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Microbial Nutrition and Growth

CHAPTER SUMMARY

Growth Requirements (pp. 166–174)

Microbiologists use the term *growth* to indicate an increase in a *population* of microbes rather than an increase in size. Microbial growth depends on the metabolism of nutrients, and results in the formation of a discrete **colony**, an aggregation of cells arising from a single parent cell. A **nutrient** is any chemical required for growth of microbial populations. The most important of these are compounds containing carbon, oxygen, nitrogen, and/or hydrogen.

Nutrients: Chemical and Energy Requirements

All cells require three things to conduct metabolism: a carbon source, a source of energy, and a source of electrons or hydrogen atoms.

Sources of Carbon, Energy, and Electrons

Organisms can be categorized into one of four groups based on their source of carbon and their use of either chemicals or light as a source of energy:

- **Photoautotrophs** use carbon dioxide as a carbon source and light energy from the environment to make their own food.
- **Chemoautotrophs** use carbon dioxide as a carbon source but catabolize organic molecules for energy.
- **Photoheterotrophs** are photosynthetic organisms that acquire energy from light and acquire nutrients via catabolism of organic compounds.
- **Chemoheterotrophs** use organic compounds for both energy and carbon.

In addition, **organotrophs** acquire electrons from organic sources, whereas **lithotrophs** acquire electrons from inorganic sources.

Oxygen Requirements

Obligate aerobes require oxygen as the final electron acceptor of the electron transport chain, whereas **obligate anaerobes** cannot tolerate oxygen and use an electron acceptor other than oxygen. Toxic forms of oxygen are highly reactive and cause a chain of vigorous oxidation. Four forms of oxygen are toxic:

- **Singlet oxygen** ($^1\text{O}_2$) is molecular oxygen with electrons that have been boosted to a higher energy state, typically during aerobic metabolism. Phototropic microorganisms often contain pigments called **carotenoids** that prevent toxicity by removing the excess energy of singlet oxygen.
- **Superoxide radicals** (O_2^-) are formed during the incomplete reduction of oxygen during electron transport in aerobes and during metabolism by anaerobes in the presence of oxygen. They are detoxified by superoxide dismutase.

- **Peroxide anion** (O_2^{2-}) is a component of hydrogen peroxide, which is formed during reactions catalyzed by superoxide dismutase. The enzymes catalase and peroxidase detoxify peroxide anion.
- **Hydroxyl radicals** ($OH\cdot$) result from ionizing radiation and from the incomplete reduction of hydrogen peroxide. Hydroxyl radicals are the most reactive of the four toxic forms of oxygen, but because hydrogen peroxide does not accumulate in aerobic cells, the threat of hydroxyl radicals is virtually eliminated in aerobic cells.

Not all organisms are either strict aerobes or anaerobes. **Facultative anaerobes** can maintain life via fermentation or anaerobic respiration, though their metabolic efficiency is often reduced in the absence of oxygen. **Aerotolerant anaerobes** prefer anaerobic conditions, but can tolerate oxygen because they have some form of the enzymes that detoxify oxygen's poisonous forms. **Microaerophiles** require low levels of oxygen. **Capnophiles** grow best with high carbon dioxide levels in addition to low oxygen levels.

Nitrogen Requirements

Nitrogen is a growth-limiting nutrient for many microorganisms, which acquire it from organic and inorganic nutrients. Though nitrogen constitutes about 79% of the atmosphere, relatively few organisms can utilize nitrogen gas. A few bacteria reduce nitrogen gas to ammonia via a process called **nitrogen fixation**, which is essential to life on Earth.

Other Chemical Requirements

In addition to the main elements found in microbes, very small amounts of **trace elements** such as selenium, zinc, etc., are required. Most microorganisms also require small amounts of certain organic chemicals that they cannot synthesize. These are called **growth factors**. For example, vitamins are growth factors for some microorganisms.

Physical Requirements

In addition to chemical nutrients, organisms have physical requirements for growth, including specific conditions of temperature, pH, osmolarity, and pressure.

Temperature

Since both proteins and lipids are temperature-sensitive, different temperatures have different effects on the survival and growth rates of microbes. Though microbes survive within the limits imposed by a minimum growth temperature and a maximum growth temperature, an organism's metabolic activities produce the highest growth rate at the **optimum growth temperature**.

Microbes are described in terms of their temperature requirements as (from coldest to warmest):

- **Psychrophiles** require temperatures below 20°C.
- **Mesophiles** grow best at temperatures ranging between about 20°C and 40°C.
- **Thermophiles** require temperatures above 45°C.
- **Hyperthermophiles** require temperatures above 80°C.

pH

Organisms are sensitive to changes in acidity because hydrogen ions and hydroxyl ions interfere with hydrogen bonding within the molecules of proteins and nucleic acids;

as a result, organisms have ranges of acidity that they prefer and can tolerate. Most bacteria and protozoa are called **neutrophiles** because they grow best in a narrow range around a neutral pH, between 6.5 and 7.5. By contrast, other bacteria and many fungi are **acidophiles**, and grow best in acidic environments where pH can range as low as 0.0. In contrast, **alkalinophiles** live in alkaline soils and water up to pH 11.5.

Physical Effects of Water

Microorganisms require water to dissolve enzymes and nutrients and to act as a reactant in many metabolic reactions. Osmotic pressure restricts cells to certain environments. Whereas the cell walls of some microbes protect them from osmotic shock, osmosis can cause other cells to die from either swelling and bursting, or shriveling (**crenation**). **Obligate halophiles** require high osmotic pressure such as exists in salt water. *Facultative halophiles* do not require but can tolerate salty conditions.

Water exerts pressure in proportion to its depth, and the pressure in deep ocean basins and trenches is tremendous. Organisms that live under extreme pressure are called **barophiles**. Their membranes and enzymes depend on pressure to maintain their three-dimensional functional shapes, and typically they cannot survive at sea level.

Ecological Associations

Relationships in which one organism harms or even kills another are considered *antagonistic*. In *synergistic* relationships, members of an association cooperate such that each receives benefits that exceed those that would result if each lived separately. In *symbiotic* relationships, organisms live in close nutritional or physical contact, becoming interdependent.

Biofilms are an example of complex relationships among numerous individuals, which are often different species, that together attach to surfaces and display metabolic and structural traits different from those expressed by any of the microorganisms alone. They often form as a result of **quorum sensing**, a process in which bacteria respond to the density of nearby bacteria by utilizing signal and receptor molecules.

Culturing Microorganisms (pp. 174–184)

Microbiologists culture microorganisms by transferring an **inoculum**—a sample—from a clinical or environmental specimen into a **medium**, a collection of nutrients. Liquid media are called **broths**. Microorganisms that grow from an inoculum are called a **culture**. Cultures visible on the surface of solid media are called *colonies*.

Clinical Sampling

A **clinical specimen** is a sample of human material such as feces, saliva, cerebrospinal fluid, or blood, that is examined and tested for the presence of microorganisms. Clinical specimens must be properly labeled and transported to a microbiological laboratory to avoid both death of the pathogens and growth of normal organisms.

Obtaining Pure Cultures

Suspected pathogens must be isolated from the normal microbiota in culture. Scientists use several techniques to isolate organisms in **pure cultures** (*axenic cultures*) composed of cells arising from a single progenitor called a **colony-forming unit** (CFU). To obtain pure cultures, all media, vessels, and instruments must be **sterile**; that is, free of any microbial contaminants. The use of aseptic techniques is critical as well.

The most commonly used isolation technique in microbiological laboratories is the **streak plate** method. In this technique, a sterile inoculating loop is used to spread an inoculum across the surface of a solid medium in *Petri dishes*. After an appropriate period of time called **incubation**, colonies develop from each isolate and are distinguished from one another by differences in characteristics.

In the **pour plate** technique, CFUs are separated from one another using a series of dilutions. The final dilutions are mixed with warm agar in Petri dishes. Individual CFUs form colonies in and on the agar.

Culture Media

A variety of media are available for microbiological cultures. A common example is *nutrient broth*. **Agar**, a complex polysaccharide, is a useful compound because it is difficult for microbes to digest, solidifies at temperatures below 40°C, and does not melt below 100°C. Still-warm liquid agar media can be poured into Petri dishes, which once the agar solidifies are then called **Petri plates**. When warm agar media are poured into test tubes that are then placed at an angle and left to cool until the agar solidifies, the result is **slant tubes**, or **slants**. In addition, a variety of culture media are available:

- A medium for which the precise chemical composition is known is called a **defined medium** (or **synthetic medium**).
- **Complex media** contain a variety of growth factors and can support a wider variety of microorganisms than defined media.
- **Selective media** typically contain substances that either favor the growth of particular microorganisms or inhibit the growth of unwanted ones.
- **Differential media** are formulated such that either the presence of visible changes in the medium or differences in the appearances of colonies helps microbiologists differentiate among the different kinds of bacteria growing on the medium. One example involves the differences in organisms' utilization of the red blood cells in blood agar.
- **Reducing media** provide conditions conducive to culturing anaerobes. They contain compounds that chemically combine with free oxygen and remove it from the medium.
- **Transport media** are used by health care personnel to move specimens safely from one location to another while maintaining the relative abundance of organisms and preventing contamination of the specimen or environment.

Special Culture Techniques

Special culture techniques include the following:

- **Animal and cell cultures** allow for the growth of microorganisms for which artificial media are inadequate. Mammals, bird eggs, and cultures of living cells are used.
- **Low-oxygen cultures** favor the growth of microorganisms that thrive in environments intermediate between strictly aerobic and anaerobic, such as within the respiratory or intestinal tract of mammals. Candle jars, chemical packets, or carbon dioxide incubators are used to remove oxygen from the environment.
- **Enrichment cultures** use a selective medium designed to increase very small numbers of a chosen microbe to observable levels.
- **Cold-enrichment cultures** require the incubation of a specimen in a refrigerator, allowing for the enrichment of the culture with cold-tolerant species.

Preserving Cultures

Refrigeration at 4°C is often the best technique for storing bacterial cultures for short periods of time. Deep-freezing and lyophilization are used for long-term storage of bacterial cultures. **Deep-freezing** involves freezing the cells at temperatures from -50°C to -95°C. **Lyophilization** is freeze drying; that is, removal of water from a frozen culture via an intense vacuum.

Growth of Microbial Populations (pp. 184–192)

Most unicellular microorganisms reproduce by *binary fission*, a process in which a cell grows to twice its normal size and then divides in half to produce two equally sized daughter cells.

Mathematical Considerations in Population Growth

With binary fission, any given cell divides to form two cells; then each of these new cells divides in two, to make four, and then four becomes eight, and so on. This type of growth, called **logarithmic growth** or **exponential growth**, produces dramatically greater yields than simple addition, known as *arithmetic growth*. Microbiologists use *scientific notation* to deal with the huge numbers involved in expressing microbial population size.

Generation Time

The time required for a bacterial cell to grow and divide is its **generation time**. Viewed another way, generation time is the time required for a population of cells to double in number. Most bacteria have a generation time of 1–3 hours.

Phases of Microbial Growth

A graph that plots the number of bacteria growing in a population over time is called a **growth curve**. When microbial growth is plotted on a *semilogarithmic scale* (which uses a logarithmic scale for the y-axis), the plot of the population's growth results in a straight line. When bacteria are grown in a broth, the typical microbial growth curve has four distinct phases:

- In the **lag phase**, the organisms are adjusting to their environment.
- In the **log phase**, the population is most actively growing.
- In the **stationary phase**, new organisms are being produced at the same rate at which they are dying.
- In the **death phase**, the organisms are dying more quickly than they can be replaced by new organisms.

Measuring Microbial Growth

Because of each cell's small size and incredible rate of reproduction, it is not possible to count every one in a population. Thus, microbiologists estimate population size by counting the number in a small, representative sample, and then multiplying. Microbiologists use either direct or indirect methods to estimate the number of cells.

Among the many direct methods are the following:

- In **viable plate counts**, microbiologists base an estimate of the size of a microbial population upon the number of colonies formed when diluted samples are plated onto agar media.

- In **membrane filtration**, a large sample is poured through a filter small enough to trap cells.
- In **microscopic counts**, a sample is placed on a *cell counter*, a glass slide with an etched grid, and viewed through a microscope. A microbiologist can count the number of bacteria in several of the large squares and then calculate the mean number of bacteria per square.
- **Electronic counters** are devices that count cells as they interrupt an electrical current flowing across a narrow tube held in front of an electronic detector. *Flow cytometry* is one variation.
- The **most-probable number (MPN) method** is a statistical estimating technique based on the fact that the more bacteria in a sample, the more dilutions are required to reduce their number to zero.

Among the indirect methods are measurements of metabolic activity, measurements of a population's dry weight, and measurement of the turbidity of a broth, especially by a device known as a *spectrophotometer*.

KEY THEMES

In the last chapter we learned about metabolism, an intricate assortment of chemical pathways essential to life. In Chapter 3 we learned how microbes bring in nutrients from the environment to fuel metabolism. In this chapter we focus on those nutrients from two perspectives: what the microbes need to have present in their natural environment to sustain themselves, and what materials need to be present in the laboratory to mimic nature. While studying this chapter, focus on the following:

- *A microbe must either obtain the nutrients it needs from the environment or be able to produce everything it needs itself:* Though each microbe is different, all must obtain the essential materials needed for survival; if they do not, they die.
- *Growth of populations proceeds through phases:* Nutrients are not constant in the environment; how microbes grow will therefore vary depending on many parameters.

QUESTIONS FOR FURTHER REVIEW

Answers to these questions can be found in the answer section at the back of this study guide. Refer to the answers only after you have attempted to solve the questions on your own.

Multiple Choice

1. An organism that uses glucose for carbon and sulfur compounds for energy is a:

a. Photoautotroph	c. Chemoautotroph
b. Photoheterotroph	d. Chemoheterotroph
2. Which of the groups of microbes below is most metabolically diverse in terms of nutritional classes?

a. Algae	c. Fungi
b. Prokaryotes	d. Protozoa

3. Which of the oxygen species below is not toxic to cells?
 - a. O_2
 - b. O_2^-
 - c. O_2^{2-}
 - d. All are toxic to cells
4. Hydroxyl radicals are neutralized in the cell by the action of:
 - a. Carotenoids
 - b. Superoxide dismutase
 - c. Peroxidase
 - d. All of the above work to neutralize hydroxyl radicals
5. Which element listed below is often growth limiting in the environment?
 - a. Carbon
 - b. Hydrogen
 - c. Nitrogen
 - d. Oxygen
6. Vitamins are examples of:
 - a. Trace elements
 - b. Growth factors
 - c. Antioxidants
 - d. Both b and c
7. A microbe's optimum growth temperature is generally closer to:
 - a. The minimum growth temperature
 - b. The maximum growth temperature
 - c. The midpoint between the minimum and maximum growth temperatures
 - d. The optimum temperature is the same as the maximum temperature
8. An organism living at the bottom of the ocean would have to be:
 - a. Barophilic
 - b. Psychrophilic
 - c. Anaerobic
 - d. All of the above
9. Biofilms are best described as:
 - a. Antagonistic relationships between microbes
 - b. Synergistic relationships between microbes
 - c. Symbiotic relationships between microbes
 - d. Complex relationships showing features of synergy and symbiosis
10. Of the methods used to isolate CFUs, which one would allow you to isolate microaerophilic microbes?
 - a. Streak plate
 - b. Pour plate
 - c. Streak plate incubated in a candle jar
 - d. Both b and c
11. On what type of media would you try to grow an organism whose nutritional requirements were not known?
 - a. Defined media
 - b. Complex media
 - c. Anaerobic media
 - d. Selective media
12. On what type of media would you try to grow and differentiate between two species of digestive tract microorganisms?
 - a. Defined media
 - b. Complex media
 - c. Anaerobic media
 - d. Selective media
13. What type of special culture technique would need to be employed to propagate viruses in the laboratory?
 - a. Cell culture
 - b. Low-oxygen culture
 - c. Enrichment culture
 - d. None of the above could be used to propagate a virus

14. To preserve a bacterial specimen for years at a time, it should be stored:
 - a. At room temperature
 - b. In a refrigerator
 - c. In a deep freezer
 - d. As a lyophilized sample
15. If a population of bacteria is growing in a sewer line that sees a continuous flow of wastes, which phase of the growth cycle will the population remain in most of the time?
 - a. Lag phase
 - b. Log phase
 - c. Stationary phase
 - d. Death phase
16. In which phase of the growth curve are antimicrobial treatments most successful?
 - a. Lag phase
 - b. Log phase
 - c. Stationary phase
 - d. Death phase
17. A person with an active infection begins to take an antibiotic. The drug should push the bacterial population into which phase of the growth curve?
 - a. Lag phase
 - b. Log phase
 - c. Stationary phase
 - d. Death phase
18. Which of the following techniques would be best to use to determine the number of living cells in a culture?
 - a. Viable plate count
 - b. Microscopic count
 - c. Coulter counter
 - d. Most Probable Number

Fill in the Blanks

1. Organisms must acquire _____ from the environment to provide energy and building materials for anabolism.
2. Facultative anaerobes _____ (do/do not) have to have mechanisms to protect against the effects of toxic oxygen species. An example of a facultative anaerobe is _____.
3. Nitrogen gas in the atmosphere is turned into useful biological forms by prokaryotes performing this process: _____.
4. _____ grow best at temperatures lower than 15°C; thermophiles, on the other hand, grow best above _____°C.
5. Fungi are generally _____ (acidophiles/neutrophiles/alkalinophiles).
6. Microbes can be grown in liquid _____ cultures or as _____ on solid agar plates.

7. The most common isolation technique used in the microbiology laboratory is the _____ method.
8. _____ cultures are designed to increase the numbers of a desired microbe that is present in a sample in very small numbers.
9. A bacterial culture starts with five cells. The bacteria reproduce every 35 minutes. At the end of 6 hours, you would have _____ cells in your culture.
10. _____ is a/an _____ (direct/indirect) method for estimating the growth of a microbial population in broth that uses spectrophotometry.

Short-Answer Questions for Thought and Review

1. What is a limiting nutrient? Is hydrogen a good example of a limiting nutrient? Why?
2. Explain why aerotolerant and microaerophilic microbes can't survive high oxygen levels but they can survive low levels.
3. When looking at the environmental requirements of microbes (pH, oxygen, etc.), the terms "tolerant" and "obligate" are often used. Give one example of why being tolerant is better than being an obligate.
4. How could the routine ingestion of over-the-counter antacids contribute to the formation of ulcers by *Helicobacter pylori*?
5. Clinical samples from patients must be handled in such a way as to avoid contamination. What are some of the ways a clinical sample can be contaminated from the time it is taken from the patient to the time it is analyzed in the laboratory?

Critical Thinking

1. There is a very large difference between the amount of carbon comprising a prokaryotic cell (50%) and the amount of iron (0.25%). Both elements are necessary to the cell and the cell would die without either. Why, then, is there such a disparity in how much of each is needed? (Hint: Think of what the elements are used for when composing your answer.)
2. Which type of microbe, a single-celled microbe or a multicellular microbe, will have the broader temperature range over which it can survive? Why?
3. Where might you expect to find a fastidious microorganism living?

Concept Building Questions

1. Explain how limiting nutrients in the environment affect the growth potential of microbes. Relate your answer to transport mechanisms across the cytoplasmic membrane (Chapter 3) and to metabolism (Chapter 5).
2. When isolating white blood cells from blood products it is sometimes desired to remove the red blood cells. If the blood cells have been resuspended in a saline solution (0.9% NaCl), should the solution added to lyse the red blood cells have a higher or lower concentration of NaCl? Why?
3. Figure 6.7 presents some of the characteristics of bacterial colonies. These descriptions are often useful when trying to determine the identity of a bacterial sample. In Chapter 4 we learned that certain identification is generally not done using morphological characteristics alone. Explain why the information in Figure 6.7 can help narrow down possible identities for a microbe, but can't be used to make a definitive identification.