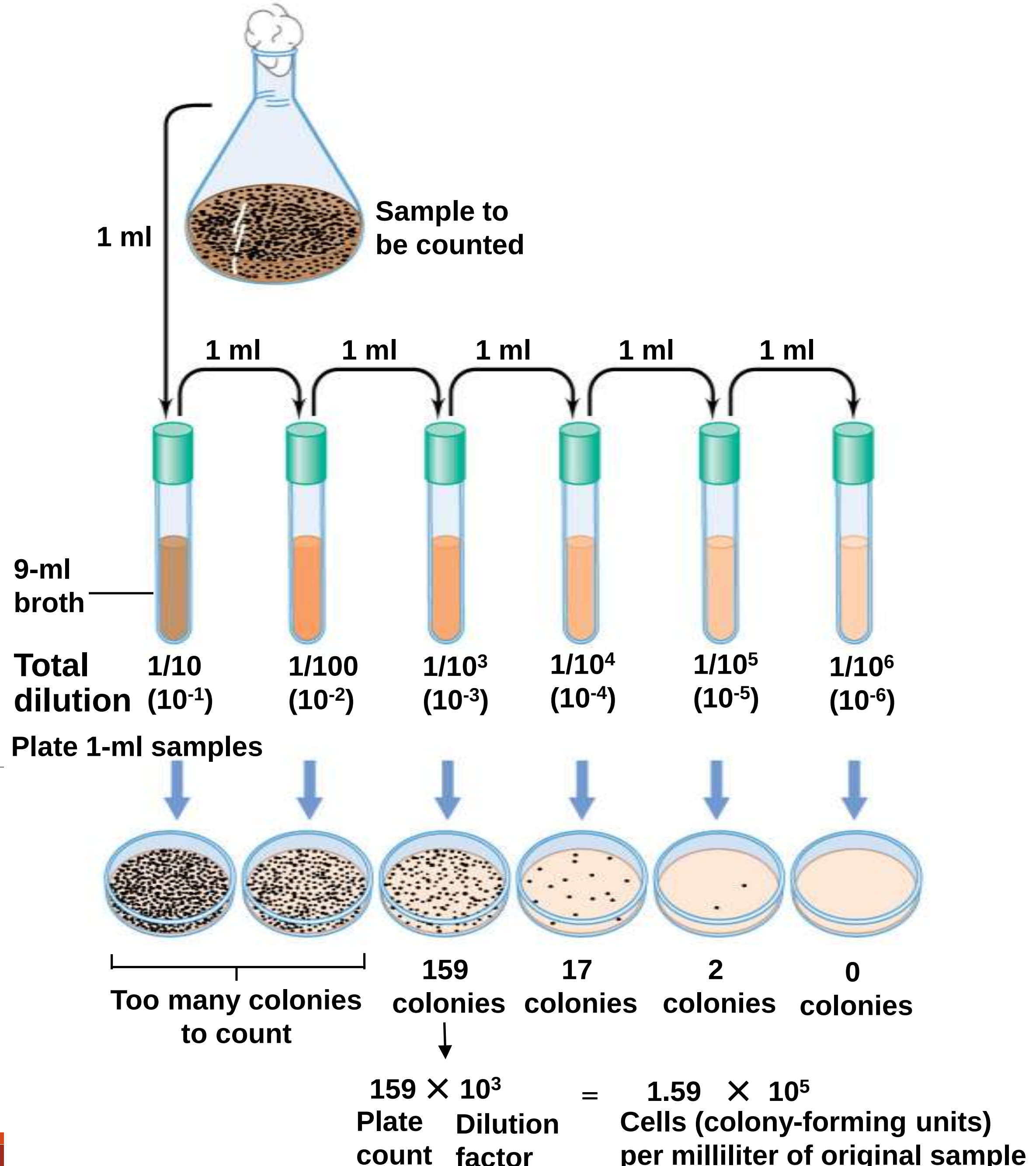


Sample is pipetted into sterile plate

Sterile medium is added and mixed well with inoculum

Typical pour-plate results

Figure 5.16



1 ml
Sample to be counted
1 ml
1 ml
1 ml
1 ml
1 ml
9-ml broth
Total dilution
1/10 (10^{-1})
1/100 (10^{-2})
$1/10^3$ (10^{-3})
$1/10^4$ (10^{-4})
$1/10^5$ (10^{-5})
$1/10^6$ (10^{-6})
Plate 1-ml samples
Too many colonies to count
159 colonies
17 colonies
2 colonies
0 colonies
159×10^3 = 1.59×10^5
Plate count
Dilution factor
Cells (colony-forming units) per milliliter of original sample

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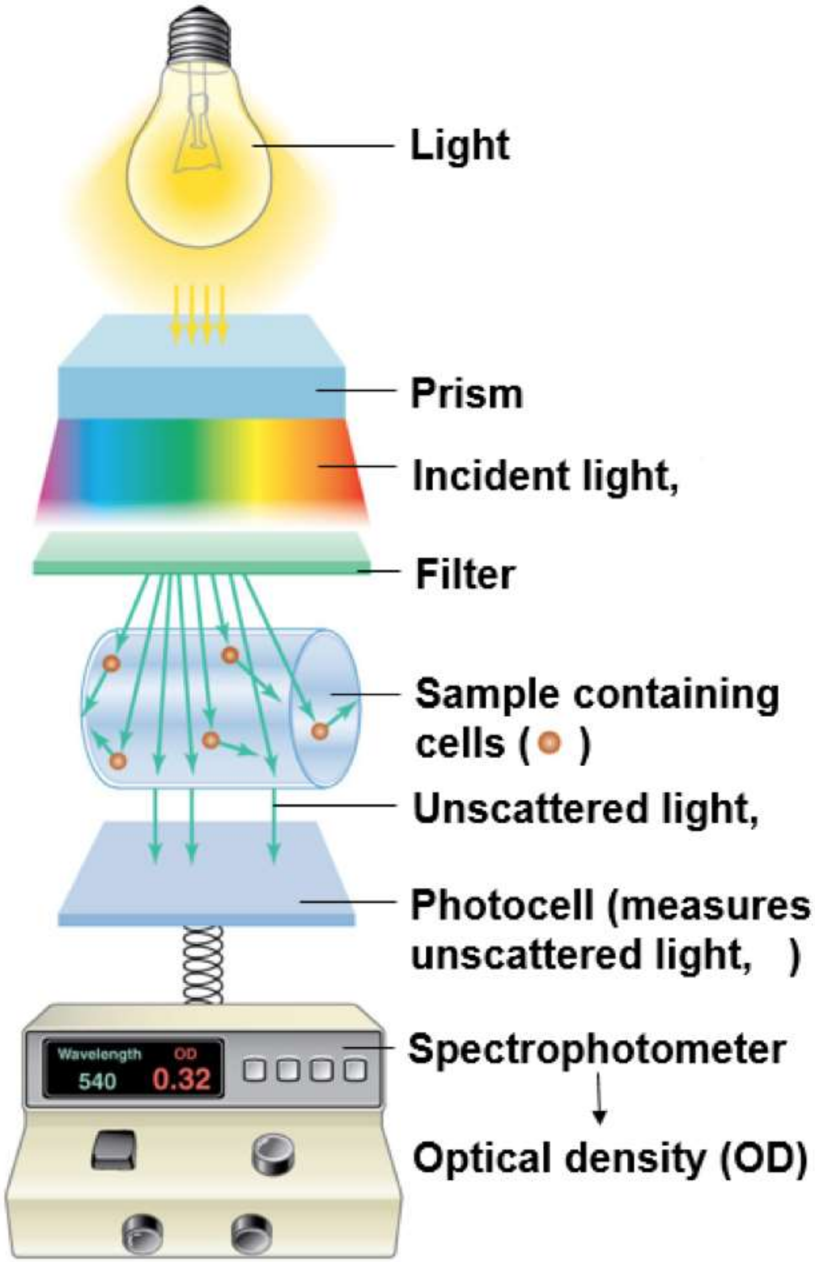
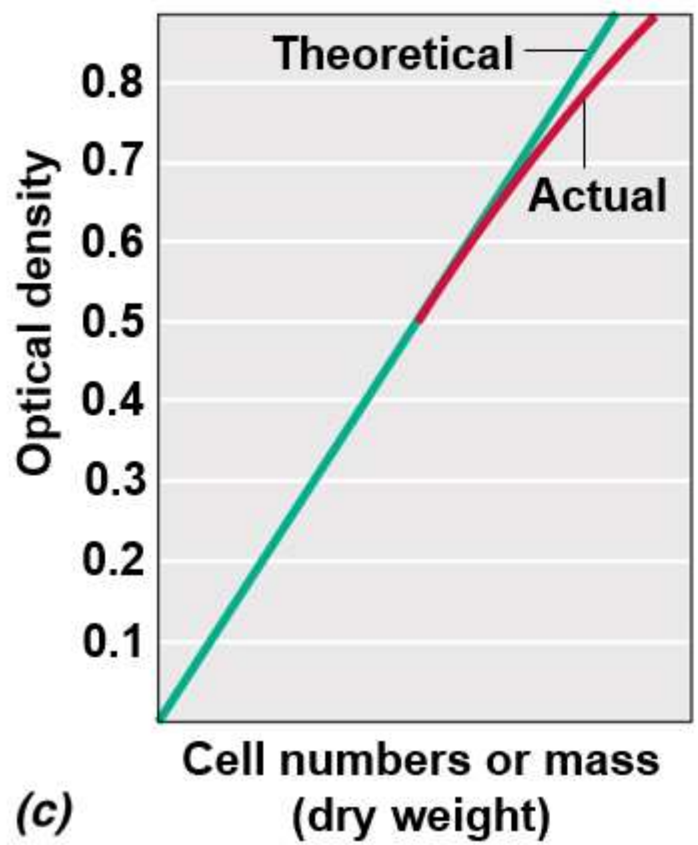
Turbidimetric Methods

- Turbidity measurements are an indirect, rapid, and useful method of measuring microbial growth
- Most often measured with a spectrophotometer and measurement referred to as optical density (O.D.)
- To relate a direct cell count to a turbidity value, a standard curve must first be established
- Quick and easy to perform
- Typically do not require destruction or significant disturbance of sample
- Sometimes problematic (e.g., microbes that form clumps or biofilms in liquid medium)


Light
Prism
Incident light,
Filter
Sample containing cells ( )
Unscattered light,
Photocell (measures unscattered light,)
Spectrophotometer
Wavelength
540
OD
0.32
Optical density (OD)

(a)


Theoretical
Actual
Optical density
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Cell numbers or mass (dry weight)

(c)

Environmental Factors Affecting Growth

- Temperature
- Acidity and Alkalinity
- Osmotic Effects on Microbial Growth
- Oxygen and Microorganisms
- Toxic Forms of Oxygen

Effect of Temperature on Growth

Temperature is a major environmental factor controlling microbial growth

Cardinal temperatures: the minimum, optimum, and maximum temperatures at which an organism grows.

Microorganisms can be classified into groups by their growth temperature optima.

- Psychrophile: low temperature
- Mesophile: midrange temperature
- Thermophile: high temperature
- Hyperthermophile: very high temperature

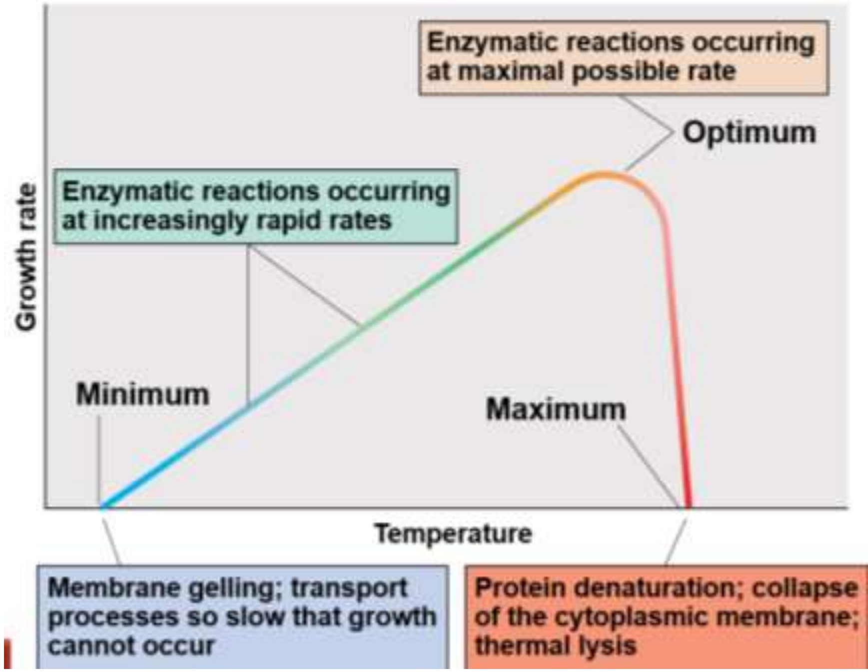
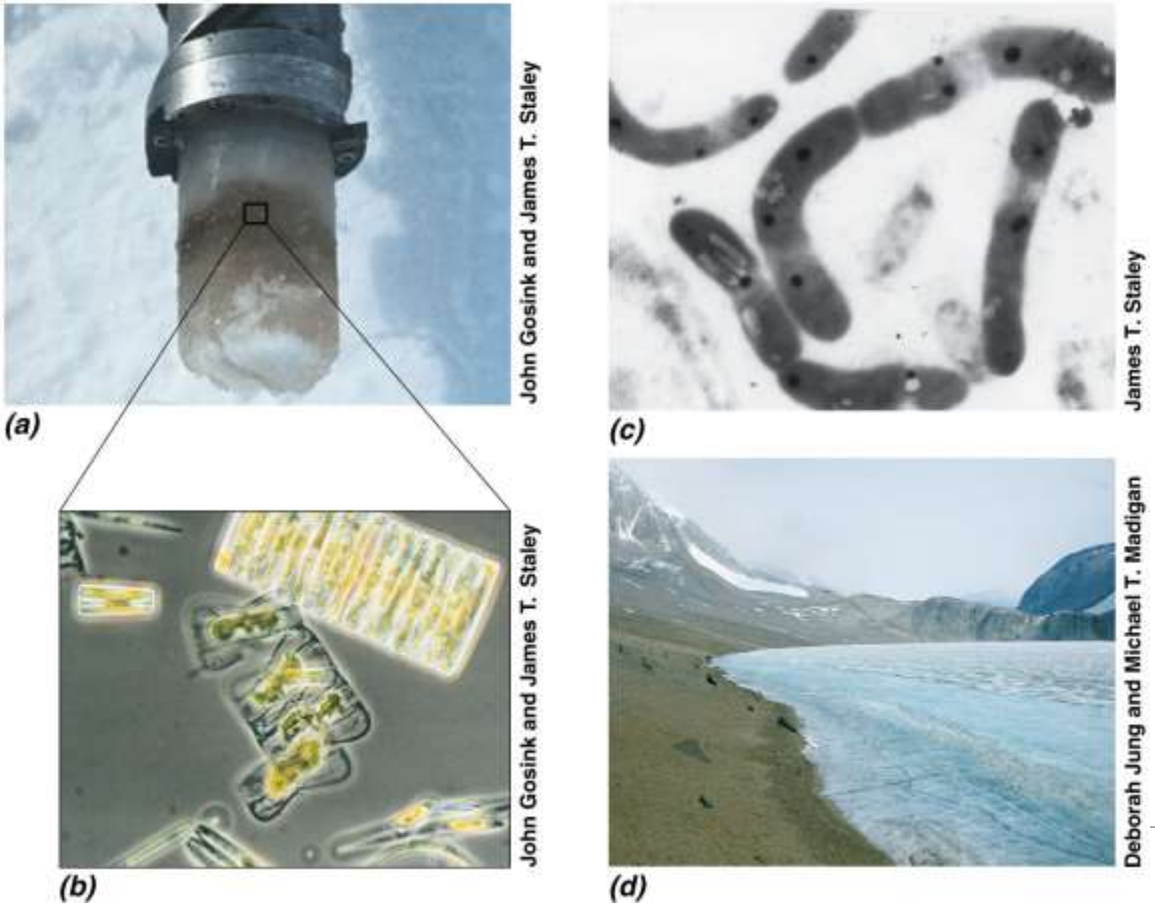
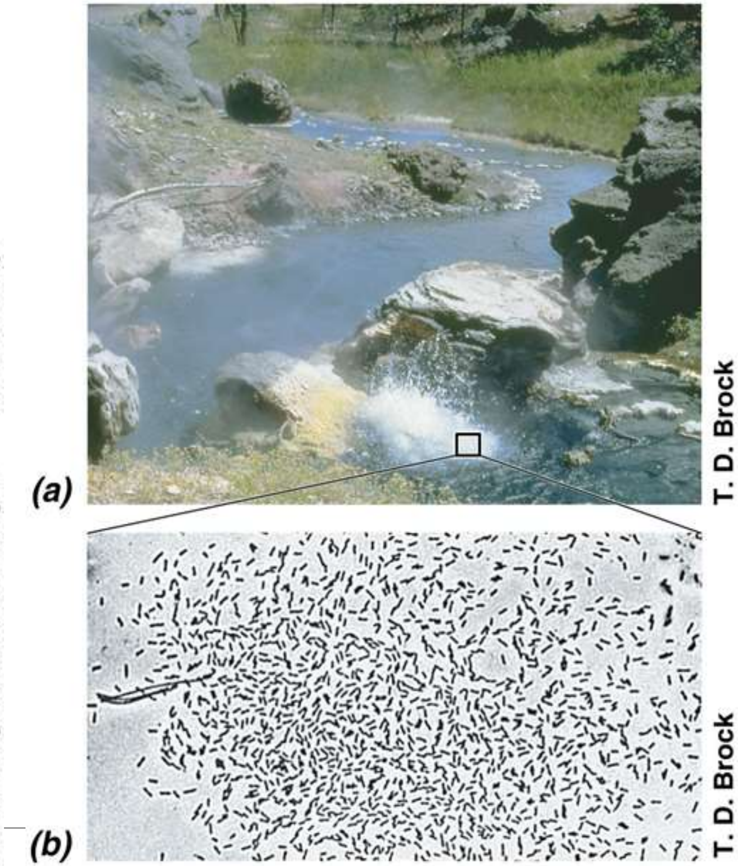


Figure 5.20


(a)
John Gosink and James T. Staley
(b)
John Gosink and James T. Staley
(c)
James T. Staley
(d)
Deborah Jung and Michael T. Madigan


(a)
T. D. Brock
(b)
T. D. Brock

Acidity and Alkalinity

The pH of an environment greatly affects microbial growth .

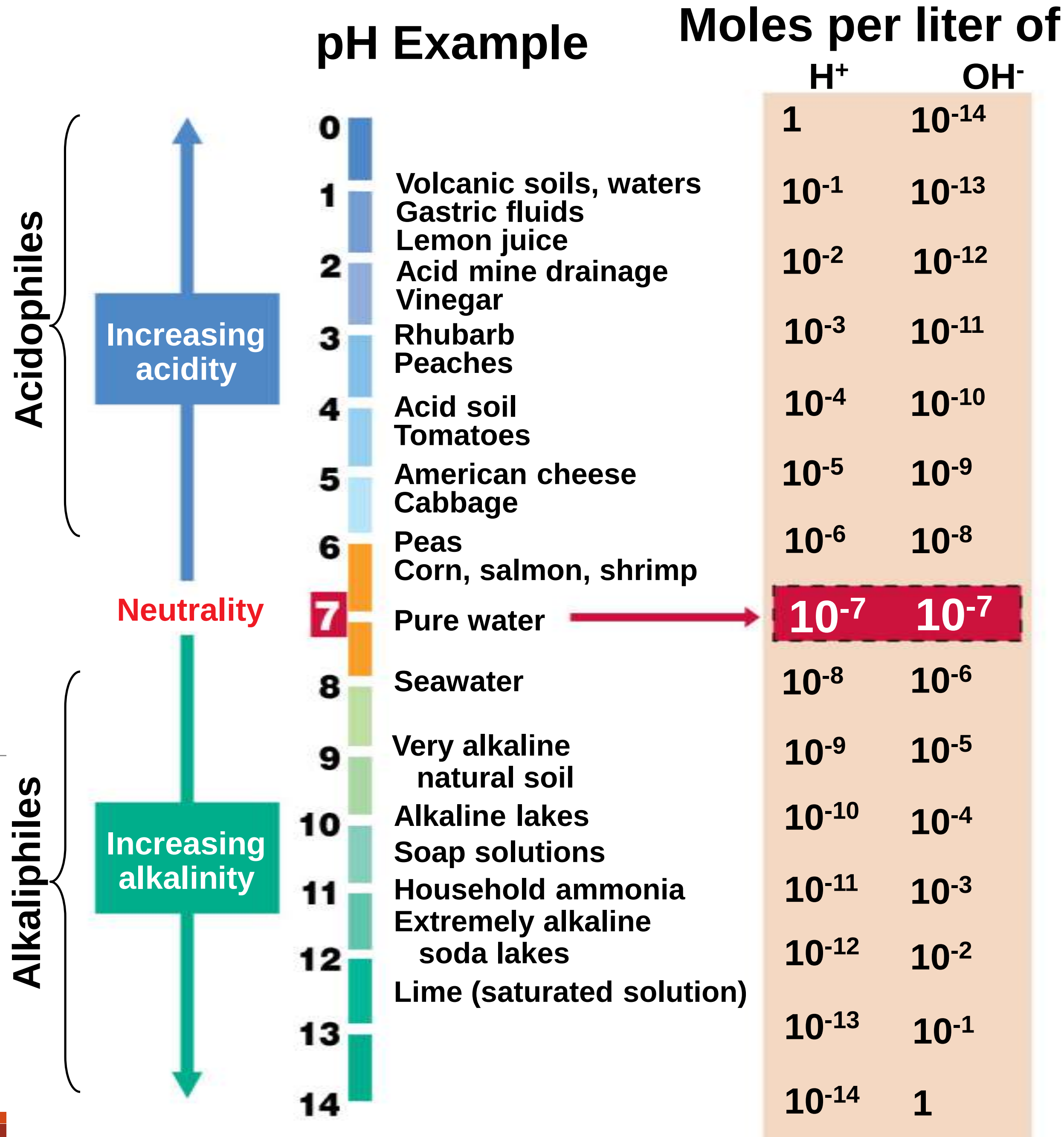
Some organisms have evolved to grow best at low or high pH, but most organisms grow best between pH 6 and 8 (**neutrophiles**)

- ***Acidophiles***: organisms that grow best at low pH (<6)
  - Some are obligate acidophiles; membranes destroyed at neutral pH
  - Stability of cytoplasmic membrane critical
- ***Alkaliphiles***: organisms that grow best at high pH (>9)
  - Some have sodium motive force rather than proton motive force

The internal pH of a cell must stay relatively close to neutral even though the external pH is highly acidic or basic

Microbial culture media typically contain buffers to maintain constant pH

Figure 5.24



pH Example
Moles per liter of:
H+
OH-
Acidophiles
Increasing acidity
Neutrality
Increasing alkalinity
Alkaliphiles
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Volcanic soils, waters
Gastric fluids
Lemon juice
Acid mine drainage
Vinegar
Rhubarb
Peaches
Acid soil
Tomatoes
American cheese
Cabbage
Peas
Corn, salmon, shrimp
Pure water
Seawater
Very alkaline natural soil
Alkaline lakes
Soap solutions
Household ammonia
Extremely alkaline soda lakes
Lime (saturated solution)
1 10-14
10-1 10-13
10-2 10-12
10-3 10-11
10-4 10-10
10-5 10-9
10-6 10-8
10-7 10-7
10-8 10-6
10-9 10-5
10-10 10-4
10-11 10-3
10-12 10-2
10-13 10-1
10-14 1

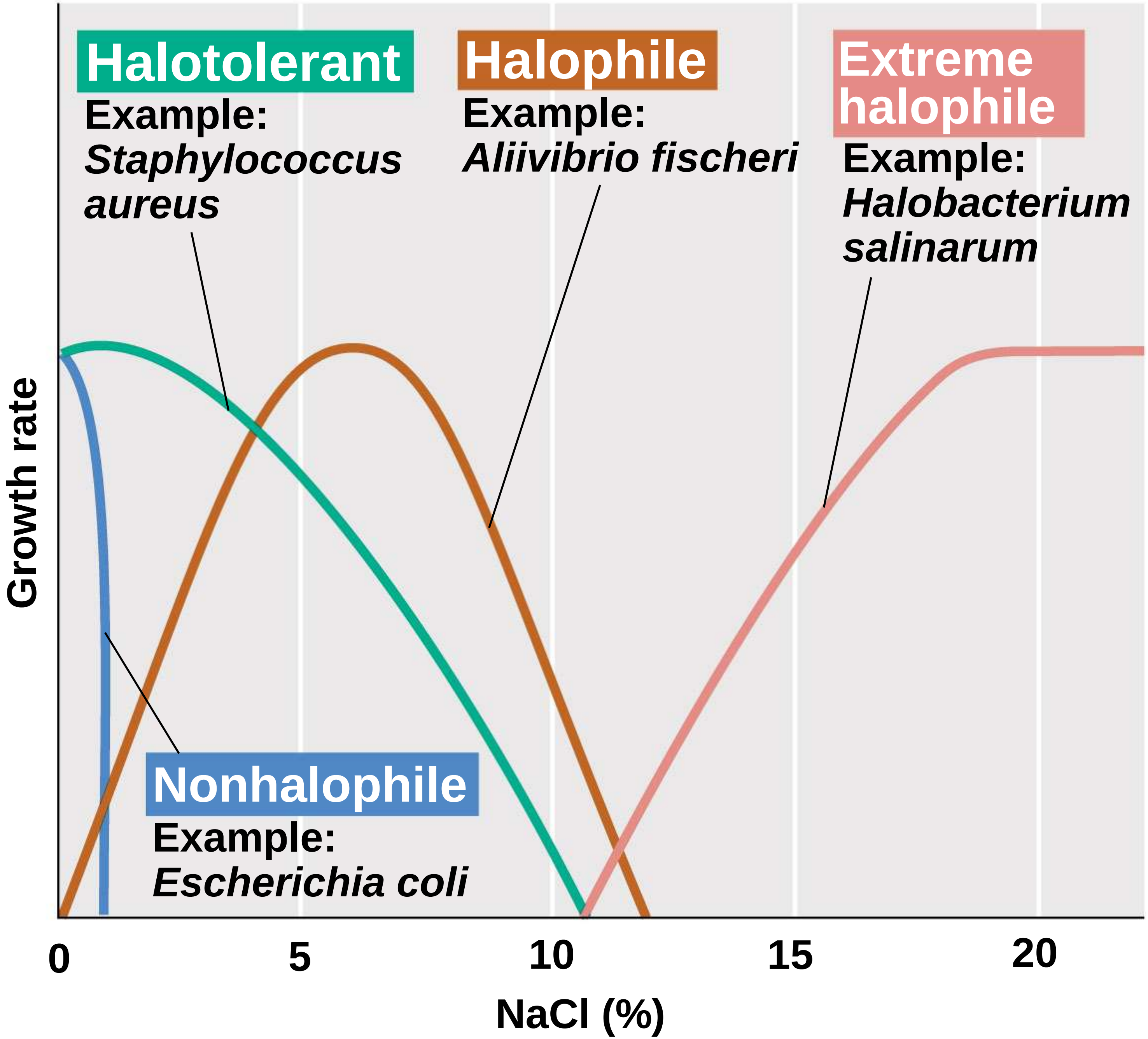
Osmotic Effects on Microbial Growth

Typically, the cytoplasm has a higher solute concentration than the surrounding environment, thus the tendency is for water to move into the cell (*positive water balance*)

When a cell is in an environment with a higher external solute concentration, water will flow out unless the cell has a mechanism to prevent this

- ***Halophiles***: organisms that grow best at reduced water potential; have a specific requirement for NaCl.
- ***Extreme halophiles***: organisms that require high levels (15–30%) of NaCl for growth
- ***Halotolerant***: organisms that can tolerate some reduction in water activity of environment but generally grow best in the absence of the added solute
- ***Nonhalophile***

Figure 5.25



Halotolerant
Example:
Staphylococcus aureus
Halophile
Example:
Aliivibrio fischeri
Extreme halophile
Example:
Halobacterium salinarum
Nonhalophile
Example:
Escherichia coli
Growth rate
0
5
10
15
20
NaCl (%)

Oxygen and Microorganisms

Aerobes: require oxygen to live

Anaerobes: do not require oxygen and may even be killed by exposure

Facultative organisms: can live with or without oxygen

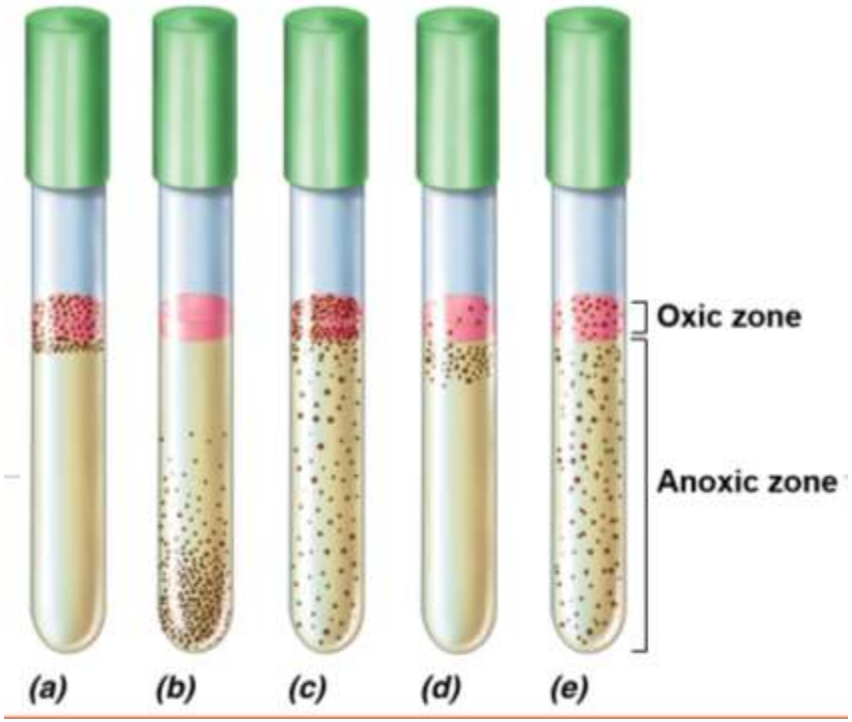
Aerotolerant anaerobes: can tolerate oxygen and grow in its presence even though they cannot use it

Microaerophiles: can use oxygen only when it is present at levels reduced from that in air

Oxygen and Microorganisms

Thioglycolate broth : Complex medium that separates microbes based on oxygen requirements.

- Reacts with oxygen so oxygen can only penetrate the top of the tube.



Growth versus oxygen concentration.
(a) aerobic, (b)anaerobic, (c) facultative, (d)microaerophilic, (e) aerotolerant anaerobe


ANY
QUESTIONS
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