

5

Cultivation of

Microorganisms

CHAPTER

Cultivation is the process of propagating organisms by providing The proper environmental conditions. Growing microorganisms are making replicas of themselves, and they require the elements present in their chemical composition. Nutrients must provide these elements in metabolically accessible form.

In addition, organisms require metabolic energy to synthesize

macromolecules and maintain essential chemical gradients across their membranes. Factors that must be controlled during growth include the nutrients, pH, temperature, aeration,

salt concentration, and ionic strength of the medium.

REQUIREMENTS FOR GROWTH

Most of the dry weight of microorganisms is organic matter containing the elements carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur. In addition, inorganic ions such as potassium, sodium, iron, magnesium, calcium, and chloride are required to facilitate enzymatic catalysis and to maintain chemical gradients across the cell membrane.

For the most part, the organic matter is in macromolecules formed by **anhydride bonds** between building blocks.

Synthesis of the anhydride bonds requires chemical energy, which is provided by the two phosphodiester bonds in ATP (adenosine triphosphate; see Chapter 6). Additional energy required to maintain a relatively constant cytoplasmic composition

during growth in a range of extracellular chemical environments is derived from the **proton motive force** . The proton motive force is the potential energy that can be derived by passage of a proton across a membrane. In eukaryotes, the membrane may be part of the mitochondrion or the chloroplast. In prokaryotes, the membrane is the cytoplasmic membrane of the cell.

The proton motive force is an electrochemical gradient with two components, a difference in pH (hydrogen ion concentration) and a difference in ionic charge. The charge on the outside of the bacterial membrane is more positive than the charge on the inside, and the difference in charge contributes to the free energy released when a proton enters the cytoplasm from outside the membrane. Metabolic processes that generate the proton motive force are discussed in Chapter 6 . The free energy may be used to move the cell, to maintain ionic or molecular gradients across the membrane, to synthesize anhydride bonds in ATP, or for a combination of these purposes. Alternatively, cells given a source of ATP may use its anhydride bond energy to create a proton motive force that in turn may be used to move the cell and to maintain chemical gradients.

To grow, an organism requires all of the elements in its organic matter and the full complement of ions required for energetics and catalysis. In addition, there must be a source of energy to establish the proton motive force and to allow macromolecular synthesis. Microorganisms vary widely in their

nutritional demands and their sources of metabolic energy.

NUTRITION

Nutrients in growth media must contain all the elements necessary for the biologic synthesis of new organisms. In the following discussion, nutrients are classified according to the elements they supply.

Carbon Source

As already mentioned, plants and some bacteria are able to use photosynthetic energy to reduce carbon dioxide at the expense of water. These organisms belong to the group of **autotrophs**, creatures that do not require organic nutrients for growth. Other autotrophs are the **chemolithotrophs**, organisms that use an inorganic substrate such as hydrogen or thiosulfate as a reductant and carbon dioxide as a carbon source.

Heterotrophs require organic carbon for growth, and the organic carbon must be in a form that can be assimilated. Naphthalene, for example, can provide all of the carbon and energy required for respiratory heterotrophic growth, but very few organisms possess the metabolic pathway necessary for naphthalene assimilation. Glucose, on the other hand, can support the fermentative or respiratory growth of many organisms. It is important that growth substrates be supplied at levels appropriate for the microbial strain that is being grown: Levels that will support the growth of one organism may inhibit the growth of another organism.

Carbon dioxide is required for a number of biosynthetic reactions. Many respiratory organisms produce more than enough carbon dioxide to meet this requirement, but others require a source of carbon dioxide in their growth medium.

Nitrogen Source

Nitrogen is a major component of proteins, nucleic acids, and other compounds, accounting for approximately 5% of the dry weight of a typical bacterial cell. Inorganic dinitrogen (N_2) is very prevalent, comprising 80% of the earth's atmosphere. It is also a very stable compound, primarily because of the high activation energy required to break the nitrogen–nitrogen triple bond. However, nitrogen may be supplied in a number of different forms, and microorganisms vary in their abilities to assimilate nitrogen (Table 5-1). The end product of all pathways for nitrogen assimilation is the most reduced form of the element, ammonia (NH_3). When NH_3 is available, it diffuses into most bacteria through transmembrane channels as dissolved gaseous NH_3 rather than ionic ammonium ion (NH_4^+). The ability to assimilate N_2 reductively via NH_3 , which is called **nitrogen fixation**, is a property unique to prokaryotes, and relatively few bacteria are capable of breaking the nitrogen–nitrogen triple bond. This process (see Chapter 6) requires a large amount of metabolic energy and is readily inactivated by oxygen. The capacity for nitrogen fixation is found in widely divergent bacteria that have evolved quite different biochemical strategies to protect their nitrogen-fixing enzymes from oxygen.

Sulfur Source

Similar to nitrogen, sulfur is a component of many organic cell substances. It forms part of the structure of several coenzymes and is found in the cysteinyl and methionyl side chains of proteins. Sulfur in its elemental form cannot be used by plants or animals. However, some autotrophic bacteria can oxidize it to sulfate (SO_4^{2-}). Most microorganisms can use

sulfate as a sulfur source, reducing the sulfate to the level of hydrogen sulfide (H₂S). Some microorganisms can assimilate H₂S directly from the growth medium, but this compound can be toxic to many organisms.

Phosphorus Source

Phosphate (PO₄³⁻) is required as a component of ATP; nucleic acids; and such coenzymes as NAD, NADP, and flavins. In addition, many metabolites, lipids (phospholipids, lipid A), cell wall components (teichoic acid), some capsular polysaccharides, and some proteins are phosphorylated. Phosphate is always assimilated as free inorganic phosphate (Pi).

Mineral Sources

Numerous minerals are required for enzyme function. Magnesium ion (Mg²⁺) and ferrous ion (Fe²⁺) are also found in porphyrin derivatives: magnesium in the chlorophyll molecule, and iron as part of the coenzymes of the cytochromes and peroxidases. Mg²⁺ and K⁺ are both essential for the function and integrity of ribosomes. Ca²⁺ is required as a constituent of gram-positive cell walls, although it is dispensable for gram-negative bacteria. Many marine organisms require Na⁺ for growth. In formulating a medium for the cultivation of most microorganisms, it is necessary to provide sources of potassium, magnesium, calcium, and iron, usually as their ions (K⁺, Mg²⁺, Ca²⁺, and Fe²⁺). Many other minerals (eg, Mn²⁺, Mo²⁺, Co²⁺, Cu²⁺, and Zn²⁺) are required; these frequently can be provided in tap water or as contaminants of other medium

ingredients.

The uptake of iron, which forms insoluble hydroxides at neutral pH, is facilitated in many bacteria and fungi by their production of **siderophores**—compounds that chelate iron and promote its transport as a soluble complex. These include hydroxamates ($-\text{CONH}_2\text{OH}$) called sideramines, and derivatives

of catechol (eg, 2,3-dihydroxy-benzoylserine).

Plasmid-determined

siderophores play a major role in the invasiveness of some bacterial pathogens (see Chapter 7).

Siderophore and

nonsiderophore-dependent mechanisms of iron uptake by bacteria are discussed in Chapter 9.