1 **Chapter 6**

Microbial Growth

2 **Microbial Growth**

- Increase in number of cells, not cell size
  - Populations
  - Colonies

3 **The Requirements for Growth**

- Physical requirements
  - Temperature
  - pH
  - Osmotic pressure
- Chemical requirements
  - Carbon
  - Nitrogen, sulfur, and phosphorous
  - Trace elements
  - Oxygen
  - Organic growth factor

4 **Physical Requirements**

- Temperature
  - Minimum growth temperature
  - Optimum growth temperature
  - Maximum growth temperature

5 **Typical Growth Rates and Temperature**

6 **Psychrotrophs**

- Grow between 0°C and 20–30°C
- Cause food spoilage

7 **Food Preservation Temperatures**

8 **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

9 **Osmotic Pressure**

- Hypertonic environments, or an increase in salt or sugar, cause plasmolysis
- Extreme or obligate halophiles require high osmotic pressure
- Facultative halophiles tolerate high osmotic pressure
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10 **Plasmolysis**

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- Carbon
  - Structural organic molecules, energy source
  - Chemoheterotrophs use organic carbon sources
  - Autotrophs use CO_2

12 **Chemical Requirements**

- Nitrogen
  - In amino acids and proteins

- Most bacteria decompose proteins
- Some bacteria use NH_4+ or NO_3–
- A few bacteria use N_2 in nitrogen fixation

13 **Chemical Requirements**

- Sulfur
  - In amino acids, thiamine, and biotin
  - Most bacteria decompose proteins
  - Some bacteria use SO_4^{2-} or H_2S
- Phosphorus
  - In DNA, RNA, ATP, and membranes
  - PO_4^{3-} is a source of phosphorus

14 **Chemical Requirements**

- Trace elements
  - Inorganic elements required in small amounts
  - Usually as enzyme cofactors

15 **The Effect of Oxygen (O2) on Growth**

16 **Toxic Oxygen**

- Singlet oxygen: O_2 boosted to a higher-energy state
- Superoxide free radicals: O_2^-
- Peroxide anion: O_2^{2-}
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- Hydroxyl radical (OH•)

17 **Organic Growth Factors**

- Organic compounds obtained from the environment
- Vitamins, amino acids, purines, and pyrimidines

18 **Biofilms**

- Microbial communities
- Form slime or hydrogels
  - Bacteria attracted by chemicals via quorum sensing
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19 **Biofilms**

- Share nutrients
- Sheltered from harmful factors
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20 **Biofilms**

- Patients with indwelling catheters received contaminated heparin
- Bacterial numbers in contaminated heparin were too low to cause infection
- 84–421 days after exposure, patients developed infections
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21 **Biofilms**

- *Pseudomonas fluorescens* was cultured from the catheters
- What happened?

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22 **Culture Media**
- Culture medium: Nutrients prepared for microbial growth
- Sterile: No living microbes
- Inoculum: Introduction of microbes into medium
- Culture: Microbes growing in/on culture medium

23 **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 at ~40°C

24 **Culture Media**
- Chemically defined media: Exact chemical composition is known
- Complex media: Extracts and digests of yeasts, meat, or plants
  - Nutrient broth
  - Nutrient agar

25

26

27 **Anaerobic Culture Methods**
- Reducing media
  - Contain chemicals (thioglycolate or oxyrase) that combine O_2
  - Heated to drive off O_2

28 **Anaerobic Jar**

29 **An Anaerobic Chamber**

30 **Capnophiles**
- Microbes that require high CO2 conditions
- CO2 packet
- Candle jar
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31 **Selective Media**
- Suppress unwanted microbes and encourage desired microbes

32 **Differential Media**
- Make it easy to distinguish colonies of different microbes.

33 **Enrichment Culture**
- Encourages growth of desired microbe
- Assume a soil sample contains a few phenol-degrading bacteria and thousands of other bacteria
  - Inoculate phenol-containing culture medium with the soil, and incubate
  - Transfer 1 ml to another flask of the phenol medium, and incubate
  - Transfer 1 ml to another flask of the phenol medium, and incubate
  - Only phenol-metabolizing bacteria will be growing

34 **Obtaining Pure Cultures**
- 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)
- The streak plate method is used to isolate pure cultures
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35 **The Streak Plate Method**

36 **Preserving Bacterial Cultures**
- Deep-freezing: –50° to –95°C
- Lyophilization (freeze-drying): Frozen (–54° to –72°C) and dehydrated in a vacuum

37 **Reproduction in Prokaryotes**
- Binary fission
- Budding
- Conidiospores (actinomycetes)
- Fragmentation of filaments

38 **Binary Fission**

39 **Binary Fission**

40

41 **Generation Time**
- If 100 cells growing for 5 hours produced 1,720,320 cells:

42 **Bacterial Growth Curve**

43 **Phases of Growth**

44 **Measuring Microbial Growth**

1 Direct Methods
- Plate counts
- Filtration
- MPN (most probable #)
- Direct microscopic count

2 Indirect Methods
- Turbidity
- Metabolic activity
- Dry weight

45 **Serial Dilutions**

46 **Plate Counts**

47 **Plate Counts**
- After incubation, count colonies on plates that have 25–250 colonies (CFUs)

48 **Counting Bacteria by Membrane Filtration**

49 **Most Probable Number**
- Multiple tube MPN test
- Count positive tubes

50 **Most Probable Number**
- Compare with a statistical table.

51 **Direct Microscopic Count**

52 **Direct Microscopic Count**

53 **Turbidity**

54 **Turbidity**