### Chapter II

# THEORETICAL CONSIDERATIONS

Oxidation ditches are shallow basins used for the purpose of purifying sewage or other liquid waste by storage under climatic conditions that favour the growth of algae. The conversion of organics to inorganics or stabilization in such ponds results from the combined metabolic activity of bacteria and algae.

The ponds are usually 2 to 4 ft. deep, although deeper ponds have been used. The minimum depth is controlled by weed growth. It has been found that depth is affected to light penetration, mixing and surface reaeration.

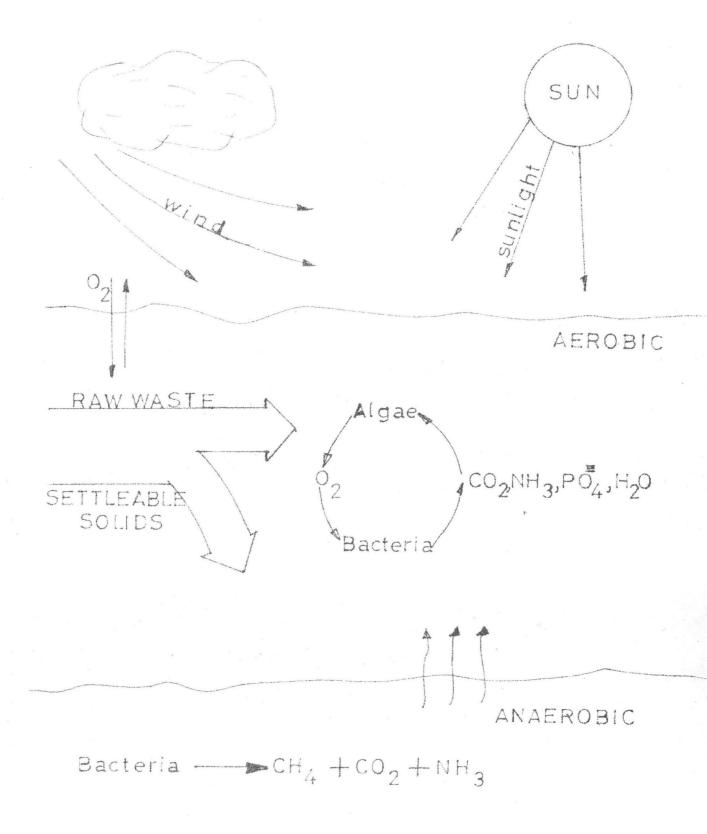
Oxidation ponds were originally used for secondary treatment but in the past few years a large number of ponds have been constructed to treat raw sewage. Most oxidation ponds to day are designed for complete treatment of raw sewage.

Oxidation ditch depends upon two primative forms of life, algae & bacteria. The primary source of energy, other than the waste being treated is the sun. Energy from the sun as converted by the photosynthetic properties of the algae makes it possible for the latter to use the metabolic end products of bacteria; primarily, carbon dioxide to produce more algae cells and free  $O_2$ . The oxygen is then available to aerobic bacteria as in figure 3.

The bacteria utillize sewage organics for growth and energy, the latter being provided through oxidation of the organic carbon to carbon dioxide along with other FIGURE 3

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Schematic diagram of oxidation pond symbiosis between bacteria and algae (After Mc.Kinney, 1962)



metabolic end products is utilized in algae growth through photosynthesis O<sub>2</sub> is produced in the process and is available for continuing bacterial oxidation. This oxygen, along with the oxygen dissolved through surface aeration, keeps the process aerobic under ideal conditions.

Decomposition of the organic material by the bacteria is carried on in three distinct zones :

a. Aerobic bacteria in the zone of DO

b. Facultative anaerobes operating near the bottom or in the upper portions of the bottom sediments

c. Anaerogic bacteria in the interior of the bottom sediments in the absence of  $0_2$ 

It is desirable to maintain aerobic conditions because aerobic microorganisms bring about a more complete reduction of organic material. The zone of facultative anaerobes will be aerobic or anaerobic, depending upon the presence or absence of molecular oxygen. Anaerobic biological activity will prevail under the surface of the bottom sediments. If the pond is not overloaded for the existing conditions the anaerobic metabolic end products will be utilized and further reduced in the aerobic zone and never reach the liquid surface. If the pond is overloaded for too long period of time, the odours and effects of anaerobic digestion will occur.

As stated above that aerobic conditions are desirable to be maintained so in this experiment, rotor was fixed to the laboratory model oxidation ditch as a source of oxygen supply.

## Biological Consideration

One of the fundamental objects attributes of living organisms is their ability to utilie and convert energy. One gram of E. coli bacteria develops energy at the rate of 0.6 watt and is a not unusual rate for bacteria, (Clark & Viessman, 1970) It is essential in water and waste treatment for the engineer to have some knowledge of the way in which energy is transfered from one form to another and from one place to another. The biochemical processes involved in these energy transfers are known collectively as metabolism. Those involved in the breakdown of complex matter to simpler forms are known as <u>katabolism</u>, and the reverse processes of synthesis of complex matter from simpler substances is called <u>anabolism</u>.

The decomposition of organic matter in polluted water is directly linked to the presence of bacteria and other organisms. Althrough some bacteria are responsible for causing disease, most are beneficial in the decomposition of organic matter :- for example, fertility of soil depends on this decomposition. The filamentous fungi also are important members of the population of organisms occurring in polluted waters. Like many bacteria, most fungi have simple cells without chlorophyll. Protozoa and other plankton are present in polluted waters, but their growth rates are much slower than those of bacteria. They are significant in the life **cycle** of polluted water because they use bacteria as food. A community of organisms having varying degrees of complexity extents through all forms of aquatic life. Bacteria are by far the most influential organisms in waste water treatment processes.

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Chemical reactions involved in metabolism

proceed at a much greater rate than that in a normal environment. These high reaction rates are made possible by the presence of numerous catalysts. Catalysts are substances that affect the rate of a chemical reaction but which themselves remain unchanged at the end of the reaction. Organic catalysts produced by bacteria are called enzymes. These enzymes are rather specific in the reactions they promote.

The growth and multiplication of bacteria in water is dependent upon the presence of compounds containing C & N<sub>2</sub> in a form capable of being synthesized in to new cells and use to furnish energy for the cells. Ingestion of food by bacteria is accomplished by the pas. sage of compounds through the cell walls. Small bacterial cells require food that is in true solution; some of larger bacteria are capable of ingesting more complex molecules. Organic wastes in true solution, or which are finely divided, are immediately available to the bacteria as food. Insoluble larger particles of organic matter must be chemically acted upon with the aid of enzymes to bring them in to solution prior to their being available as bacterial food. This reaction takes place outside the cell wall.

## Microorganisms

The microorganisms in oxidation ponds are the same as those existing in the other treatment processes with the bacteria and algae predominants and the protozoa

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and rotifers being present under certain loading condition. The predomination of various species of microorganisms will depend on the loading factors and the physical design of the pond. An oxidation pond at a low organic loading will have a different microbial population than one at high organic loading will have a different microbial population than one at high organic loading. (Mc. Kinney, 1962)

The predominate bacteria will be the Pseudomonas, Flavobacterium and Alcaligens, It has been said by some that the rapid die-off of coliform bacteria points to the production of antibiotics by the algae or other bacteria Actually, there has been no evidence of antibiotic production in oxidation ponds, but there is a rapid die-off of Coliforms. The season for this lies in the competition of the predominating microorganisms for food and the high population of predatory protozoa. Coliforms have been shown to lose out in the competition for food with the bacteria in the above three genera. (Mc. Kinney, 1962)

The aerobic system are the primary systems for waste treatment and this includes activated sludge, trickling filter, oxidation pond, and compositing. The basic equation is expressed directly in terms of dissolved oxygen uptake as long as system retain truly aerobic (Mc. Kinney, 1962)

The oxidation of inorganic or organic compounds to obtain energy for synthesis is called chemosynthesis and is the most common method of bacterial metabolism. The bacteria which oxidize inorganic compounds utilize carbon dioxide for their photoplasm and are called autotrophic bacteria.

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The bacteria which oxidize organic compounds for energy obtain the carbon for synthesis from the same organic compound used for energy. A portion of the organic compound is used for energy and a portion is used for synthesis. These bacteria are known as heterotrophic. Both the autotrophic and heterotrophic bacteria are important to sanitary microbiology.

The heterotrophic bacteria are the most important group of bacteria. They require organic compounds which supply their carbon and their energy. The heterotrophic bacteria can be broken in to three groups based on their relationships with oxygen in to the energy reaction. The heterotrophs which utilize free dissolved oxygen are known as aerobes.

The heterotrophs which oxidize organic matter in the complete absence of dissolved oxygen are known as anaerobes. There is a group of bacteria which utilize free oxygen when it is present but which can also carry on metabolism in the absence of free oxygen. This latter group of bacteria is .. known as facultative bacteria have somtimes been designated as facultative aerobes or facultative anaerobes. All three terms are synonymous (Mc. Kinney, 1962)

#### Decomposition of sewage

The decomposition of sewage may be divided in to anaerobic and aerobic stages. These conditions are usually, but not always, distinctly separate. The growth of certain forms of bacteria is concurrent while the growth of some other forms is dependent on the results of the life processes of other bacteria in the early stages of decomposition.

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When sewage is fresh it may contain some oxygen. This oxygen is quickly exhausted, so that the first important stop in the decomposition of sewage is carried on under anaerobic conditions. It may be accompanied by the creation of foul odors of compounds containing sulfur, particularly hydrogen sulfide; odorless gases such as carbon dioxide and methane; and other, more complicated organic compounds. An exception to the rule that putrefaction takes place only in the absence of oxygen is the production of foul-smelling substances by the putrefactive activity of obligatory and facultative anaerobes. Hydrogen sulfide can be produced, apparently, in the presence of oxygen, the action that takes place not being thoroughly under stood.

The biolysis of sewage is the term applied to the changes through which its organic constituents pass owing to the metabolism of bacterial life. Organic matter is composed almost exclusively of the four elements carbon oxygen, hydrogen, and nitrogen (COHN); sometimes sulfur and phosphorus are also present. The organic constituents of sewage can be divided into protiens, carbohydrates, and fats. The protiens are constituents principally of animals tissue, but they are found also in the seeds of plants. The chief distinguishing characteristic of the protiens is the possession of 15 to 16 per cent of nitrogen. To this group belong the albumens and casein. The carbohydrates are organic compounds in which the ratio of hydrogen to oxygen is the same as in water, and the number of carbon atoms is 6 or a multiple of 6. To this group belong the sugars, starches, and celluloses. The fats and

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salts are formed, together with water, by the combination of the fatty acids with the tri-acid base glycerol. The more common fats are stearin, palmatin, olein, and butyrine. The soaps are mineral salts of the fatty acids formed by replacing the weak base glycerol with some of the stronger alkalies.

The first state in the biolysis of sewage is marked by the rapid disappearance of the available oxygen present in the water mixed with the organic matter to form sewage. In this state the urea, ammonia, and other products of digestive putrefactive decomposition are partially oxidized, and in this oxidation the available of oxygen present is rapidly consumed. the conditions in the sewage becoming anaerobic. The second state is putrefaction in which the action is under anaerobic conditions. The protiens are broken down to form urea. ammonia, the foul-smelling mercaptans, hydrogen sulfide, and so forth, and fatty and aromatic acids. The carbohydrates are broken down in to their orginal fatty acids, water, carbon dioxide, hydrogen, methane, and other substances. Cellulose is also broken down but much more slowly. The fats and soaps are affected somewhat similarly to the hydrocarbons and are broken down to form the original acid of their make-up. together with carbondioxide, hydrogen, methane, and so forth. The bacterial action on fats and soaps is much slower than an protiens, and the active biological agents in the biolysis of the hydrocarbons, fats, and soaps are not so closely confined to anaerobes as in the biolysis of the protiens. The third state in the biolysis of sewage is the oxidation or nitrification of the products of decomposition resulting from the

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putrefactive state. The products of decomposition are converted to nitrites and nitrates, which are in stable condition and are available for plant food. (Babbitt & Baumann, 1967)

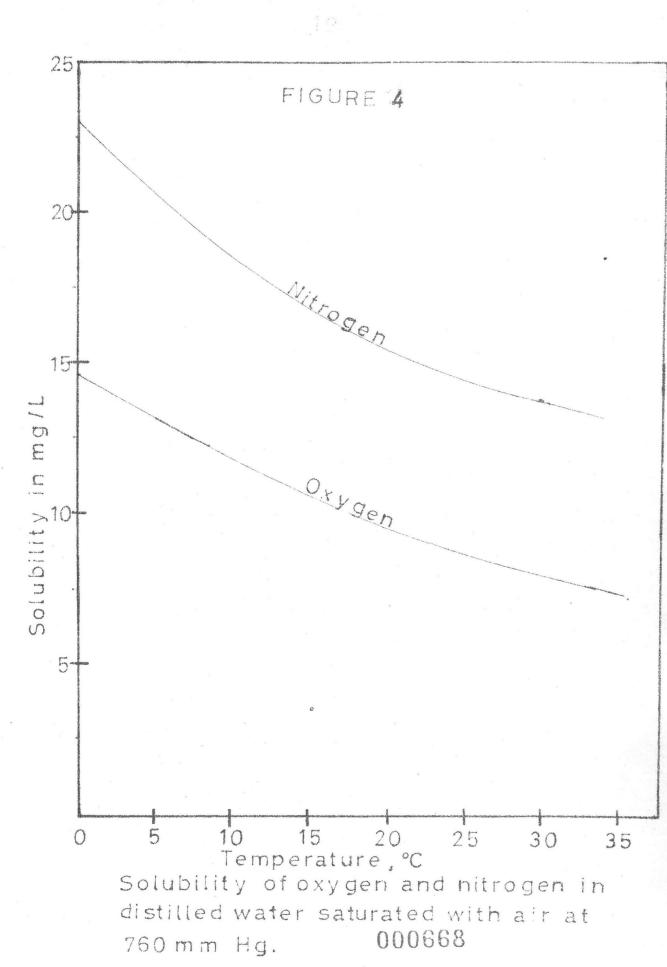
## Dissolved Oxygen.

All living organisms are dependent upon oxygen in one form or another to maintain the metabolic processes that produce energy for growth and reproduction. Aerobic processes are the subject of greatest interest because of their need for free oxygen.

The sanitary engineers, of course, interested in atmospheric conditions in relation to man, but in addition, he is vitally concerned with the "atmospheric conditions" that exist in liquids, water being the liquid in greatest abundance and importance.

All the gases of the atmosphere are soluble in water to some degree. Both nitrogen and oxygen are classed as poorly soluble, and since they do not react with water chemically, their solubility is directly proportional to their partial pressures. Hence Henry's law may be used to calculate the amounts present at saturation at any given temparature. The solubility of both nitrogen and oxygen varies greatly with the temperature over the range of interest to sanitary engineers. Figure 4 shows solubility curves for the two gases in distilled or low solide content water in equilibrium with air at 760 m.m. pressure. The solubility is less in saline waters. It will be noted that under the partial-pressure conditions that exist in the

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atmosphere, more nitrogen the oxygen dissolves in water. At saturation, the dissolved gases contain about 38 per cent oxygen, or nearly twice as much oxygen as in the normal atmosphere.

The solubility of atmospheric oxygen in fresh waters ranges from 14.6 mg/L at 0 c to about 7 mg/L at 35 c under atmospheric pressure. Since it is a poorly soluble gas, its solubility varies directly with the atmospheric pressure at any given temperature. This is an important consideration at high altitudes. Because rates of biological oxidation increase with temperture, and oxygen demand increases accordingly, high temperature conditions, where dissolved oxygen is least soluble, are of greatest concerned to sanitary engineers. Most of the critical conditions related to dissolved oxygen deficiency in sanitary engineering practice occur during the summer months when temperatures are high and solubility of oxygen is at a minimum. For this reason it is customary to think of dissolved oxygen levels of about 8 mg/L as being the maximum available under critical conditions.

The low solubility of oxygen is the major factor that limits the purification capacity of natural waters and necessitates treatment of wastes to remove pollutional matter before discharge to receiving streams. In aerobic biological treatment processes, the limited solubility of oxygen is of great importance because it governs the rate at which oxygen will be absorbed by the medium and therefore the cost of operation.

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Sanitary significance of dissolved oxygen. In liquid wastes, dissolved oxygen is the factor that determines whether the biological changes are brought about by aerobic or anaerobic organisms the former use free oxygen for oxidation of organic and inorganic matter and produce innocuous end products, where as the latter bring about such oxidation through the reduction of certain inorganic salts such as sulfates, and the end products are often very obnoxious. Since both types of organisms are ubiquitous in nature, it is highly important that conditions favorable to the aerobic organisms (aerobic conditions) be maintained; othermise the anaerobic organisms will take over, and development of nuisance conditions will result. Thus dissolved oxygen measurements are vital for maintaining aerobic conditions in natural waters that receive pollutional matter and in aerobic treatment processes intended to purify domestic and industrial waste waters.

Dissolved oxygen determination are used for a wide variety of other purposes. It is one of the most important single tests that the sanitary engineer uses. In most instances involving the control of stream pollution, it is desirable to maintain conditions favorable for the growth and reproduction of normal population of fish and other aquatic organisms. This condition requires the maintenance of dissolved oxygen levels that will support the desired aquatic life in a healthy condition at all times.

Determinations of dissolved oxygen serve as the basis of the BOD test; thus they are the foundation of the most important determination used to evaluate the pollutional strength of sewages and industrial wastes. The rate of biochemical oxidation can be measured by determining residual dissolved oxygen in a system at various intervals of time.

All aerobic treatment processes depend upon the presence of dissolved oxygen, and tests for it are indispensable as a means of controlling the rate of aeration to make sure that adequate amounts of air are supplied to maintain aerobic conditions and also to prevent excessive use of air (SAWYER & Mc CARTY, 1967)

The greatest demand for oxygen occurs at the head end of the aeration tank when the food and microorganisms are mixed together (Mc. Kinney, 1962)

## Organic compounds

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All organic compounds contain carbon in combination with one or more elements. The hydrocarbons contain only carbon and hydrogen. A great many compounds contain carbon, hydrogen and oxygen, and they are considered to be the major elements. Minor elements in naturally occuring compounds are nitrogen, phosphorus, and sulfur, compounds produced by synthesis may contain, in addition, halogens, certain metals, and a wide variety of other elements.

There are three major types of organic compounds, the aliphatic, aromatic, and heterocyclic. The aliphatic compounds are those in which the characteristic groups are linked to a straight or branched carbon chain. The aromatic compounds have these groups linked to a particular type of six member carbon ring which contains three double honds. Such rings have peculiar stability and chemical character,

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and are present in an important group of compounds. The heterocyclic compounds have a ring structure in which one member is an element other than carbon

Most organic compounds can serve as a source of food for bacteria.

#### Carbohydrates

The term carbohydrate is applied to a large group of compounds of carbon, hydrogen, and oxygen in which the hydrogen and oxygen are in the same ratio as in water, i.e., two atoms of hydrogen for each atom of oxygen, the processing of carbohydrate materials occurs in the lumber, paper, and textile industries, as well as in food industry. Wastes from these industries are major problems and tax the ingenuity of sanitary engineers to find satisfactory solutions.

Carbohydrates may be grouped in three general classifications, depending upon the complexity of their structure

1. simple sugars, or monosaccharides

2. complex sugars, or disaccharides

3. polysaccharides

Sucrose is the common sugar of commerce. It is derived largely from sugar cane and sugar beets. The sap of such trees as the sugar maple contains considerable sucrose. Hydrolysis of the sucrose molecule results in the formation of one molecule of glucose and one molecule of fructose.

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Sucrose  $(C_{12} H_{22} O_{11}) + H_2 O$ 

OH OH OH OH OH O CH<sub>2</sub>-CH-CH-CH-CH-C-H.....glucose +

OH OH OH OH O CH2-CH-CH-CH-C-CH2-OH....fructose

Glucose is the most common of the aldohexose sugars. It is found naturally in fruit juices and in honey. It is manufactured in great quantity by the hydrolysis of corn starch. It is the principal component of corn syrup. Both corn syrup and glucose are used extensively in candy manufacture. Glucose is much less sweet than ordinary sugar and replaces it for many purposes. Glucose is the only hexose sugar that can be prepared in relatively pure form by hydrolysis of dissaccharides or polysaccharides. All the other hexose sugars occur in combination with glucose.

Fractose is the only significant ketohexose and occurs naturally in honey. When cane or beet sugar is hydrolyzed, one molecule of fractose and one molecule of glucose are formed each molecoule of sucrose.

Glucose and galactose are of particular interest to sanitary engineers. Glucose is always one of the products and may be the sole product when di-or polysaccharides are hydrolyzed. It is there fore found in a wide variety of industrial wastes. Galactose is formed from the hydrolysis

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of lactose, or milk sugar, and is found in wastes from the dairy industry. Both sugars are readily oxidized by aerobic bacteria to form acids, and the oxidation amy stop at that point because of the unfavorable pH conditions produced by the acids unless precautions are taken to control the pH by means of buffers or alkaline materials. Lactic acid is an important intermediat in the oxidation of galactose. Both sugars are fermented rapidly under anaerobic conditions, with acid formation.

 $C_{12}$   $H_{22}$   $O_{11}$  +  $H_2O$  <u>invertase</u>  $C_6$   $H_{12}$   $O_6$  +  $C_6$   $H_{12}$   $O_6$ sucrose Glucose Fructose

# Biochemistry of carbohydrates

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The primary function of carbohydrate matter in higher animals is to serve as a source of energy. With microscopic organisms, however, the differentiation of foods for particular purposes is not a rigid matter. Bacteria, for example, utilize carbohydrate matter for the synthesis of fats and protiens as well as for energy. In addition, of course, carbohydrate is also used in building cell tissue and may be stored as polysaccharide in side or out side the cell wall.

The mechanisms by which bacteria and other microorganisms transform carbohydrates are believed to be essentially the same as those occuring in plants and animals. The first stage in carbohydrate metabolism involves hydrolysis. This degradation must progress to at least the disaccharide stage before transfer through the cell wall can occur. Once within the cell wall, the simple sugars are used for energy or synthesis.

The path way by which bacteria metabolize simple sugars for energy depends upon whether the conditions are aerobic or anaerobic in either case, the initial conversion is to pyruvic acid as fallows a simple hexose:

C6<sup>H</sup>12<sup>O</sup>6 <u>bacteria</u> 2CH<sub>3</sub>CCOOH + 4 H enzymes Pyruvic acid

Under aerobic conditions, the pyruvic acid and hydrogen are oxidized to carbon dioxide and water for energy:

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$$2 \text{ CH}_3 \text{ CCOOH} + 4 \text{ H} + 6 \text{ O}_2 \frac{\text{Bacteria}}{\text{enzymes}} 6 \text{ CO}_2 + 6 \text{ H}_2\text{O}$$

under anaerobic condition, however, this oxidation is not possible, and so the bacteria discharge various fermentation end products back in to solution. The nature of the end products depends upon the bacterial species involved, as well as the environmental conditions imposed. A few possibilities are indicated below: OH

Decteria 2 CH3 CHCOOH 0 2 CH<sub>3</sub> CCOOH + 4 H bacteria CH<sub>3</sub> CH<sub>2</sub> COOH + CH<sub>3</sub> COOH+HCOOH enestics is a ■ 2 CH<sub>3</sub> CH<sub>2</sub> OH + 2 CO<sub>2</sub>

Other acids, alcohols, and ketones may also be formed under anaerobic conditions. The large quantities of organic acids formed from anaerobic carbohydrate metabolism can overtake the buffering capacity of a waste, resulting in a low pH and a cessation of biological activity. This is a distinct possibility even with normally aerobic systems, which may suddenly be over loaded with a carbohydrate waste, resulting in temporary buildup of acid intermediates and a resulting drop in pH (SAWYER & mc LARTY, 1967)

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