CHAPTER-5

MICROBIAL METABOLISM

Microbial growth is essential for soil ecosystem function. Microbial growth in soil is transitory and depends on the availability of nutrients and adequate physiochemical conditions. Different conditions of nutrient availability occur in soil and, as a result, different growth strategies are required. For example, under dry surface soil conditions, biodegradable nutrients (fecal material, animal and microbial corpses, plant residues, seeds and fruits) might accumulate because there is insufficient water to support microbial activity. Upon the return of water, a flush of rapid microbial growth occurs. This scenario of transient, luxuriant nutrient availability can be contrasted with the rhizosphere where a more consistent supply of nutrients is available or with the large pool of less labile soil organic matter, or humus, which can be digested only by microorganisms equipped with the enzymes necessary to attack these complex substrates.

Mathematical Concepts of Microbial Growth

Under favourable environmental conditions of initially nonlimiting nutrients, microbial growth proceeds through various phases shown in figure below. Briefly, after an adaptive or lag phase (a), the organism enters a period of unlimited growth (b), eventually, nutrients become limiting, toxic metabolites accumulate, and growth slows down (c). Finally, growth ceases (d) and is followed by cell death (e) (Figure 5-1).

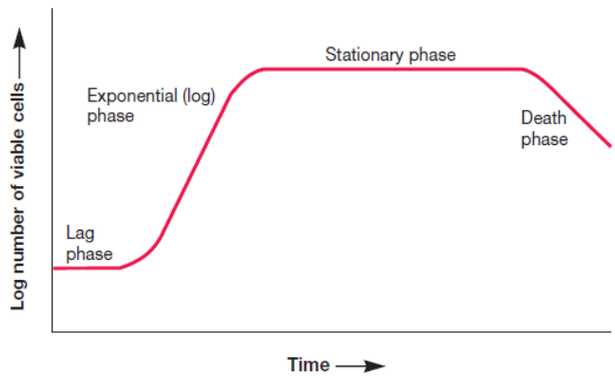

Figure 5-1: Generalized microbial growth curve:(a) adaptive or lag phase, (b) logarithmic or exponential growth, (c) limited growth, (d) stationary phase, (e) death phase.

During the unlimited growth phase, cell numbers increase exponentially or double per constant interval of time. This time interval is referred to as *doubling time*, or *generation time* (often abbreviated as t_d). The number of cells per unit volume (N_t) after a period of growth (t) depends upon the number of cells per unit volume in the initial population of cells (N_o) and the number of doublings (n) that have occurred during the time interval (t). Mathematically this can be expressed as:

$$N_t = (N_o) \text{ X } 2^n \qquad (1)$$

Because n = t/td, equation (1) can be rearranged as:

$$N_t = (N_o) \text{ X } 2^{t/td} \qquad (2)$$

It is more convenient to consider the linear form of this equation (2):

$$\ln N_t = \ln N_o + \frac{\ln 2 \text{ X } t}{t_d} \qquad (3)$$

Because it is more convenient to work with logarithms of base 10 than with natural logarithms, the latter are divided throughout by 2.303 to give:

$$\text{Log}_{10} N_t = \log_{10} N_o + \frac{\log_{10} 2 \text{ X } t}{t_d} \qquad (4)$$

During the unlimited growth phase, a plot of Log_{10} N_t against t will produce a straight line with an intercept on the y-axis at log_{10} N_o and a slope of $log_{10}2$

This type of microbial cell growth is referred to as logarithmic or *exponential growth*.

The term $\frac{log_{10}2}{}$ is a constant, which is called the growth rate consent (often t_d abbreviated to "μ"). In some textbooks μ is expressed in the natural logarithm form and associated with the denominator 2.303 as follows:

$$\mu = \frac{\ln 2}{2.303 \text{ X } t_d} \qquad (5)$$

The magnitude of the growth rate constant is inversely proportional to the doubling time. Some bacteria can achieve amazingly short generation times (< 1 hour) when nutrients are non-limiting. As a result, they can generate populations of astronomical proportions in short periods of time. Soil conditions are rarely conducive for unlimited growth. Most microbial growth in soil probably occurs under substrate-limited conditions, and for long periods of time microorganisms exist in a state of starvation-induced dormancy.

The term metabolism refers to the sum total of the biochemical reactions that occur within living cells. It is simply impossible to appreciate fully the discipline of soil microbiology without a fundamental understanding of the underlying biochemical principles. In discussing metabolic diversity, a central concept is that the over all goal of all forms of metabolism is essentially the same; to obtain energy or carbon in the production of cellular constituents necessary for growth, survival and reproduction.

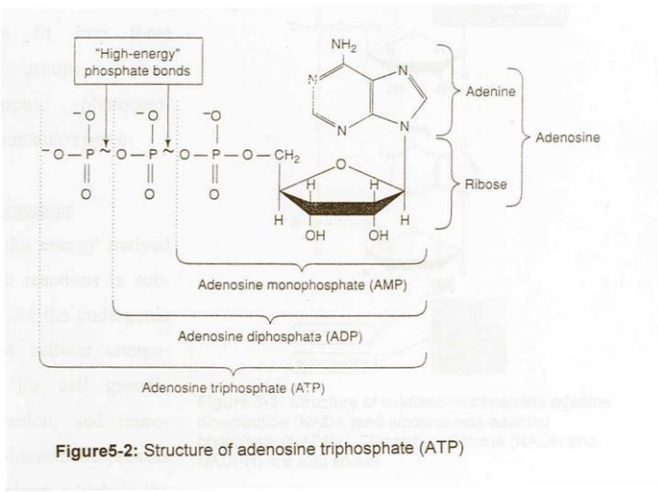

Figure5-2: Structure of adenosine triphosphate (ATP)

An Overview of Microbial Metabolism

Biochemical reactions in metabolism either yield energy (*exergonic*) or consume energy (*endergonic*). Exergonic reactions result in the production of energy needed to support cellular processes. They are often referred to as "spontaneous" because, given the proper conditions; they can be sustained without the addition of external energy. Enderognic activities result in the biosynthesis of cellular components and microbial biomass. These reactions are not spontanous and require a supply of energy from exergonic reactions to proceed. Although such a separation of exergonic and endergonic reactions is helpful for purposes of discussion, metabolism involves both types of reactions and is a highly integrated and interdependent system.

Exergonic Reactions

Microbes obtain energy from a large number of sources, including organic compounds, inorganic substances, and light. Furthermore, microorganisms as a group, and some times as individuals as well, can use these sources under both aerobic and anaerobic conditions. Thus, to a large extent, the presence of microorganisms every where reflects their diverse strategies for obtaining energy.

Two general types of energy-rich compounds are derived from the exergonic reactions of metabolism:

- High-energy phosphate compounds and
- Stored electrons associated with specialized carrier molecules.

Adenosine triphosphate (ATP) is the dominant high-energy phosphate compound in cells (Figure 5-2). The potential energy stored in the "high-energy phosphate bonds of ATP is useful in wide variety of biosynthetic reactions. Electrons released during exergonic metabolism are generally captured by oxidized electron carriers

such as nicotinamide adenine dinucleotide (NAD+), NAD phosphate (NADP+) or related compounds (Figure 5-3). The reduced forms of these carriers will be referred to as NADH and NADPH, respectively.

An additional but less energy-rich electron carrier is flavin adenine dinucleotide (FAD/FADH2). The electrons stored in these compounds are often called *reducing equivalents* or *reducing power*.

Most organisms obtain cellular energy from the biodegradation of energy-rich organic compounds, such as carbohydrates, proteins, and lipids; this "dismantling" of organic substances is *catabolism*. Alternatively, some organisms obtain energy from transformations of various inorganic compounds, whereas others use light as an energy source. Organisms that derive energy from organic or inorganic substances are called *chemotrophs*, while *photorophs* convert light energy into chemical energy to support metabolic processes. Organisms that obtain reducing equivalents from organic compounds are referred to as *organotrophs*, and those that use inorganic substan-ces are called *lithotrophs*. The vast majority of microorganisms encountered by soil microbiologists fit into three metabolic groups i.e., chemoheterotrophs, photoauto-trophs, and chemoautotrophs.

Enderogonic Reactions

A portion of the energy derived from exergonic reactions is sub-sequently used for the endergonic biosynthesis of cellular compo-nents needed for cell growth, biomass production, and repro-duction. This biosynthetic process is called *anabolism*, which is the functional opposite of catabolism.

One fundamental source of diversity among microorganisms relates to carbon nutrition. Most microorganisms obtain the carbon intermediates, or "building blocks," needed for biosynthesis from the degradation of organic compounds; these organisms are *heterotrophs*. The remaining organisms (algae and certain bacteria) can convert inorganic carbon from carbon dioxide (or carbonates) or other one-carbon substances (e.g., methane) into organic compounds by the endergonic process known as carbon fixation; organisms are called autotrophs.

Microorganism can be categorized with respect to three important metabolic requirements (Figure 5-4)

- Source of energy (chemotrophs or phototrophs),
- Source of reducing equivalents (organotrophs or lithotrophs), and
- Source of carbon for anabolism (heterotrophs or autotrophs)

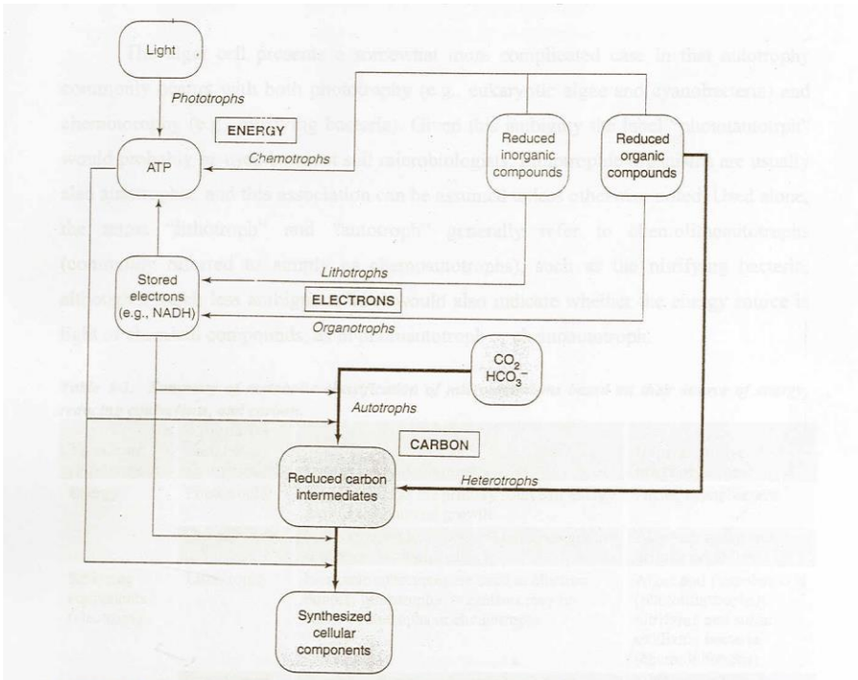

Figure 5-4: Overview of different metabolic pathways used by soil microorganisms for acquiring cellular energy (primarily ATP) for anabolic processes, storing electrons (reducing equivalents) such as NADH, and reducing carbon intermediates for the synthesis of other cellular compounds and polymers. Bolded arrows and shaded boxes show flow of carbon; other arrows and boxes represent energy and electron flow.

According to this system (Table 5-1) a filamentous fungus might be called a "chemoorganoheterotroph" or perhaps a "heterotrophic chemooganotrophs," whereas as photosynthetically active alga could be referred to as an "photolithoautototroph." In practice however many soil microbiologists have informally adopted an abbreviated system for referring to these metabolic categories that recognizes that certain combinations of these

traits nearly always occur together. For example, the description of the fungus given above is typically reduced to simple “heterotroph” because the vast majority of heterotrophic organisms are also chemoorganotrophs.

The algal cell presents a somewhat more complicated case in that autotrophy commonly occurs with both phototrophy (e.g., eukaryotic algae and cyanobacteria) and chemotorophy (e.g., nitrifying bacteria). Given this ambiguty the label “phototautotrph” would probably be used by most soil microbiologists. Lithotrophic organisms are usually also autotrophic, and this association can be assumed unless otherwise noted. Used alone, the terms “lithotroph” and “autotroph” generally refer to chemolithoautotrophs (commonly referred to simply as chemoautotrophs), such as the nitrifying bacteria, although a much less ambiguous label would also indicate whether the energy source is light or chemical compounds, as in photoautotroph or chemoautotroph.

Table 5-1. Summary of metabolic classification of microorganisms based on their source of energy, reducing equivalents, and carbon.

Metabolic requirement	Alternative metabolic strategies	Definition and comments	Representative microorganisms
Energy	Phototrophy	Light is used as the primary source of energy for metabolism and growth	Algae, cyanobacteria
	Chemotrophy	Light-independent chemical reactions are the source of metabolic energy	All nonphotosynthetic cellular organisms
Reducing equivalents (electrons)	Lithotrophy	Inorganic substances are used as electron donors; lithrotrophic organisms may be either phototrophs or chemotrophs	Algae and cyanobacteria (photolithotrophs); nitrifying and sulfur oxidizing bacteria (chemolithotrohs)
	Organotrophy	Organic substances are used as electron donors; normally associated with chemotrophy, although also exhibited by certain photosynthetic bacteria (phototrophs).	Fungi, most bacteria, and protozoa
Carbon	Autotrophy	Cellular carbon is derived mostly or entirely from CO2 associated primarily with lithotrophy.	Algae, cyanobacteria, nitrifying and sulfur-oxidizing bacteria
	Heterotrophy	Cellular carbon is derived from preformed organic compounds; generally associated with chemoorganotrophy	Fungi, most bacteria and protozoa

The Role of Enzymes in Metabolism

Although redox chemistry and free energy principles are useful in predicting energy relation-ships in metabolic processes, they provide no information concerning the rate at which reactions proceed. Certain reactions may release energy too rapidly for cellular processes and are incompatible with living cells, generally because of heat production. Conversely, many chemical reactions do not occur at appreciable rates even though the corresponding free energy change is highly favorable. This results from the need to satisfy the *activation energy* for the reaction- the energy input necessary to break the existing chemical bonds within each reactant. For a reaction to proceed, its activation energy must either by met by an external energy source or reduced in magnitude by the presence of a catalyst (Figure 5-5).

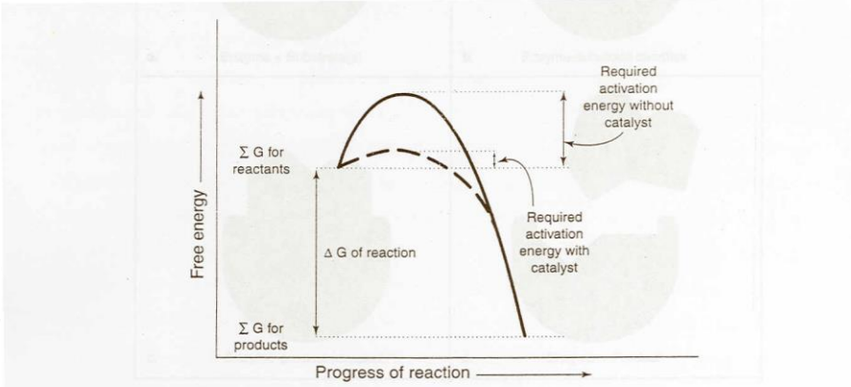

Figure 5-5: Effect of a catalyst on the activation energy required for initiating an exergonic chemical reaction.

A *catalyst* is a substance that promotes a reaction by reducing the required energy of activation without itself being altered by the ensuring reaction. This catalytic role in living cells is provided by specialized proteins called *enzymes*. Unlike most nonbiological catalysts, enzymes are highly specific in that they generally promote only one particular reaction or class of reaction. Enzymes promote efficient metabolism not only by facilitating the initiation of biochemical reactions but also by controlling the rate and extent of these reactions. Enzymes do this by temporarily binding the reactants at the enzyme's active site and thereby physically orienting them so as to both promote and control their interaction. In this way, enzymes allow reaction to proceed in a step-by-step fashion with minimum energy input.

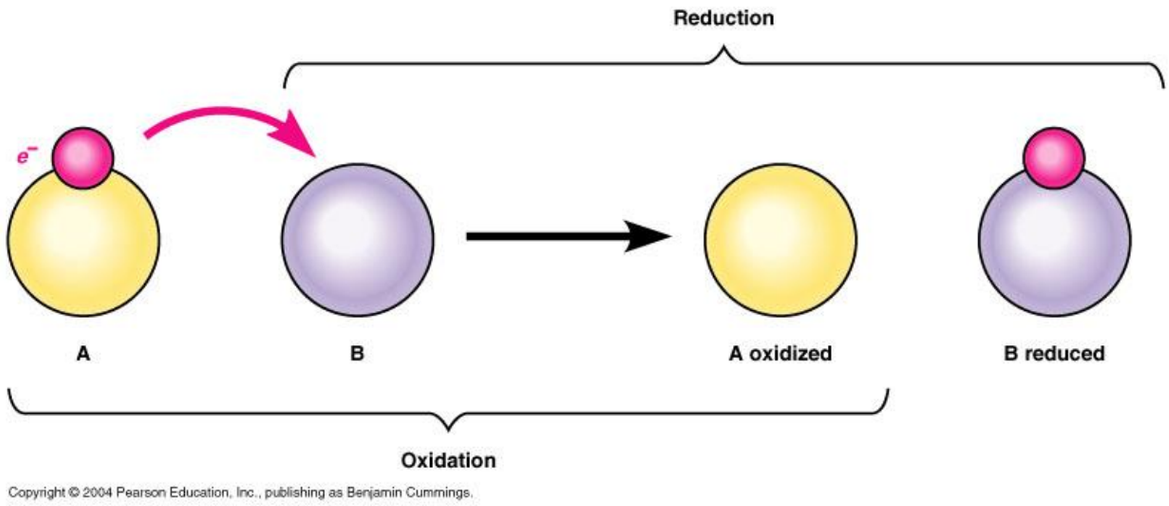

Figure 5-6: Major steps in an enzymatic reaction.

Enzymes often require the presence of various nonprotein substances called coenzymes to carry out their catalytic functions. The noncomplexed protein component is referred to as the *apoenzyme*, whereas the apoenzyme-coenzyme complex is called the *holoenzyme*. Coenzymes may bind either covalently or noncovalently to the apoenzyme. Covalently bound coenzymes are called *prosthetic groups*. For example, the heme group in many enzymes is usually present as a prosthetic group. Noncovalently bound coenzymes (e.g., NADH) are only transiently associated with the enzyme and may be thought of as cosubstrates. Many substances serve as coenzymes, including metal ions (e.g., iron, molybdenum, and magnesium) and carrier molecules (e.g., NADH, $FADH_2$, and various vitamins). The carrier molecules function to supply substances required by the reaction, such as electrons and hydrogens, or to aid in the transfer of chemical groups, such as phosphate, between molecules.

Microorganims need control of enzyme production both to promote balanced cell growth and to minimize unnecessary energy expenditure for protein production. Therefore, various strategies have evolved to regulate the production and activity of cellular enzymes. Enzymes needed for fundamental cellular processes are generally maintained at constant levels in the cell, and their production is unaffected by concentrations of substrate or products; such enzymes are called *constitutive enzymes*. Alternatively, enzyme production may be *inducible*, when production occurs in the presence of an inducer molecule, or derepressible, when production occurs in the absence of a repressor molecule. Many cataboloic enzymes are inducible in that the proper degradative enzymes are only produced in response to the presence of the corresponding substrate, which functions as an inducer; for example, lactose (milk sugar) acts as an inducer for the production of its degradative enzymes. Conversely, some anabolic enzymes are derepressible so as to limit enzyme production to periods when a specific enzymatic product has dropped below some critical concentration in the cell; that

product functions as a repressor. The enzymes responsible for the conversion of dinitrogen gas to ammonia are example of derepressible enzymes in that their production is inhibited by the presence of the product.

Enzyme activity can be controlled not only at the level of enzyme production, but also by modifying the activity of existing enzymes in the cell. This general type of altered activity is known as alosteric control, meaning that molecule affects a metabolic reaction by binding to the corresponding enzyme at an *allosteric site* distinct from the active site (Fig. 5-7). The most common form of allosteric control is allosteric inhibition, also referred to as *feedback inhibition* or end *product inhibition*. Alloesteric inhibitors are

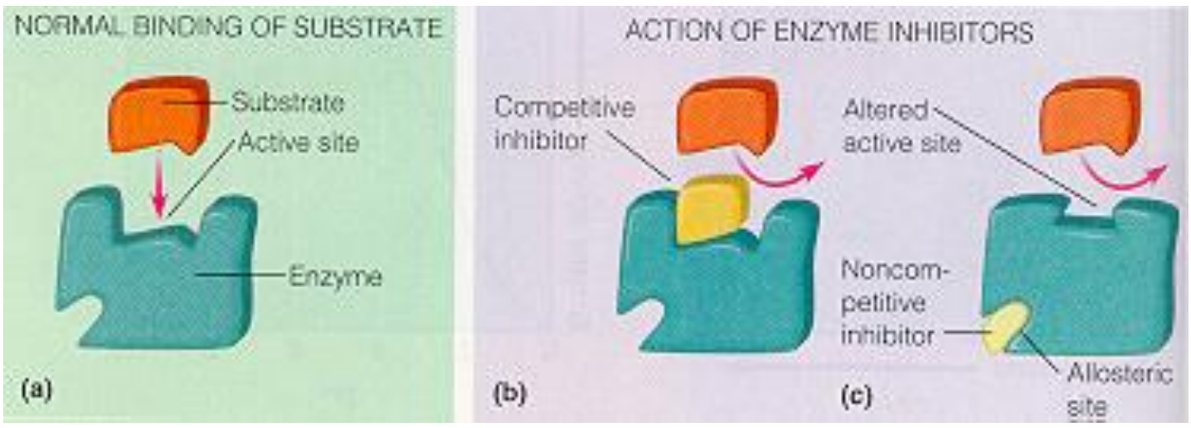

Figure 5-7: Allosteric inhibitors decrease the rate of enzymatic reactions by binding to a separate allosteric site on the enzyme and thereby indirectly altering the configuration of the active site to prevent substrate binding.

generally metabolic end products which weaken the metabolic pathway that produced them by inhibiting an early step in the pathway. Attachement of the inhibitor at the allosteric site reduces enzymatic activity by altering the conformation of the active site, thereby preventing attachment of the substrate (s). In other cases, the attachment of a substance at the allosteric site may result in increased enzyme activity.

Enzymes promote many types of reactions within living cells and generally named for the particular reaction catalyzed. Enzyme names are further designated by the suffix "-ase." Table 5-2 summarizes the kinds of reactions carried out by different classes of enzymes.

Specific examples of enzymes important in soil microbiology include:

- Cellulases, which degrade the polymer cellulose into smaller constituents,
- Nitrogenase, which converts dinitrogen gas into biologically available ammonia,
- Sulfatase, which release sulfate from protein and certain other organic compounds, and
- Phosphatases, which remove phosphate groups from organic compounds such as nucleic acids.

Table 5-2. Major classes and subclasses of enzymes and the corresponding types of reactions catalysed.

Class	Representative subclass	Types of reactions catalysed
Oxidoreductases	Dehydrogenases Oxidases Reductases Peroxidases Catalases	Catalyse oxidation reduction reactions. Important in fermentation and respiration pathway.
Transferases	Aminotransferases Kinases	Catalyse the transfer of molecular substituents among molecules.
Hydrolases	Glycosidases Peptidases Phosphatases Ribonucleases	Catalyse the hydrolytic cleavage of chemical bonds.
Lyases	Decarboxylases Synthases Lyases	Catalyse the addition or removal of chemical groups such as carbon dioxide, ammonia and water
Isomerases	Racemases Isomerases	Catalyse inversions at asymmetric carbon atoms and the intramolecular transfer of molecular substituents.
Ligases	Synthetases Carboxylases	Catalyse the binding of two molecules with the expenditure of ATP. Important in anabolic pathways.

All enzymes are not intracellular, meaning that they are produced and are active within the cell. Enzymes that catalyze the degradation of polymeric substances added to soil, such as cellulose from crop residues, are necessarily extracellular because the polymer is too large to be transported across the cellular membrane. However, once the polymer has been reduced to its smaller subunits, subsequent catabolism may proceed intracellularly. Because many enzymes are important in nutrient cycling and other soil-related processes, standardized assays have been developed for measuring their activities.