NUTRISI DAN PERTUMBUHAN MIKROORGANISME

Microbial Nutrition

Chapter Overview

This chapter describes the basic nutritional requirements of microorganisms. Cells must have a supply of raw materials and energy in order to construct new cellular components. This chapter also describes the processes by which microorganisms acquire nutrients and provides information about the cultivation of microorganisms.

Chapter Objectives

After reading this chapter you should be able to:

1. list the ten elements that microorganisms require in large amounts (macronutrients/macroelements) and the six elements that they require in trace amounts (micronutrients)
2. list the major nutritional categories and give the source of carbon, energy, and hydrogen/electrons for each of the categories
3. compare the various processes (passive diffusion, facilitated diffusion, active transport, group translocation) by which cells can obtain nutrients from the environment
4. describe the various types of culture media for microorganisms (synthetic, complex, selective, differential) and tell how each is normally used in the study of microorganisms
5. describe the techniques used to obtain pure cultures (spread plate, streak plate, pour plate)

These are the most important concepts you are learning in this chapter:

1. Microorganisms require about 10 elements in large quantities. All microorganisms can be place in one of a few nutritional categories on the basis of their requirement for carbon, energy, and hydrogen atoms or electrons.
2. Nutrient molecules frequently cannot cross selectively permeable plasma membranes through passive diffusion.
3. Nutrient molecules must be transported by one of three major mechanisms involving the use of membrane carrier proteins. Eucaryotic microorganisms also employ endocytosis for nutrient uptake.
4. Culture media are needed to grow microorganisms in the laboratory. Culture media is used to carry out microbial identification, water and food analysis, and isolation of particular microorganisms.
5. Pure cultures can be obtained through the use of spread plates, streak plates, or pour plates.
6. Pure cultures are required for the study of an individual microbial species.

Study Outline

I. The Common Nutrient Requirements

A. Macroelements, also known as macronutrients (C, O, H, N, S, P, K, Ca, Mg, Fe), are required by microorganisms in relatively large amounts
B. Trace elements or micronutrients (Mn, Zn, Co, Mo, Ni, Cu) are required in trace amounts by most cells and are often adequately supplied in the water used to prepare the media or in the regular media components
C. Other elements may be needed by particular types of microorganisms

II. Requirements for Carbon, Hydrogen, and Oxygen
A. Autotrophs use carbon dioxide as their sole or principal carbon source
B. Heterotrophs use reduced, preformed organic molecules (usually from other organisms) as carbon sources

III. Nutritional Types of Microorganisms
A. All organisms need a source of energy and electrons
1. Energy
a. Phototrophs use light as their energy source
b. Chemotrophs obtain energy from the oxidation of organic or inorganic compounds
2. Electrons
a. Lithotrophs use reduced inorganic compounds as their electron source
b. Organotrophs use reduced organic compounds as their electron source

IV. Most microorganisms can be categorized as belonging to one of four major nutritional types depending on their sources of carbon, energy, and electrons:
A. Photolithotrophic autotrophs
B. Chemoorganotrophic heterotrophs
C. Photoorganotrophic heterotrophs
D. Chemolithotrophic autotrophs

V. Some organisms show great metabolic flexibility and alter their metabolic patterns in response to environmental changes; mixotrophic organisms combine autotrophic and heterotrophic metabolic processes, relying on inorganic energy sources and organic carbon sources

VI. Requirements for Nitrogen, Phosphorus, and Sulfur
A. Nitrogen is needed for the synthesis of amino acids, purines, pyrimidines and other molecules; depending on the organism, nitrogen can be supplied by organic molecules, by assimilatory nitrate reduction, or by nitrogen fixation
B. Phosphorus is present in nucleic acids, phospholipids, nucleotides and other molecules; most microorganisms use inorganic phosphate to meet their phosphorus needs
C. Sulfur is needed for the synthesis of certain amino acids and other molecules; most microorganism meet their sulfur needs by assimilatory sulfate reduction

VII. Growth Factors
A. Growth factors are organic compounds required by the cell because they are essential cell components (or precursors of these components) that the cell cannot synthesize; there are three major classes:
1. Amino acids-needed for protein synthesis
2. Purines and pyrimidines-needed for nucleic acid synthesis
3. Vitamins-function as enzyme cofactors

B. Knowledge of specific growth factor requirements makes possible quantitative growth-response assays

VIII. Uptake of Nutrients by the Cell

A. Passive diffusion-a phenomenon in which molecules move from an area of high concentration to an area of low concentration because of random thermal agitation
   1. Requires a large concentration gradient for significant levels of uptake
   2. Limited to only a few small molecules (e.g., glycerol, H_2O, O_2, and CO_2)

B. Facilitated diffusion-a process that involves a carrier molecule (permease) to increase the rate of diffusion; net effect is limited to movement from an area of higher concentration to an area of lower concentration
   1. Requires a smaller concentration gradient than passive diffusion
   2. The rate plateaus when the carrier becomes saturated (i.e., when it is binding and transporting molecules as rapidly as possible)
   3. Generally more important in eucaryotes rather than procaryotes

C. Active transport-a process in which metabolic energy is used to move molecules to the cell interior where the solute concentration is already higher (i.e., it runs against the concentration gradient)
   1. Characteristics of active transport
      a. Saturable uptake rate
      b. Requires an expenditure of metabolic energy
      c. Can concentrate molecules inside the cell even when the concentration inside the cell is already higher than that outside the cell
   2. ATP-binding cassette transporters (ABC transporters) use ATP to drive transport against a concentration gradient; they are observed in bacteria, archaea and eucaryotes
   3. Proton motive forces can also be used to power active transport
   4. Types of active transport
      a. Symport is the linked transport of two substances in the same direction
      b. Antiport is the linked transport of two substances in opposite directions

D. Group translocation-a process in which molecules are modified as they are transported across the membrane

E. Iron uptake-the organism secretes siderophores that complex with the very insoluble ferric ion, which is then transported into the cell

IX. Culture Media

A. A culture medium is a solid or liquid preparation used to grow, transport, and store microorganisms

B. Synthetic (defined) media are media in which all components and their concentrations are known

C. Complex media are media that contain some ingredients of unknown composition and/or concentration; this type supplies amino acids, vitamins, growth factors, and other nutrients

D. Types of Media
   1. General purpose media will support the growth of many microorganisms

2. Enriched media are supplemented by blood or other special nutrients to encourage the growth of fastidious heterotrophs
3. Selective media favor the growth of particular microorganisms and inhibit the growth of others
4. Differential media distinguish between different groups of bacteria on the basis of their biological characteristics

X. Isolation of Pure Cultures
   A. A pure culture is a population of cells arising from a single cell
   B. The spread plate and streak plate methods separate cells on an agar surface such that each cell grows into a completely isolated colony (a macroscopically visible growth or cluster of microorganisms on a solid medium)
   C. The pour plate method involves diluting a sample to decrease the number of microorganisms, mixing the dilution with agar, and then pouring the mixture into a petri dish
   D. Colony morphology helps microbiologists identify bacteria because individual species often form colonies of characteristic size and appearance

Chapter Web Links

The Microbe Zoo
(http://commtechlab.msu.edu/sites/dlc-me/zoo/)
"A virtual zoo filled with exotic specimens arranged in pavilions representing the habitats in which they naturally dwell, such as Water World, DirtLand, and the Animal Pavilion. Subsections of the zoo pavilions representing specific microbial habitats include the compost heap, the toxic waste dump, habitat on humanity, the termite gut, the house of horrors, and several others".

Astrobiology Web
(http://www.astrobiology.com/extreme.html)
Life in Extreme Environments

Chapter 6: Microbial Growth

Chapter Overview

This chapter describes the basic nature of microbial growth in the presence of an adequate nutrient supply. Several methods for the measurement of microbial growth are described and different systems used for microbial growth are also described. The chapter finishes with a mengetahuiion of the influence of various environmental factors on the growth of microorganisms.

Chapter Objectives

After reading this chapter you should be able to:

1. name the various phases of growth that occur in closed culture systems and describe what is occurring in each phase

2. determine from experimental data the various parameters (number of generations, specific growth rate constant, mean generation time) that describe microbial growth in mathematical terms
3. menjelaskan the influence of various environmental factors (water availability, pH, temperature, oxygen concentration, pressure, radiation) on the growth of microorganisms
4. categorize microorganisms according to the environmental factors that are conducive to optimal growth of the organism

These are the most important concepts you are learning in this chapter:

1. Growth is defined as an increase in cellular constituents and may result in an increase in a microorganism's size, population number , or both.
2. When microorganisms are grown in a closed system, population growth remains exponential for only a few generations and then enters a stationary phase due to factors such as nutrient limitation and waste accumulation. In an open system with continual nutrient addition and waste removal, the exponential phase can be maintained for long periods.
3. A wide variety of techniques can be used to study microbial growth by following changes in the total cell number, the population of viable microorganisms, or the cell mass.
4. Water availability, pH, temperature, oxygen concentration, pressure, radiation, and a number of other environmental factors influence microbial growth. Yet many microorganisms, and particularly bacteria, have managed to adapt and flourish under environmental extremes that would destroy most higher organisms.

Study Outline

I. Growth
   A. Growth is an increase in cellular constituents that may result in an increase in cell size, an increase in cell number, or both
   B. Because observing growth of single cells is difficult, microbiologists usually study growth of a population of microbes

II. The Growth Curve
   A. Population growth is usually analyzed in a closed system called a batch culture; it is usually plotted as the logarithm of cell number versus the incubation time
   B. Lag phase-the period of apparent inactivity in which the cells are adapting to a new environment and preparing for reproductive growth, usually by synthesizing new cell components; it varies considerably in length depending upon the condition of the microorganisms and the nature of the medium
   C. Exponential (log) phase-the period in which the organisms are growing at the maximal rate possible given their genetic potential, the nature of the medium, and the conditions under which they are growing; the population is most uniform in terms of chemical and physical properties during this period
   D. Stationary phase-the period in which the number of viable microorganisms remains constant either because metabolically active cells stop reproducing or because the reproductive rate is balanced by the rate of cell death

1. Microbial populations enter stationary phase for several reasons including nutrient limitation, toxic waste accumulation, and possibly cell density
2. Responses to starvation conditions are of practical importance for medical and industrial microbiology; these responses include morphological changes and changes in gene expression and physiology

E. Death phase-the period in which the cells are dying at an exponential rate

F. The mathematics of growth-microbial growth can be described by certain mathematical terms:

1. Mean growth rate constant is the number of generations per unit time, often expressed as generations per hour
2. Mean generation (doubling) time is the time required for the population to double
3. Generation times vary markedly with the species of microorganism and environmental conditions; they can range from 10 minutes for some bacteria to several days for some eucaryotic microorganisms

III. Measurement of Microbial Growth

A. Measurement of cell numbers

1. Direct count methods do not distinguish between living and dead cells, and may be accomplished by direct microscopic observation on specially etched slides (such as Petroff-Hausser chambers or hemacytometers) or by using electronic counters (such as Coulter Counters, which count microorganisms as they flow through a small hole or orifice)
2. Viable cell counts involve plating diluted samples (using a pour plate or spread plate) onto suitable growth media and monitoring colony formation; this type of method counts only those cells that are reproductively active; because it is not possible to be certain that each colony arose from a single cell, results are usually expressed as colony forming units (CFU); analysis of aquatic samples is frequently done by counting colonies growing on membrane filters having pores small enough to trap bacteria

B. Measurement of cell mass-may be used to approximate the number of microorganisms if a suitable parameter proportional to the number of microorganisms present is used (suitable parameters may be dry weight, light scattering in liquid solutions, or biochemical determinations of specific cellular constituents such as protein, DNA, or ATP)

IV. The Continuous Culture of Microorganisms

A. Used to maintain cells in the exponential growth phase at a constant biomass concentration for extended periods of time (these conditions are met by continual provision of nutrients and removal of wastes)

B. A chemostat-a continuous culture device that maintains a constant growth rate by supplying a medium containing a limited amount of an essential nutrient at a fixed rate and by removing medium that contains microorganisms at the same rate

C. A turbidostat-a continuous culture device that regulates the flow rate of media through the vessel in order to maintain a predetermined turbidity or cell density; there is no limiting nutrient

V. The Influence of Environmental Factors on Growth

A. Microorganisms grow in a variety of environmental conditions; certain microorganisms, referred to as extremophiles, grow under harsh conditions that would kill most other organisms

B. Solutes and water activity

1. If a microorganism is placed in a hypotonic solution (one with a lower solute concentration), water will enter the cell and cause it to burst, unless the microorganism has a protective mechanism to reduce the osmotic concentration of the cytoplasm
2. If a microorganism is placed in a hypertonic solution (one with a higher solute concentration), water will leave the cell causing dehydration, unless the microorganism has a protective mechanism to increase the osmotic concentration of the cytoplasm
3. Water activity (aw) is the amount of water available to microorganism; it is reduced by the interaction of water with solute molecules; osmotolerant organisms can grow in solutions of both high and low water activity; halophiles require environments of low water activity (high osmotic pressure due to saline conditions) in order to grow

C. pH

1. pH is the negative logarithm of the hydrogen ion concentration
2. Each species has a pH growth range and pH growth optimum a. Acidophiles grow best between pH 0 and 5.5 b. Neutrophiles grow best between pH 5.5 and 8.0 c. Alkalophiles grow best between pH 8.5 and 11.5 d. Extreme alkalophiles grow best at pH 10.0 or higher
3. Microorganisms can usually adjust to changes in environmental pH by maintaining an internal pH that is near neutrality; some bacteria also synthesize protective proteins (acid shock proteins) in response to pH

D. Temperature

1. Temperature has a profound effect on microorganisms; as the temperature rises, there is an increase in the growth rate due to increasing the rates of enzyme reactions; eventually a temperature becomes too high and microorganisms are damaged by enzyme denaturation, membrane disruption, and other phenomena
2. Organisms exhibit distinct cardinal temperatures (minimal, maximal, and optimal growth temperatures)
    a. Psychrophiles can grow well at 0°C, have optimal growth at 15°C or lower, and usually will not grow above 20°C
    b. Psychrotrophs (facultative psychrophiles) can also grow at 0°C, but have growth optima between 20°C and 30°C, and growth maxima at about 35°C
    c. Mesophiles have growth optima of 20 to 45°C, minima of 15 to 20°C, and maxima of about 45°C or lower
    d. Thermophiles have growth optima of 55 to 65°C, and minima around 45°C
    e. Hyperthermophiles have growth optima of 80 to 110°C and minima around 55°C
3. Oxygen concentration
    a. An organism able to grow in the presence of O2 is an aerobe; one that cannot is an anaerobe

b. Obligate aerobes are completely dependent on atmospheric O2 for growth
c. Facultative anaerobes do not require O2 for growth, but do grow better in its presence
d. Aerotolerant anaerobes ignore O2 and grow equally well whether it is present or not
e. Obligate (strict) anaerobes do not tolerate O2 and die in its presence
f. Microaerophiles require lower levels (2 to 10%) for growth because normal atmospheric levels of O2 (20%) are damaging to the cell
g. The different relationships with O2 are due to several factors including inactivation of proteins and the effect of toxic O2 derivatives (superoxide radical, hydrogen peroxide, and hydroxyl radical), which oxidize and destroy cellular constituents; many microorganisms possess enzymes that protect against toxic O2 derivatives (superoxide dismutase and catalase)

4. Pressure
   a. Barotolerant organisms are adversely affected by increased pressure, but not as severely as are nontolerant organisms
   b. Barophilic organisms require, or grow more rapidly in the presence of, increased pressure
5. Radiation
   a. There are many types of electromagnetic radiation, including visible light, ultraviolet light (UV), infrared rays, radio waves, and ionizing radiation; some of these can be harmful to organisms
   b. Ionizing radiation such as X rays or gamma rays is very harmful to microorganisms; low levels produce mutations and may indirectly result in death, whereas high levels are directly lethal by direct damage to cellular macromolecules or through the production of oxygen free radicals
   c. Ultraviolet radiation damages cells by causing the formation of thymine dimers in DNA; this damage can be repaired by photoreactivation (repairs thymine dimers by direct splitting when the cells are exposed to blue light) or by dark reactivation (repairs thymine dimers by excision and replacement in the absence of light)
6. Many microorganisms that are airborne or live on exposed surfaces use carotenoid pigments for protection against photooxidation

VI. Microbial Growth in Natural Environments
   A. Growth limitation by environmental factors
      1. Microbial environments are complex, constantly changing, and may expose a microorganism to overlapping gradients of nutrients and environmental factors
         a. Liebigís law of the minimum states that the total biomass of an organism will be determined by the nutrient present in the lowest concentration relative to the organismís requirements.

b. Shelfordís law of tolerance states that there are limits to environmental factors below and above which microorganisms cannot survive and grow, regardless of the nutrient supply

2. In response to low nutrient level (oligotrophic environments) and intense competition, many microorganisms change their morphology or physiology or both

B. Counting viable but nonculturable vegetative procaryotes

1. When microorganisms are stressed they can remain viable but lose the ability to grow on media normally used for their cultivation (viable but nonculturable cells)
2. Numerous microscopic and isotopic procedures to identify and count viable but nonculturable cells have been developed

C. Quorum sensing and microbial populations

1. Quorum sensing (autoinduction) is a process by which bacteria can communicate and behave cooperatively
2. Chemical signals are secreted by bacteria and used to communicate with each other; gram-negative bacteria use acyl homoserine lactones (HSLs) as signals; gram-positive bacteria often use oligopeptide signals

Chapter Web Links

<u>Microbial Population Explosion from the Why Files</u>
(http://whyfiles.org/shorties/count_bact.html)