

# BIOCHEMISTRY AND WASTE TREATMENT

*by E. D. Schroeder*

The purpose of this paper is to indicate the manner in which the familiar aspects of process engineering are related to the biochemical aspects of biological waste treatment processes. A detailed description of the biochemical reactions and mechanisms involved in metabolism will not be given. Instead an effort will be made to describe different aspects of biological systems in terms familiar to engineers and to discuss the advantages and limitations of biological systems which are related to biochemistry.

Biological processes are much more complicated than ordinary chemical reaction processes. Although the same fundamental laws apply to both biological and nonbiological systems, the complexity of biological systems often prevents the direct application of normal engineering procedures and methods of design and control. An example of this type of problem is the growth of cells in biological systems. The metabolism of an actively growing bacterial culture is different from that of a culture in endogenous respiration; that is, a population of cells which is utilizing stored materials for food in a manner analogous to a man on a diet. A change in metabolism has an effect upon the characteristics of the culture and thus when a change in the environment occurs both the physical and chemical systems can be upset. Often a biological system is greatly affected by small changes in the environment, and in addition the range of environmental conditions such as pH and temperature in which biological systems can operate is very small. An understanding of this involves the biochemistry of bacterial systems.

All biological processes have more similarities than differences in their reaction systems and environmental requirements. Waste treatment systems are more general in nature than biological processes for the production of particular products such as alcohol or antibiotics. Waste treatment is usually accomplished through the use of heterogeneous bacterial populations. Heterogeneous means that many bacterial species are present within the culture. The heterogeneous population gives the

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treatment system a great deal of flexibility, since essentially all organic compounds are attacked by one or more groups of bacteria. The fact that a heterogeneous population is desirable in biological waste treatment systems is fortunate because maintenance of pure cultures would be difficult and costly due to the sources of organic wastes and the quantities that must be treated.

Most bacterial species utilize many different organic materials (substrates) as energy and carbon sources. An example is glucose which can be used as the sole carbon source by a wide variety of bacteria. However, the oxidation of glucose will follow slightly different "reaction pathways" in different species of bacteria, and the "reaction pathways" will also vary with environmental conditions in a given species.

The term "reaction pathway" indicates that more than one reaction is taking place in the course of oxidation of a compound. In fact, nearly every detectable change in a biological system is due to a large number of interrelated reactions. Many of these are coupled in series and are then grouped and spoken of as "reaction pathways."

### *Biochemical Reactions*

Individual biochemical reactions almost always involve enzyme catalysts.<sup>1</sup> Enzymes are always proteins although they may have non-protein prosthetic or coenzyme associated groups. The structure of an enzyme is essential to its activity, and the fragility of enzyme structure explains many characteristics of biological systems. All proteins, and therefore all enzymes, have a basic or primary structure consisting of amino acids linked by peptide bonds (Fig. 1).<sup>2</sup> Enzymes are formed of long chains with molecular weights from 10,000 to over 1,000,000. Hydrogen bonding between the amino group and carboxyl group of adjacent amino acid residues causes a helical "secondary" structure (Fig. 2). The side chains of the amino acids interact to bring the chain,

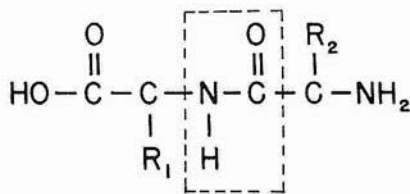


FIG. 1 - THE PEPTIDE BOND

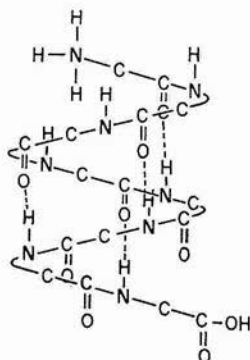


FIG. 2 - HELICAL PROTEIN STRUCTURE

or sometimes several chains, into a "globular" structure. The bonding of this globular "tertiary" structure consists of disulfide bridges and sulfhydryl bonds, among others.

The tertiary structure is the most important factor in the maintenance of enzyme activity.<sup>3</sup> A substrate molecule attaches to a specific site on an enzyme. The three-dimensional structure of this active site is determined by the tertiary structure of the enzyme. When the tertiary structure is disturbed, even minutely, the activity of the enzyme is changed or is lost.<sup>4</sup> Usually there is only one active site per molecule. Thus enzymes are a particular type of catalyst, and their characteristics are different from those catalysts used in nonbiological systems.

The disturbance of enzyme structure, or denaturation of the enzyme, may be accomplished by chemical or physical means.<sup>5</sup> Chemically the most common method of denaturation is by forcing the environmental pH below five or above nine. There are enzymes which operate outside of this range, but most do not. Because enzyme activity is dependent upon pH, bacterial activity is also dependent upon pH. While extreme pH values denature enzymes in general, certain chemicals are more specific. Many compounds inhibit the action of a specific enzyme, or a specific group of enzymes. These compounds may act as substrate analogues, may react with the groups forming the active site, or may affect the enzyme molecular structure by attaching to the enzyme at another point and altering the bonding.<sup>6</sup> Another possibility is the blocking of the active site by an inhibitor which attaches to the enzyme at a point near the active site.<sup>7</sup>

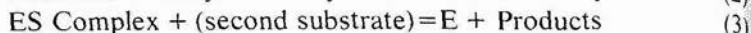
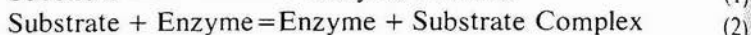
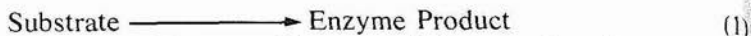
Physical changes in the environment also cause enzyme denaturation. A primary example is temperature. Most enzymes are heat denatured at temperatures approaching 50°C. As with pH, exceptions are known.

Other physical effects such as high or low osmotic pressure may cause enzyme denaturation. However, these effects often destroy other parts of cell structure such as the membrane and cell wall, making their effect on enzyme structure less important.

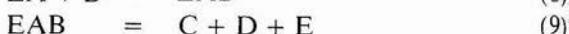
Denaturation may be irreversible or reversible. When denaturation is caused by high temperature or by extremes in environmental pH, the tertiary structure is usually irreversibly destroyed. Specific chemical inhibitors may or may not have an irreversible effect. The inhibition is usually a function of the affinity of the inhibitor and substrate molecules for the enzymes.

In recent years several models of biological systems have been proposed which assume that the kinetic properties of enzymatic reactions should also govern the kinetic characteristics of the overall biological system. The assumption is based upon the theory that the rate of metabolism, or synthesis, is controlled by a rate-limiting biochemical reaction,

and that this reaction involves an enzyme. Usually a model of this type makes use of the Michaelis-Menten<sup>8</sup> model for enzymatic reactions (equations 1, 2, and 3).



The use of the Michaelis-Menten model also includes the assumptions that reaction (3) is much slower than reaction (2) and that (2) is reversible, while (3) is essentially irreversible. Thus the overall production of product, or use of the first substrate, is rate controlled by reaction (3). Reaction (3) is assumed to be first order with respect to the ES Complex concentration. This model is one of the least complex that could be reasonably proposed. A slightly more complex model, proposed by Briggs and Haldane,<sup>9</sup> postulates the formation of an enzyme complex with two substrates. This model seems to be generally more correct, although rarely used in practice, and is described by the following equations:



The assumptions made in using the Briggs-Haldane model are that the rate is proportional to the complex (EAB) concentration and that reaction (9) is slow with respect to reactions (5), (6), (7) and (8).

One of the most important considerations in biochemistry is the role of inhibitors as agents of intracellular control. The effects of inhibitors on rate processes within a bacterial cell have also been considered in describing the kinetics of enzymatic reactions<sup>10</sup>. Of particular interest is the fact that reaction products are utilized by the cell to control the rate of certain processes within the cell. This is due to inhibition of some reactions by their reaction products.<sup>11</sup>

The validity of the assumption that a single rate-limiting step controls the overall kinetic properties of a biological system may be questioned. Certainly other processes must be taken into consideration before making such an assumption. This analysis is discussed by a subsequent paper at this conference.

### *Metabolism*

Reproduction is the primary "objective" of bacteria in any biological

process. Thus the trend in any biological system is toward a maximum energy for synthesis. As was stated earlier there are many similarities between the numerous species of microorganisms. Because of these similarities and the fact that the heterogeneous population present in a waste treatment process can be expected to obtain the maximum energy for synthesis available under the particular environmental conditions, the energy metabolism of bacteria can be discussed in a general manner, rather than by giving a discussion of specific species.

Usually bacterial processes are divided by engineers into two groups, aerobic and anaerobic. The line between the two groups is often pictured as being a sharp division between types of metabolism and species of bacteria present. An introductory knowledge of biochemistry shows that there should be little fundamental differences between anaerobic and aerobic systems and that in some cases the two types of environments should and do achieve the same or quite similar results. Understanding the biochemistry allows the choice of an anaerobic system or an aerobic system to be made on the basis of reasonable parameters rather than on traditional concepts.

The relationship between aerobic and anaerobic metabolism may best be illustrated through the use of carbohydrate metabolism as an example. Figure 3 gives an overall view of carbohydrate breakdown and the process is explained in the following paragraphs.

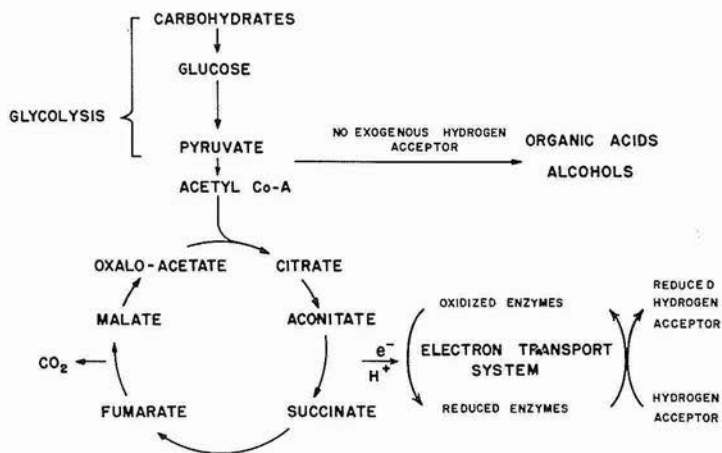


FIG. 3 - CARBOHYDRATE METABOLISM

Glucose, a six carbon monosaccharide sugar, is the central compound in carbohydrate metabolism. Glucose makes up the repeating units of many polysaccharides including glycogen, starch, and cellulose. The sugar is easily converted by microorganisms into many related com-

pounds, and a great deal of work has been expended in the study of its role in metabolism. Indicative of the importance of glucose and its position in metabolism is that large numbers of bacterial species are able to utilize glucose as their sole source of carbon and energy.

The initial series of reactions in glucose metabolism is generally grouped under the term glycolysis. Although all species of bacteria do not follow this reaction sequence, it is a very common one. The terminal point in glycolysis involves a branch point. The further metabolism of glucose, which at this point has been converted to two moles of pyruvic acid for each mole of glucose entering the pathway, depends upon the environment. If oxygen is present terminal oxidation of the sugar will proceed with the production of carbon dioxide.

Terminal oxidation takes place through a cyclic reaction system known as the Krebs Cycle, or the Citric Acid Cycle. The pyruvic acid resulting from glycolysis is decarboxylated, yielding  $\text{CO}_2$ , two hydrogen ions, two electrons, and an acetyl-coenzyme complex. The acetyl group is then added onto oxaloacetic acid furnished by the Krebs Cycle to form citric acid. Subsequent reactions decarboxylate the citric acid twice, producing two moles of  $\text{CO}_2$ , oxaloacetic acid and eight hydrogen ions and eight electrons. The oxaloacetic acid is able to accept additional acetyl groups because it has been "regenerated" by the cycle. Carbon dioxide is a waste product. The hydrogen ions and electrons are involved with the primary energy-producing system of the cell, the electron transport system, sometimes called the cytochrome system.

The cytochrome system involves a series of enzymatic reactions in which a compound is alternately reduced and oxidized. Some of the oxidation reactions are highly exothermic and release several thousand calories per mole. This energy is used in a coupled reaction system which produces adenosine triphosphate (ATP) from adenosine diphosphate and inorganic phosphate. The phosphate ester bond formed is a very high energy bond. The terminal step in electron transport involves a hydrogen acceptor from outside the cell, that is, exogenous to the cell. In the case of aerobic systems the hydrogen acceptor is molecular oxygen and its reduced form is water.<sup>12</sup>

Adenosine triphosphate is formed during glycolysis and as the result of other processes. However, the amount produced as the result of electron transport is much greater than that resulting from other methods of production; about ten times as much as glycolysis, for example.

When molecular oxygen is not present the electron transport system is blocked because there is no way to reoxidize the reduced coenzymes. As a result the Krebs Cycle is also inoperative and pyruvic acid must be oxidized in some other manner, or released as a waste product. Some organisms, and these would include most of the common bacteria found



in waste treatment, utilize the pyruvic acid or subsequent reaction products to oxidize reduced coenzymes similar to those in the electron transport system. The result of this process is that reduced waste products such as acids, alcohols, and ketones are produced with the oxidation of enzymes necessary for cell functioning. This is done without production of ATP and the process is much less efficient than the oxygen reaction.

Synthesis is underway within the cell concurrent with substrate oxidation. Many of the products formed from the intermediate oxidation reactions are diverted into synthesis pathways and only a fraction of the initial substrate actually goes through the complete oxidation process in either aerobic or anaerobic metabolism.

Fat and protein metabolism is tied to carbohydrate metabolism primarily through Krebs Cycle. In the breakdown of fats, successive two-carbon groups are removed from the carbon chain by a process known as Beta oxidation. The two-carbon pieces are used in the formation of acetyl coenzyme A, the compound intermediate between pyruvic acid and the Krebs Cycle.<sup>13</sup>

Protein metabolism involves two basic steps: peptide breakdown and transamination. Because proteins are made up of long chains of amino acids joined by peptide bonds, the breakdown of the chain into the amino acid subunits occurs first. The second step is removal of the alpha-amino group by a process known as transamination. The product of transamination is usually very similar to a glycolysis or Krebs Cycle intermediate.

Oxygen has been spoken of as an "exogenous hydrogen acceptor." This is because molecular oxygen is the most generally used of a group of compounds furnished from the environment which act as terminal oxidants of the cytochrome system. Other prominent members of the group are nitrogen and sulfur, in various valence states.<sup>14</sup> Nitrate ion is probably the most generally used exogenous hydrogen acceptor other than molecular oxygen. Here it is important to point out that the use of an exogenous hydrogen acceptor is a specific reaction, and that there must be present, as part of the electron transport system, an enzyme which specifically reacts with the acceptor in question. A wide number of organisms make use of molecular oxygen. A smaller group make use of both oxygen and nitrate ion.

Some of the most common bacteria in waste treatment can use both oxygen and nitrate ion as a hydrogen acceptor, and the relationship between these two acceptors can be used to develop the following ideas about the characteristics of biological systems.

Oxygen and nitrate ion have been shown to act in an analogous manner in several different bacterial species.<sup>15</sup> For both acceptors the electron transport system is involved and adenosine triphosphate is produced in

reactions coupled to the electron transport system. However, the reaction involving nitrate is essentially completely inhibited by the presence of molecular oxygen. The reverse case is not true. Although the reactions are analogous they are not the same, nor does the nitrate furnish oxygen for the aerobic reaction.

Inhibition of nitrate reduction by oxygen indicates that a biological system must be anaerobic if nitrate ion is to be used as a hydrogen acceptor. This subject has been discussed in the literature with suggestions that the addition of nitrate ion would keep the system from going "anaerobic." Knowledge of the role of hydrogen acceptors and the specificity of enzymes in general, coupled with specific information about the nitrate respiration system, leads one to an essentially opposite conclusion. As a practical matter, the end products are qualitatively the same and the nitrate system, even though anaerobic, is not objectionable.

#### *Effects of Biochemical Considerations*

The analogy between nitrate and oxygen respiration is useful in discussing energy relationships in anaerobic and aerobic systems. The nitrate respiration system is truly anaerobic in that molecular oxygen is absent from the system, yet the reactions involved are analogous to aerobic respiration and the fate of the substrate is the same. Since the electron transport systems of the two respiration processes are different in some reactions at least, there is a possibility that the number of ATP molecules generated per mole of substrate sent through the Krebs Cycle is different.

In the case where an exogenous hydrogen acceptor is totally lacking the end products will differ from either the aerobic or the nitrate respiration scheme. Also, the amount of ATP produced will be considerably less, and thus the amount of synthesis will be less also.

Note that another process or reaction sequence is normally considered in the case of anaerobic metabolism, i. e., methane fermentation. Bacteria have been found which utilize the organic acid end products of "normal anaerobic metabolism," such as that described previously, for the formation of additional bacteria and methane. The overall anaerobic reaction sequence is usually visualized as shown below.



The particular organisms producing methane have been very hard to isolate, which is peculiar since relatively large amounts of methane are produced in anaerobic waste treatment processes. There are several possible reasons for this. One is that the organisms have very strict



environmental and nutritional requirements. A second possibility is that most of the organisms belong to a more general class which utilizes other substrates and produces other end products as well. A symbiotic relationship between two or more species might also account for methane fermentation.

Obviously the presence of exogenous hydrogen acceptors in an anaerobic system would lead to oxidation of the substrate to carbon dioxide by the Krebs Cycle with the release of relatively large amounts of energy for cell synthesis. This means that the traditional goal of anaerobic treatment of wastes would not be met by systems with exogenous hydrogen acceptors. These goals are minimum cell production and methane fermentation. Because of the problems associated with the traditional anaerobic "digestion" process it would seem that consideration of the methods and goals is needed.

As one would suspect from equations (11) and (12), more energy per mole of substrate is available for cell synthesis during the first state of the process. This indicates that the rate of acid production may be greater than the rate of methane production and could lead to excess substrate for the second stage and a problem in maintaining a suitable environment for the organisms.

### *Physical and Chemical Control of Biological Systems*

Biological systems may be controlled by both physical and chemical means. Physical environment factors such as temperature, liquid flow rate, mixing and pH play an important role in determining the overall rate in a biological process. In addition the process can be controlled through chemical response by nutrient concentration, substrate concentration, and, as mentioned previously, the hydrogen acceptor.

Temperature directly affects the rate of biochemical reactions just as it does chemical reactions. Although the Arrhenius relationship for relating the reaction rate to temperature has been applied to biological systems in general, as well as to biochemical reactions, there is little reason to suppose that there is a sound basis for the application. Even the simplest enzymatic reaction has a large number of temperature dependent factors that affect the rate. Certainly the entire cell has a large number of factors, other than reaction, which may control the overall rate of synthesis. The most likely explanation for the acceptance of the Arrhenius relationship is that since it has an exponential term a wide range of data may be fitted to the equation.

The biochemical reaction rate does increase with temperature over a certain range. Biological reactions seldom proceed at a measurable rate below 0°C and there is an upper limit at which enzymes are denatured.

This limit is about 50°C but there are exceptions which go nearly to 100°C.

In continuous flow systems the washout rate must be kept below the growth rate. Thus, hydraulic design of the system is affected by metabolic characteristics of the bacteria involved. Mixing is not likely to cause physical damage to bacteria in biological processes. However, mixing can change the physical environment of the bacteria and thus change the metabolic characteristics of the system.

Fluidized biological treatment processes such as "activated sludge" make use of a flocculant suspension of bacteria. The floc particles are large, relative to the size of individual cells. Breaking up of the particles would set up a different type of environment for these bacteria originally within the floc particles. There is a good possibility that a change from anaerobic to aerobic metabolism might take place with those organisms originally inside of a particle, making the system more complicated than would appear at first glance.

The control of pH has been mentioned previously. In anaerobic metabolism utilizing "endogenous" hydrogen acceptors, organic acids are produced. These acids must be removed in some manner or the pH will drop to a point at which the biological system will not operate. Many industrial wastes are initially outside of the acceptable pH region. In some cases a specific bacterial culture may develop which will operate under the conditions imposed by the waste. In some instances the pH of the waste has to be changed before biological treatment can take place. However, CO<sub>2</sub> production as a consequence of organic destruction provides pH adjustment in a well-designed process.

Nutrients other than the substrate are important in any biological system. Although carbon is the most general of the elements making up the cell many other elements are needed. One of the most important is nitrogen. Nitrogen is necessary for the production of amino acids and proteins. Proteins are the largest single constituent of the cell by weight. Nitrogen is also necessary for the construction of nucleic acids and several polysaccharides synthesized by the cell.

Phosphorous is important in the synthesis of nucleic acids as well as in the energy transport system in the cell. Sulfur is a side chain component of three important amino acids. Magnesium, molybdenum, and aluminum act as chelating agents in several essential reactions. Iron is contained in the porphyrin prosthetic group of several proteins including the cytochromes. Several other nutrients are necessary in trace quantity also. Work is being done on the minimum concentrations necessary to maintain a system; however, there has been some difficulty in measuring the small concentrations involved. In some cases the presence of a nutrient in any measurable quantity is enough.

*Summary*

Biological systems are made up of complicated structures and reaction sequences. In order to make the best possible use of biological methods of waste treatment it is wise to study the way in which these structures and reactions fit together, the manner in which they may be controlled, and the effects of the environment upon them. This paper has described some of the more important features of metabolism and biological reactions. Hopefully this information will be useful in considering new approaches to studying biological waste treatment systems.

## NOTES

1. Abraham White et al., *Principles of Biochemistry* (2nd ed.; New York, 1959).
2. Malcolm Dixon and E. C. Webb, *Enzymes* (2nd ed.; New York, 1964).
3. See E. E. Kosland, Jr., *Science*, CXLII (1963), 1533, and W. C. Deal et al., *Biochemistry*, II (1963), 246.
4. J. Monod et al., *J. Mol. Biology*, VI (1963), 306.
5. Dixon and Webb, *op. cit.*
6. Monod et al., *op. cit.*
7. Dixon and Webb, *op. cit.*
8. *Ibid.*
9. *Ibid.*
10. *Ibid.*
11. Monod et al., *op. cit.*
12. White et al., *op. cit.*
13. Eric Conn and Paul Stumpf, *Outlines of Biochemistry* (New York, 1963).
14. I. C. Gunsalas and R. Y. Stanier, eds., *The Bacteria*, Vol. II (New York, 1961).
15. W. D. McElroy and B. Glass, eds., *Inorganic Nitrate Metabolism* (Baltimore, Md., 1956).