There are only two sources of energy available to organisms: (1) light energy, and (2) the energy derived from oxidizing organic or inorganic molecules. **Phototrophs** use light as their energy source; **chemotrophs** obtain energy from the oxidation of chemical compounds (either organic or inorganic).
Microorganisms also have only two sources for electrons. **Lithotrophs** (i.e., “rock-eaters”) use reduced inorganic substances as their electron source, whereas **organotrophs** extract electrons from organic compounds.

Table 5.1 Sources of Carbon, Energy, and Electrons

Carbon Sources	
Autotrophs	CO_2 sole or principal biosynthetic carbon source (*pp. 207–8*)[a]
Heterotrophs	Reduced, preformed, organic molecules from other organisms (*chapters 9 and 10*)
Energy Sources	
Phototrophs	Light (*pp. 195–201*)
Chemotrophs	Oxidation of organic or inorganic compounds (*chapter 9*)
Electron Sources	
Lithotrophs	Reduced inorganic molecules (*pp. 193–94*)
Organotrophs	Organic molecules (*chapter 9*)

[a]For each category, the location of material describing the participating metabolic pathways is given within the parentheses.

Despite the great metabolic diversity seen in microorganisms, most may be placed in one of four nutritional classes based on their primary sources of carbon, energy, and electrons (**table 5.2**).

Table 5.2 Major Nutritional Types of Microorganisms

Major Nutritional Types[a]	Sources of Energy, Hydrogen/Electrons, and Carbon	Representative Microorganisms
Photolithotrophic autotrophy (Photolithoautotrophy)	Light energy Inorganic hydrogen/electron (H/e⁻) donor CO_2 carbon source	Algae Purple and green sulfur bacteria Cyanobacteria
Photoorganotrophic heterotrophy (Photoorganoheterotrophy)	Light energy Organic H/e^- donor Organic carbon source (CO_2 may also be used)	Purple nonsulfur bacteria Green nonsulfur bacteria
Chemolithotrophic autotrophy (Chemolithoautotrophy)	Chemical energy source (inorganic) Inorganic H/e^- donor CO_2 carbon source	Sulfur-oxidizing bacteria Hydrogen bacteria Nitrifying bacteria Iron-oxidizing bacteria
Chemoorganotrophic heterotrophy (Chemoorganoheterotrophy)	Chemical energy source (organic) Organic H/e^- donor Organic carbon source	Protozoa Fungi Most nonphotosynthetic bacteria (including most pathogens)

[a]Bacteria in other nutritional categories have been found. The categories are defined in terms of energy, electron, and carbon sources. Condensed versions of these names are given in parentheses.

The large majority of microorganisms thus far studied are either photolithotrophic autotrophs or chemoorganotrophic heterotrophs.

- **Photolithotrophic autotrophs** (often called **photoautotrophs** or photolithoautotrophs) use light energy and have CO2 as their carbon source. Eucaryotic algae and cyanobacteria employ water as the electron donor and release oxygen. Purple and green sulfur bacteria cannot oxidize water but extract electrons from inorganic donors like hydrogen, hydrogen sulfide, and elemental sulfur.
- **Chemoorganotrophic heterotrophs** (often called **chemoheterotrophs,** chemoorganoheterotrophs, or even heterotrophs) use organic compounds as sources of energy, hydrogen, electrons, and carbon. Frequently the same organic nutrient will satisfy all these requirements. It should be noted that essentially all pathogenic microorganisms are chemoheterotrophs.
- The other two nutritional classes have fewer microorganisms but often are very important ecologically. Some purple and green bacteria are photosynthetic and use organic matter as their electron donor and carbon source. These **photoorganotrophic heterotrophs** (photoorganoheterotrophs) are common inhabitants of polluted

Zinc is an essential regulatory element for eucaryotic genetics. It is a major component of "zinc fingers"—binding factors that help enzymes adhere to specific sites on DNA. Copper, cobalt, nickel, molybdenum, manganese, silicon, iodine, and boron are needed in small amounts by some microbes but not others. A discovery with important medical implications is that metal ions can directly influence certain diseases by their effects on microorganisms. For example, the bacteria that cause gonorrhea and meningitis grow more rapidly in the presence of iron ions.

Element	Cellular function
Boron (B)	Present in an auto inducer for quorum sensing in bacteria. Also found in poly ketide antibiotics.
Chromium (Cr)	Required by mammals for glucose metabolism
Cobalt (Co)	Vitamin B12; Transcarboxylase (propionic acid bacteria)
Copper (Cu)	Respiration, Cytochrome C oxidase, photosynthesis, plastocyanin, Some superoxide dismutases.
Iron (Fe)	Cytochromes, catalases, peroxidases, iron sulfur proteins, oxygenases and all nitrogenases
Manganese (Mn)	Activator of many enzymes, present in certain superoxide dismutases and in the water splitting enzyme photosystem II
Molybdenum (Mo)	Certain flavin containing enzymes, some nitrogenases, nitrate reductases, sulfite oxidases, formate dehydrogenases
Nickel (Ni)	Most dehydrogenases, Coenzyme F_{430} of methanogens, carbonmonoxide dehydrogenase, urease
Selenium (Se)	formate dehydrogenases, some hydrogenases, aminoacid selenocysteine
Tungsten (W)	Some formate dehydrogenases, oxotransferases of hyperthermophiles.
Vanadium (V)	Vanadium nitrogenase, bromo peroxidases
Zinc (Zn)	Carbonic anhydrase, alcohol dehydrogenase, RNA & DNA polymerases, many DNA binding proteins

GROWTH FACTORS AND THEIR FUNCTIONS IN METABOLISM

Microorganisms often grow and reproduce when minerals and sources of energy, carbon, nitrogen, phosphorus, and sulfur are supplied. These organisms have the enzymes and pathways necessary to synthesize all cell components required for their wellbeing. Many microorganisms, on the other hand, lack one or more essential enzymes. Therefore they cannot manufacture all indispensable constituents but must obtain them or their precursors from the environment. Organic compounds required because they are essential cell components or precursors of such components and cannot be synthesized by the organism are called **growth factors.**

There are three major classes of growth factors:

- Amino acids,
- Purines and pyrimidines, and
- Vitamins.

Amino acids are needed for protein synthesis, purines and pyrimidines for nucleic acid synthesis. **Vitamins** are small organic molecules that usually make up all or part of enzyme cofactors, and are needed in only very small amounts to sustain growth. Vitamin requirements are highly variable among microorganisms ranging from none to several. Some microorganisms require many vitamins. For example:

- *Enterococcus faecalis* needs eight different vitamins for growth. Other growth factors are also seen.
- *Haemophilus influenza*: Heme from hemoglobin or cytochromes is required for growth.
- Mycoplasmas need cholesterol.
- Lactic acid bacteria: Require multiple vitamin requirements.

The vitamins most commonly required by microorganisms are thiamine, biotin, pyridoxine and cobalamin.
Understanding the growth factor requirements of microbes has some important practical applications.
Knowledge of the specific growth factor requirements of many microorganisms makes possible quantitative growth response assays for a variety of substances. A typical assay is a growth response assay which allows the

atmosphere. A special solution is added to the pouch's reagent compartment; petri dishes or other containers are placed in the pouch; it then is clamped shut and placed in an incubator.
(5) Even very O_2 sensitive bacteria can be transferred in the open atmosphere, provided that continuous stream of O_2 free nitrogen through the culture vessel prevents contact of the medium with air. Alternatively one can use inoculation chambers which are filled with O_2 free nitrogen, argon or hydrogen. As an indicator for anaerobic conditions, resazurin can be added to media. In the presence of O_2 this is blue, if conditions are anaerobic it is colorless and after reoxidation it is red.
(6) Also a beaker with alkaline glucose-methylene blue solution, which is decolorized under anaerobic conditions, can be added to the incubation jar.

Preparation of pre reduced media for cultivation of anaerobes:

During preparation, the culture medium is boiled for several minutes to drive off most of the dissolved oxygen. A reducing agent, ex: Cysteine is added to further lower the O_2 content. O_2 free nitrogen is bubbled through the medium to keep it anaerobic. The medium is then dispensed into tubes which are being flushed with O_2 free N_2, stoppered tightly, and sterilized by autoclaving. Such tubes can be stored for many months before being used. During inoculation, the tubes are continuously flushed with O_2 free CO_2 by means of canula, restoppered and incubated.

MICROBIAL GROWTH

Growth may be defined as an increase in cellular constituents. It leads to a rise in cell number when micro-organisms reproduce by processes like budding or binary fission. In the latter, individual cells enlarge and divide to yield two progeny (daughter cells) of approximately equal size. The daughter cells are identical except for the occasional mutation. Binary fission requires: cell mass to increase, chromosome to replicate, cell wall to be synthesized and cell to divide into two cells.
Microbial growth is usually studied as a population not an individual. Growth also results when cells simply become longer or larger. If the microorganism is **coenocytic**—that is, a multinucleate organism in which nuclear divisions are not accompanied by cell divisions— growth results in an increase in cell size but not cell number. It is usually not convenient to investigate the growth and reproduction of individual microorganisms because of their small size. Therefore, when studying growth, microbiologists normally follow changes in the total population number.

Growth Curve

Population growth is studied by analyzing the growth curve of a microbial culture. When microorganisms are cultivated in liquid medium, they usually are grown in a **batch culture** or closed system— that is, they are incubated in a closed culture vessel with a single batch of medium. Because no fresh medium is provided during incubation, nutrient concentrations decline and concentrations of wastes increase. The growth of microorganisms reproducing by binary fission can be plotted as the logarithm of the number of viable cells versus the incubation time. The resulting curve has four distinct phases.

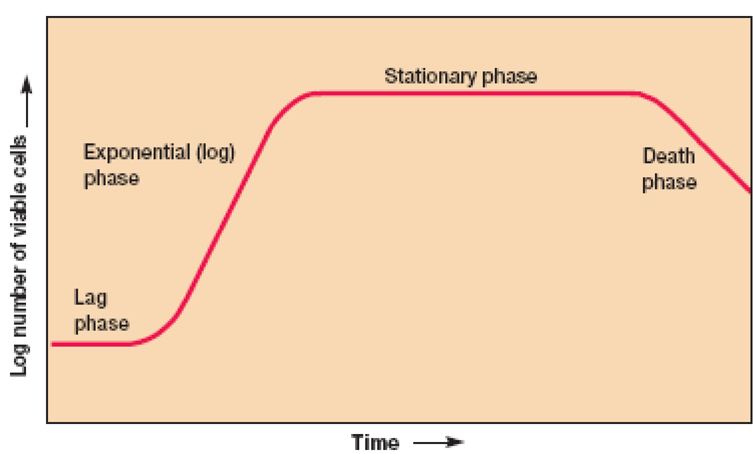

Figure: Microbial Growth Curve in a Closed System.

Buffers often are included in media to prevent growth inhibition by large pH changes. Phosphate is a commonly used buffer and a good example of buffering effect. Peptides and amino acids in complex media also have a strong buffering effect.

Temperature

Environmental temperature profoundly affects microorganisms, like all other organisms. Indeed, microorganisms are particularly susceptible because they are usually unicellular and their temperature varies with that of the external environment. For these reasons, microbial cell temperature directly reflects that of the cell's surroundings. A most important factor influencing the effect of temperature on growth is the temperature sensitivity of enzyme catalyzed reactions.

- At low temperatures a temperature rise increases the growth rate because the velocity of an enzyme-catalyzed reaction, like that of any chemical reaction, will roughly double for every 10°C rise in temperature. Because the rate of each reaction increases, metabolism as a whole is more active at higher temperatures and the microorganism grows faster. Beyond a certain point further increases actually slow growth, and sufficiently high temperatures are lethal.
- High temperatures damage microorganisms by denaturing enzymes, transport carriers, and other proteins. Microbial membranes are also disrupted by temperature extremes; the lipid bilayer simply melts and disintegrates.

Thus, although functional enzymes operate more rapidly at higher temperatures, the microorganism may be damaged to such an extent that growth is inhibited because the damage cannot be repaired. At very low temperatures, membranes solidify and enzymes don't work rapidly. Because of these opposing temperature influences, microbial growth has fairly characteristic temperature dependence with distinct **cardinal temperatures**—minimum, optimum, and maximum growth temperatures.

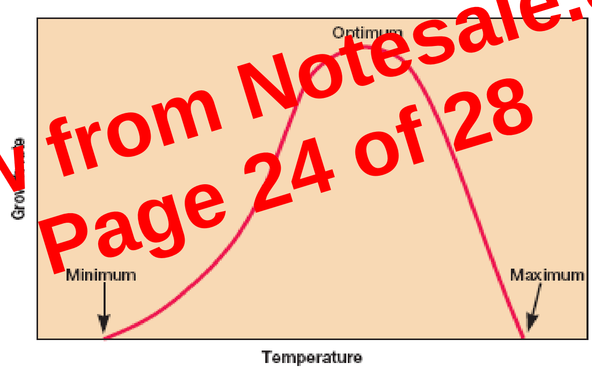

Figure: The effect of temperature on growth rate.

Although the shape of the temperature dependence curve can vary, the temperature optimum is always closer to the maximum than to the minimum. The cardinal temperatures for a particular species are not rigidly fixed but often depend to some extent on other environmental factors such as pH and the available nutrients.
Microorganisms can be placed in one of five classes based on their temperature ranges for growth.

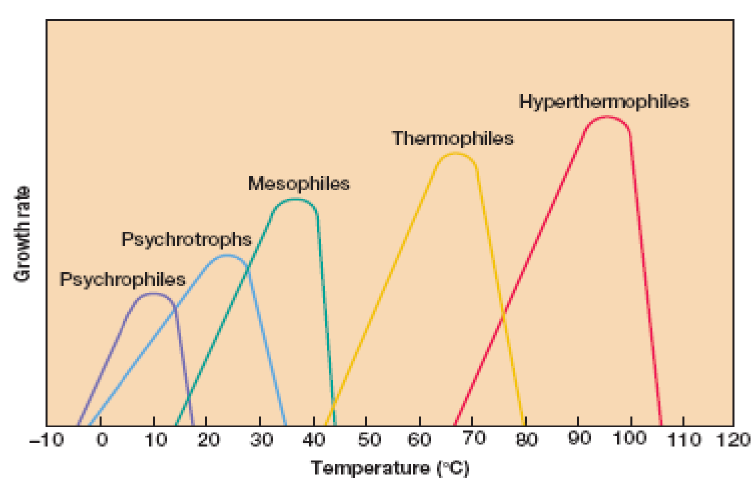

Figure: Temperature Ranges for Microbial Growth.