Cultivation of Microorganisms

Focusing

- 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.

- The organisms require metabolic energy in order 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.

Sources of Metabolic Energy

Fermentation

Fermentation is characterized by **substrate phosphorylation**, an enzymatic process in which a pyrophosphate bond is donated directly to ADP (adenosine diphosphate) by a phosphorylated metabolic intermediate.

The phosphorylated intermediates are formed by metabolic rearrangement of a fermentable substrate such as glucose, lactose, or arginine.

Respiration

Chemical reduction of an oxidant (electron acceptor) through a specific series of electron carriers in the membrane establishes the proton motive force across the bacterial membrane.

The reductant (electron donor) may be organic or inorganic: eg, lactic acid serves as a reductant for some organisms, and hydrogen gas is a reductant for other organisms.

Gaseous oxygen (O_2) often is employed as an oxidant, but alternative oxidants that are employed by some organisms include carbon dioxide (CO_2), sulfate (SO_4^{2-}) and nitrate (NO_3^-).

Photosynthesis

Photosynthesis is similar to respiration in that the reduction of an oxidant via a specific series of electron carriers establishes the proton motive force. The difference in the two processes is that in photosynthesis the reductant and oxidant are created photochemically by light energy absorbed by pigments in the membrane; thus, photosynthesis can continue only as long as there is a source of light energy.

Nutrition

Carbon Source

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.

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, as it comprises 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.

Sulfur Source

Like 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.

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 (H_2S).

Some microorganisms can assimilate H_2S directly from the growth medium, but this compound can be toxic to many organisms.

Phosphorus Source

Phosphate (PO_4^{3-}) 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 (P_i).

Mineral Sources

Magnesium ion (Mg^{2+}) and ferrous ion (Fe^{2+}) are also found in porphyrin derivatives: magnesium in the chlorophyll molecule, and iron as part of the coenzymes of the cytochromes and peroxidases.

Mg^{2+} and K^{+} are both essential for the function and integrity of ribosomes.

Ca^{2+} is required as a constituent of gram-positive cell walls, though it is dispensable for gram-negative bacteria.

Environmental Factors Affecting Growth

<u>Hydrogen Ion Concentration (pH)</u>

Most organisms have a fairly narrow optimal pH range. The optimal pH must be empirically determined for each species.

Most organisms **(neutralophiles)** grow best at a pH of 6.0–8.0, although some forms **(acidophiles)** have optima as low as pH 3.0 and others **(alkaliphiles)** have optima as high as pH 10.5.

Microorganisms regulate their internal pH over a wide range of external pH values by pumping protons in or out of their cells.

Temperature

Different microbial species vary widely in their optimal temperature ranges for growth: **Psychrophilic** forms grow best at low temperatures (15–20°C); **mesophilic** forms grow best at 30–37°C; and most **thermophilic** forms grow best at 50–60°C. Some organisms are **hyperthermophilic** and can grow at well above the temperature of boiling water, which exists under high pressure in the depths of the ocean.

Most organisms are mesophilic; 30°C is optimal for many free-living forms, and the body temperature of the host is optimal for symbionts of warm-blooded animals.

Aeration

Many organisms are obligate aerobes, specifically requiring oxygen as hydrogen acceptor; some are facultative, able to live aerobically or anaerobically; and others are obligate anaerobes, requiring a substance other than oxygen as hydrogen acceptor and being sensitive to oxygen inhibition.

Isolation of Microorganisms in Pure Culture

In order to study the properties of a given organism, it is necessary to handle it in pure culture free of all other types of organisms. To do this, a single cell must be isolated from all other cells and cultivated in such a manner that its collective progeny also remain isolated.

- Plating serial dilutions of the specimen

- **Pour plate method**
- **Spread plate method**