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#### ABSTRACT

This manual contains the textual material for a three-lesson unit which introduces students to the basic concepts applicable to all biological treatment systems. The general topic areas addressed in the lessons are: (1) the microorganisms found in biological systems; (2) the factors that affect the growth and health of biological systems; and (3) the interrelationships between groups of microorganisms, their competition, predominance, and symbiosis. A list of objectives, glossary of key terms, list of references, and stream worksheets (one for each lesson) are included. (JN)

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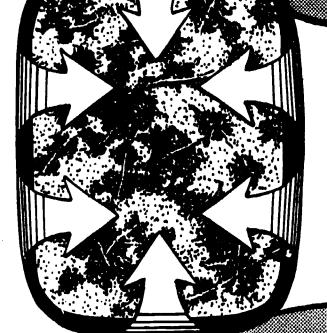
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Biological Concepts

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# BIOLOGICAL TREATMENT PROCESS CONTROL

#### CONCEPTS OF BIOLOGICAL TREATMENT

STUDENT MANUAL

# Text Written By: John W. Carnegie, Ph.D. Linn-Benton Community College Albany, Oregon

Edited By: John W. Carnegie, Ph.D. Project Director Linn-Benton Community College Albany, Oregon

.

Instructional Design By: Priscilla Hardin, Ph.D. Priscilla Hardin Instructional Services Corvallis, Oregon

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## Student Manual

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#### Objectives

Upon completion of this unit you should be able to:

- 1. State the main objective of a secondary trea nent plant.
- 2. Identify the types of microorganisms found in biological treatment systems.
- 3. Describe the structure, size and shapes of bacteria.
- 4. Describe the form and purpose of bacterial capsules.
- 5. Describe the structure and size of protozoa.
- 6. Describe the four types of protozoa.
- 7. Identify and describe viruses, fungi, and algae.
- 8. List other, higher forms of microorganisms found in biological treatment systems.
- 9. List the factors which affect microbial growth.
- 10. Draw and explain the growth curve for a population of bacteria.
- 11. Define and explain the food to microorganism (F/M) ratio.
- 12. Define hydraulic loading.
- 13. Describe and write the formulas for aerobic and anaerobic respiration.
- 14. Describe the effects of temperature and pH on microbial growth.
- 15. Name the three temperature growth zones.
- 16. State the usual optimum pH range for aquatic microorganisms.
- 17. Explain the term "population dynamics."
- 18. Name and give the formulas for the by-products of anaerobic respiration.
- 19. Explain the relative efficiency of aerobic vs anaerobic respiration.
- 20. Describe how microorganisms compete for the same food supply.



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- 21. Define the term "predominance."
- 22. Explain the effects of temperature, pH and D.O. on predominance.
- 23. List three other factors which affect predominance.
- 24. Explain the prey-predator relationship of the food chain.
- 25. Explain how protozoa can be used to assess the conditions of the bacterial population in an activated sludge system.
- 26. Explain the algae/bacteria inter-relationship.
- 27. Explain the concept of primary/secondary predominance.
- 28. Define the term "dynamic equilibrium."



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#### Glossary

bugs - the slang term used to describe the collection of microorganisms present in a biological treatment system micrometer - one thousandth of a millimeter motile - capable of moving about freely pathogenic - disease causing polluted - in reference to a stream; the condition of being undesirable from some use viewpoint; often refers to anaerobic conditions brought on by ox\_gen demanding organics. sessile - not capable of moving about freely; attached

toxic - poisonous material



# Lesson I; The Microorganisms

"You say you've got a biological treatment plant? Those 'bugs' in there are makin' the water clean? That's hard to believe! I can't see any 'bugs'. All I see is a big tank of foaming dirty water."

This is a view held by many upon seeing a biological treatment system for the first time. It is easy to see the big concrete or steel basins and the millions of gallons of water flowing through them. It is easy to hear the hum of the motors and the roar of the blowers. But it is impossible to stand by the tank of foaming, dirty looking water and see the "bugs" that are at the heart of the biological system.

One way to "see" these "bugs" is to consider the objectives of wastewater treatment and how the "bugs" accomplish the objectives. The primary objective of secondary wastewater treatment is to protect receiving streams from waste materials that lead to dissolved oxygen dreation in those streams. When untreated waste is discharged in a stream the organisms in the stream use the waste as food and consume dissolved oxygen in the process. The more waste there is, the more dissolved oxygen is consumed. Eventually, this may not be sufficient oxygen to support fish life and the "polluted" stream will be undesirable from an aesthetic point of view.

To prevent this degradation of the receiving stream the food material in the waste discharge must be reduced to a minimum. This is the objective of the secondary wastewater treatment plant.



The "bugs" or microorganisms in a biological treatment system use up the food material in the wastewater before it is released. Oxygen is supplied to the microorganisms so they can just sit there and eat and eat, and the clean water goes on out of the plant into the beautiful receiving stream

The only drawback to this process is that as they eat they grow and increase in number. Eventually, you've got quite a batch of nice happy microorganisms to deal with. In biological treatment systems any microorganisms not needed to handle the food in the waste are separated out and disposed of in a manner that does not harm the receiving stream.

The ability to treat and dispose of these excess solids (microorganisms) is a critical ingredient in the successful operation of a biological treatment system.

So as you stand at the side of an aeration basin or other biological system and look into the dirty water, what do you "see"? You should "see" microorganisms happily eating the food material in the wastewater and growing and increasing in number. And that's exactly what you, as an operator, want them to do. In fact, your main job, when you get to the bottom line, is to keep them just as comfortable and happy as possible so they can eat fast and do a good job of removing those food materials.

So, instead of a cowboy, sheepherder, or chicken farmer you're a "bug" keeper. And just like any good cowboy, sheepherder or farmer you must know all you can about these "bugs" to keep them healthy. You must know what they like to eat, what climate they like, what diseases they might get,



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what might poison them, and what predators might be a problem. We can call all of these items the environment in which the microorganisms live. You need to know everything about the microorganisms' environment so you can adjust it to keep them as healthy as possible.

Before we consider the environme of the microorganisms let's first take a closer look at these "bugs". There's really quite a mixed bag of tiny creatures in the biological system. Some of them are hard workers, some just along for the ride, and some down right troublesome. But they are all there and must be understood and dealt with continuously.

You can expend to find bacteria, protozoa, viruses, fungi, algae, and a few other forms in biological treatment systems.

The bacteria are the most numerous and, in fact, the most important to the biological treatment process. Although the other organisms, such as protozoa, fungi, and algae, also consume some of the food in the waste material it is primarily the bacteria that are responsible for the greatest share of this activity. Bacteria are tiny, single-celled organisms. An average bacterium is only about one micrometer wide. That means if you put them side by side it would take one thousand of them to stretch across the head of a pin. As tiny as they are they still carry on the activities of larger cells. They are independent, self-sufficient organisms.

Bacteria occur in three basic structural configurations: spheres, rods, spirals. The spherical bacteria are called cocci and the rods are called bacillus. The spirals or curved rods have no special name. Each



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of these types is significantly different and accounts for the wide variation in bacterial types. The bacillus or rod type can range from very short rods (<u>E. coli</u>) to rods 4 to 6 times longer than they are wide (<u>B. subtillus</u>) or very long thin rods, or anywhere in between. Some rods also form chains, end to end, to form long filaments (<u>Sphaerotilus</u> <u>natans</u>). The spiral type all look like helical spirals. Some are quite long with many spirals and some are very short; actually only part of one spiral called vibrio (Desulfovidrio).

Nearly all bacteria are motile. Some stay in contact with other objects such as rocks and plants; these bacteria move by a gliaing or sliding action across the surface. But they lack a visible means of propulsion. Some bacteria stay suspended in aquatic environments; that is, not in contact with other objects. Among these the spiral type moves like a corkscrew through the liquid. Many species of bacteria move by means of flagella. The long, whip-like flagella may appear in several different patterns depending on the species. Many bacterial species have capsules or sheaths that circle or enclose one or several bacterial cells. A capsule is a slimy, gummy coating which surrounds several or many cells. It is apparently secreted by the bacterial cells and serves as a common protective barrier against harsh environmental conditions. A sheath is similar to a capsule except it encloses the individual cells in a long filamentous chain. At times under the microscope these filaments are mistakenly identified as fungi.

The protozoa are a rather diverse group of microscopic animals. They are single-celled organisms but are several hundred times larger than bacteria. The protozoa have more complex physiological functions and



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structures without actually specialized organs such as heart, liver, and the brain. However, they do have a simple mouth in which they can ingest relatively large particles of material. Protozoa occur in varying sizes and shapes and all are motile during some part of their life cycle. Some are pathogenic to man.

There are 4 types of protozoa that will be found in biological treatment systems. These types are identified on the basis of their method of locomotion. The first group is commonly known as the amoeba (<u>Sarcodina</u>). The amoeba are unique in that they have no definite shape. They continually change from round to oval to very irregular shapes with protrusions periodically extending from the main cell mass and then being withdrawn. They move by use of these finger-like or foot-like protrusions called pseudopodia (false feet). A pseudopod is pushed out and the main cell moves b, flowing into the extended portion. This is called amoeboid movement.

Some amoeba produce a protective shell around themselves. Different substances are used for the shell depending upon the species. In any case, the cell itself is still amoeba-like and thrusts its pseudopodia out through openings in the shell in order to move and search for food.

The second group of interest is the free-swimming ciliates (<u>Ciliata</u>). The ciliates are so named because of the cilia that are distributed over the surface of the cell. Ciliates are generally ovoid or pear-shaped and maintain their shape by means of a tough but flexible coveriny. The cilia protrude from this covering in a variety of patterns. This group of free-swimming ciliates produces a rapid rhythmic movement of the cilia to



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propel themselves through the liquid. Some like the <u>Paramecium</u> are completely covered with cilia. Other free-swimming forms such as the <u>Didinium</u> have cilia in rows or spirals around the cell.

The second group of protozoa also includes ciliates classified as stalked ciliates. Most of these have a cup-snaped cell body with a stalk which attaches them to a piece of solid matter in the waver or to the sides and bottoms of tanks. Cilia are attached around the lip of the cup and the ciliary movement creates a current past the cell which brings food into contact with it. Some cells may attach by a single stalk to form a branched colony. Vorticella is a good example of a stalk ciliate. The ciliates are the most abundant protozoa in the biological systems.

The third \_roup of protozoa that you might find in the biological system is the flagellates or <u>Mastigophora</u>. The flagellates are ovoid or pear-shaped, much as the ciliates, but have flagella rather than cilia. Normally, one or more flagella is attached to one end of the cell. Many variations occur, however, with different arrangements of flagella. Some flagellates may form colonies in which the cell bodies are clumped together with their flagella projecting outward.

The last group of protozoa of interest are the suctoreans (<u>Suctoria</u>). The suctoreans have an early pre-swimming stage and an adult stalked stage. Many resemble the stalked ciliates except that no cilia are present; instead they have rigid tentacles extending from the cell body which are used to capture food particles.



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The viruses are a unique group of organisms. They are strictly parasitic. They lack the metabolic systems necessary to independently obtain energy and even to reproduce. They must use the systems of their host for these purposes. They steal the energy and raw materials of their host and take over the cellular operation. For almost any normal cell (plant, animal, or bacteria) there is a potential parasitic virus. Structurally, they are very simple. They are extremely small; most are a thousand times smaller than the common bacteria cell. Even the largest are smaller than most bacteria. They cannot be seen even with a compound light mic/oscope. They consist of little more than a bundle of genetic material with a protective covering. The protective coat can be made of different materials (such as crystalin protein or animal cell membrane) and different shapes (rods, spheres, polyhedrons, etc.). The viruses do very little as far as removing food material from the wastewater, but their presence is important since many of them are pathogenic to man.

The fungi are also of little importance as far as removing food from the wastewater. Fungi are very similar to bacteria. In fact, many microbiologists consider bacteria to be a special type of fungi.

In some biological treatment systems algae may be found. All algae are microscopic plants which carry out true photosynthesis. Although we say that algae are microscopic, many do grow to be extremely large such as the marine kelp. The green slimy growths visible in fresh water ponds and streams are examples of larger species. Some forms are single celled and require the aid of a microscope to see them clearly.

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There are a few higher forms of animal life that are significant to biological systems and are small enough to be considered as microorganisms.

There are a few higher forms of animal life that are significant to biological systems and are small enough to be considered as microorganisms.

The bio-mass on the media of trickling filters, for example, probably contains the most diverse group of higher animal forms. In addition to bacteria, fungi, protozoa, and algae; worms, snails, and fly larva can be found. In lagoons, worms and snails can be found along the bottom and edges; fly larva in the grass and weeds, and microscopic crustaceans free swimming. Rotifers, the simplest of the multicellular animals, are found in many types of systems and are common in activated sludge.

In this lesson we have taken a look at the purpose of a biological treatment system and discussed how the "bugs" are used to help prevent pollution of receiving streams. We then looked at the variety of organisms that are present in biological systems and saw that bacteria, protozoa, viruses, fungi and algae plus a few other higher forms are all living together in the large tanks of flowing water that we call the biological treatment system.



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#### Lesson II; Environmental Factors

The biological treatment system is really a tremendous number of different kinds of microorganisms consuming food material from the wastewater before it enters a receiving stream. However, the microorganisms really don't "care" about the receiving stream at all. If they "care" about anything, it is the need to obtain food and energy for survival and growth.

The plant operator, however, cares about the receiving stream and realizes that if the food and energy needs of the microorganisms can be satisfied they will perform efficiently. So you, the operator, must develop a process control strategy that responds to the needs of your microorganisms.

There are a number of factors that influence the health and growth of microorganisms. Since these factors make up the microorganisms' environment we call them environmental factors. Sometimes they are called environmental pressures because when they change they can cause the microorganisms to respond in some way.

Let's look at some of these factors and see how they might affect microorganism growth.

#### Food

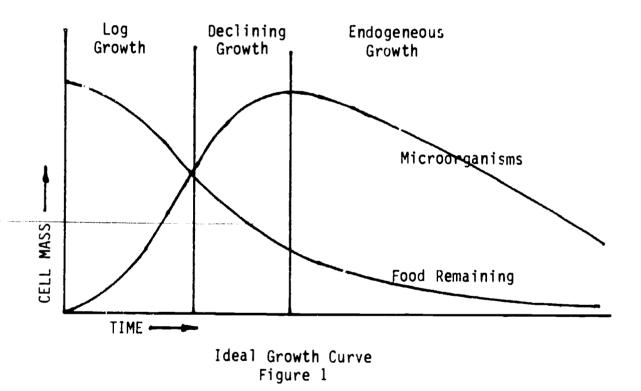
The food consumed by the microorganisms are mostly organic materials in the wastewater. These organic materials come from many places such as



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human feces, food scraps, soil and industrial wastes. The greater the organic load into the biological system the more food there is available and the faster the microorganisms will grow. However, since the organic load continually fluctuates the growth rate of the microorganisms also fluctuates in response to this particular pressure.

To illustrate this growth pattern let's look at a simple example. Figure 1 shows the pattern of growth of a population of microorganisms based on their total mass as compared to their total availability of food.



For simplicity's sake, assume that in this figure a small number (mass) of microorganisms were put into a flask containing a specific amount of well-balanced nutrients and that all other factors such as dissolved oxygen, temperature and pH are optimum. Then as time passes we can observe the change in mass of the living organisms.



In the beginning there is a great excess of food available. The microorganisms can grow and multiply as fast as their metabolic systems will allow. These are optimum growth conditions. The microorganism mass doubles every 20 - 30 minutes. This rate of mass increase is called a logarithmic increase and this phase of microorganism population growth is called the log-growth phase. The log-growth phase continues until the food available is depleted. At this point it takes the microorganisms longer to accumulate enough food for growth and multiplication. The time to double in mass increases greatly, so the rate of growth of the population slows down as more and more food is used up. Eventually the rate of growth will slow down to no growth at all. This entire period of decreasing rate of growth is appropriately called declining growth phase.

When growth ceases the concentration of food is as low as it can get. The food is not entirely gone, but it is so diluted that it is impossible for the bacteria to take it into their cells. However, at first the cells are still live and demand more food. Just as humans use stored food in their bodies in times of no food, the microorganisms are forced to use organic material within their own cells as a source of energy. This is the endogenous phase. During this phase the total mass of microorganisms will slowly decrease as the cells use up all their stored reserves and slowly begin to die. Ultimately all the cells would die.

This example is a unique condition in which food was made available only at one time. Under normal conditions food would become available either continuously or periodically and the system would be a bit more complex. This important relationship, in any case, is the balance between the amount of food and the mass of microorganisms. This is referred to as

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the F/M ratio (food/microorganism ratio). In the log-growth phase the F/M ratio is high since there is excess food. The F/M ratio decreases during the declining growth phase until in the endogenous phase it is quite low. We will discuss the importance of knowing and controlling the F/M in the operation of the activated sludge process.

The type of food is also important. Some types of organic loads are more easily utilized than others and would have the effect of decreasing growth rate.

The microorganisms also require other non-organic nutrients for a balanced diet. Just as with humans or domestic animals, minerals and trace elements must be present for the microorganisms to grow well. Some types of wastes may be deficient in certain nutrients and be stressful to the "bugs".

#### Hydraulic Load

Hydraulic load means the amount of water entering a system. As the hydraulic load or flow increases the concentration of organics may decrease. This would have the effect of decreasing the food supply and slowing the growth rate. At the same time, since the flow is up, the waste and microorganisms are moved through the system faster and the time allowed for the microorganisms to consume the food is short. They may not have time to do a good job.

#### Oxygen

The presence or absence of dissolved oxygen and, if present, the concentration of dissolved oxygen is an important factor. Most aquatic microorganisms, including protozoa, fungi, and most bacteria use free



dissolved oxygen  $(0_2)$  in the process of converting food to energy and raw materials for growth. This process is called aerobic respiration and organisms that use free dissolved oxygen are called aerobes. A few bacteria obtain the oxygen they need from chemically bound oxygen compounds such as nitrate  $(N0_3^-)$  and sulfate  $(S0_4^-)$ . This process is called anaerobic respiration and organisms that use only bound oxygen are called anaerobes. Many of the bacteria in biological process have the ability to use either free dissolved oxygen or chemically bound oxygen and are referred to as facultative organisms.

So an aquatic system with plenty of dissolved oxygen would be considered an aerobic system and would support aerobic and facultative organisms. An aquatic system with no dissolved oxygen would be considered an anaerobic system and would support anaerobic and facultative organisms.

However, the aerobic respiration process of consuming food from the wastewater is much more efficient than the anaerobic respiration process. Therefore, to keep the microorganisms working at a high efficiency it is desirable to provide plenty of dissolved oxygen to the system.

Even in aerobic systems the dissolved oxygen (D.O.) concentration can have an effect. There is usually a specific D.O. concentration range that provides optimum growth conditions.

Another important environmental factor is temperature. As with all chemical reactions temperature has an effect on the rate at which microorganisms can utilize the food material. As the temperature increases the rate of reaction increases and thus the rate of cellular growth and microorganism population growth increases. The rate of microbial growth doubles with



every 10°C increase in temperature up to some limiting temperature. At low temperatures growth is quite slow, but some organisms can exist even slightly below freezing. At higher temperatures they can be killed. Most microorganisms do best at moderate temperatures, but there are many different types of organisms and they have different optimum ranges. Microorganisms can be classified on the basis of the temperature range in which they have optimum growth.

The majority of microorganisms thrive at about  $35^{\circ}$ C. These organisms are called mesophilic organisms. They would die if the temperature reached  $40^{\circ}$  to  $45^{\circ}$  C. Microorganisms that grow best at high temperatures ( $55^{\circ}$  to  $65^{\circ}$  C) are called thermophilic organisms. But even these will die if the temperature rises much above  $65^{\circ}$ C. Most microorganisms grow very slowly, if at all, a extremely low temperatures. However, a few organisms can withstand temperatures at and slightly below freezing. These are called psychrophilic microorganisms. Psychrophiles would have very low rates of growth, thermophiles would have very high rates of growth, and mesophiles would be about average. Remember that rate of growth and removal of food from the wastewater are directly related. So thermophilic and mesophilic organisms would remove more organic food from the wastewater than psychrophilic microorganisms.

The pH of the environment also has a profound effect on the rate of microorganism growth. As with temperature, the effect is directly on the ability to convert food to energy and raw materials. Acid conditions (low pH) or basic conditions (high pH) can destroy this ability. Most microorganisms do well at pH 6.5 to 8.5. However, as with temperature some organisms can tolerate extreme pH and thus survive in basic or acidic environments. Fungi, for example, do well in acidic conditions; most



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bacteria and protozoa, however, grow best in neutral pH conditions. Abnormal pH in biological treatment processes can result in a significant decrease in the rate of removal of organic food from the wastewater.

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Some materials are toxic or poisonous to the microorganisms. If toxic materials enter the system in large amounts it could destroy all the micro-organisms. In smaller amounts it could decrease the growth rate or selectively destroy the more susceptible microorganisms and then decrease the efficiency of the food consumption.

These are the main environmental factors that can influence health and growth of microorganisms. Changes in these factors should be considered pressures that cause the microorganisms to respond in some way. If the pressures are detrimental the microorganisms will respond by growing poorly and being inefficient. If the pressures are toward optimum conditions the microorganisms will respond by growing vigorously and efficiently removing food from the wastewater.

A knowledge of the environmental factors should give you a better view of the activity within the biological treatment system. As you stand by the basin or tank you can "see" those "bugs" in there pushing and shoving to get at more food. You might also "see" some of them gasping for oxygen or dying excruciatingly because of toxic materials. You might "see" some suffering from heat prostration or shivering because of the cold.

Your reaction should be "Oh, my gosh, those poor little guys. What can I do to make things more comfortable for them." But, you still can't "see" the whole picture clearly. All of these factors and types of organisms are continually interacting and have a strong effect on one another. In the next lesson we will look at these interrelationships.



#### Lesson III; Population Dynamics

The biological treatment system is made up of many types of microorganisms living together and responding to changing conditions within the system. Each type does not live independently, but instead is affected by all the other organisms just like the people in a large city. They are all subjected to changing environmental conditions, but they may not all respond the same to the changes. Some may thrive where others may suffer. And as conditions change again a different group may thrive. The "ebb and flow" of the populations of the different microorganisms is a continuous pr .ess. The term that describes this fluctuation is population dynamics. An understanding of the principles of µopulation dynamics will help predict how the organisms will respond to different environmental conditions or pressures.

#### Predominance

Under any set of environmental conditions certain microorganisms will predominate. Predominance is established by the microorganisms that can derive energy from the available food most efficiently. Others will survive but their population will be small and not grow as rapidly.

We have discussed a number of environmental pressures in Lesson II. A change in any of the pressures will bring about a shift in the microorganism population so that a different pattern of predominance will emerge.

Before we look at how and why population predominance shifts we must consider how the organisms consume food, obtain energy and multiply. The food supply for most microorganisms is soluble or finely suspended organic material. Of course, wastewater contains large amounts of organic material and, as we have seen, it is the removal of the material that is the objective of secondary treatment. The food (organic material) is a high energy source. The microorganism uses this energy to move about, grow and multiply.



#### Patterns of Respiration

The process of converting the energy in the organics to usable energy within the microorganism is called respiration. Two common patterns of respiration exist, aerobic respiration and anaerobic respiration. Organisms undergoing aerobic respiration consume (use) free oxygen dissolved in the water. As a result energy is derived from the organic, new cells are produced as the microorganism grows and multiplies; and two by-products, carbon dioxide and water, are produced. The chemical equation expressing this process is shown below:

# Organics + $O_2 - CO_2 + H_2O + Energy$

AEROBIC RESPIRATION Figure 1

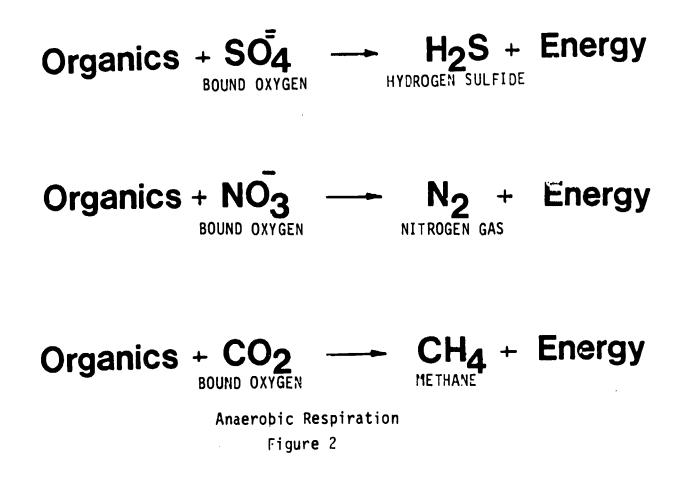
Organisms undergoing anaerobic respiration also consume oxygen but the source is chemically bound oxygen such as the oxygen found in nitrate, sulfate, phosphate and carbon dioxide. However, the microorganism still derives energy from the organics; new cells are produced as the microorganism grows and divides; and carbon dioxide is produced. However, there are a couple differences. Depending on the source of bound oxygen, several different end-products are produced, some beneficial, some troublesome. For example, if nitrate is the oxygen source, nitrogen gas  $(N_2)$  is produced; if sulfate is the oxygen source, methane gas  $(CH_4)$  is produced. Secondly, the anaerobic respiration process is not as efficient as the aerobic process.



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material as will aerobic respiration. Chemical equations expressing the anaerobic respiration process using three different sources of energy are shown below:



Organisms that carry out aerobic respiration are said to be aerobic while those utilizing anaerobic respiration are anaerobic. The protozoa and higher forms are all aerobic. Some bacteria are strictly aerobic and some are strictly anaerobic. A large number, however, are capable of utilizing free oxygen (aerobic) when it is available or chemically bound oxygen (anaerobic) if free oxygen is not available. These bacteria are called facultative.

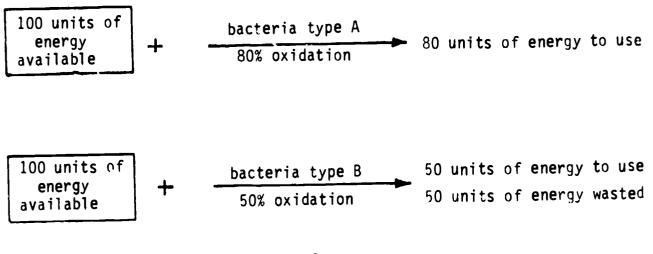
The food (organic material) not only provides energy to the microorganisms but also much of the raw building material (chemical elements) needed to produce new cells. From a predominance standpoint the population that can produce the most new cells the fastest will be the winners, predominate under the current



environmental conditions. So new cells is the "goal" and an increase in cell mass occurs during either aerobic or anaerobic respiration.

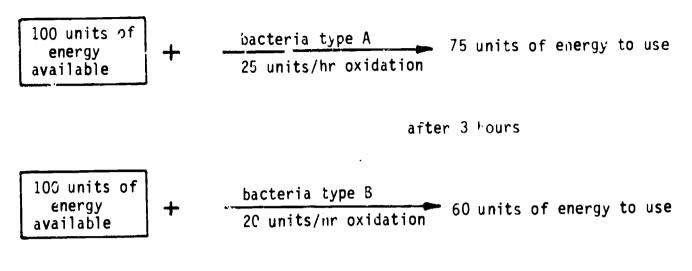
#### Competition for Food

Predominance is sometimes established as the result of successful competition for a common food source. If two types of microorganisms both utilize the same organic material, the type that can derive a larger amount of energy from the material available to it will predominate. For example, if two microorganisms use the same organics and type A is 80% efficient while type B is 50% efficient, type A will ultimately predominate because more cells will be produced from the energy obtained.





Also, if one organism can utilize the same food source faster than another, it will ultimately predominate. For example, if two microorganisms use the same organics and type A converts 25 units/hour but type B can only convert 20 units/hour, over a period of time type A will have produced more cells because it was able to utilize more energy. Type A will predominate.





The examples of predominance illustrated above are both based on how effectively a type of organism can derive energy from available food. That effectiveness is a function of the ability of adsorb and absorb food particles. Food particles must be adsorbed to the cell surface, partially broken down, and then absorbed into the cell itself where respiration occurs. Adsorption and absorption are functions of the surface of the cell, its total area and its chemical makeup. The explanation for why some organisms do better than others lies in the characteristics of the cell surface, the cell membrane, cell wall, and sheath or capsule surrounding the cell.

Most microorganisms tend to do best on one type of food material (substrate). They have systems which carry out respiration most effectively on that substrate. As we have seen, if two types of organisms are competing for the same substrate, one will tend to predominate and get the "lion's share." Some non-predominant organisms, when faced with this type of losing situation, have the ability to utilize a second choice substrate, one for which it can successfully compete. Food is not the only factor that can cause a shift in predominance. There are a number of environmental conditions that influence the effectiveness of cellular respiration. These include not only amount and type of food (substrate), but also temperature, pH, oxygen, toxic substances, as well as salinity and sunlight. These are often called growth pressures because a change in these conditions can cause population predominance to shift.

#### Effect of Temperature

Temperature has a direct effect on the rate of respiration. As a general rule an increase in temperature causes an increase in the respiration of microorganisms. However, each type of organism usually has a temperature range over which it respires most effectively.

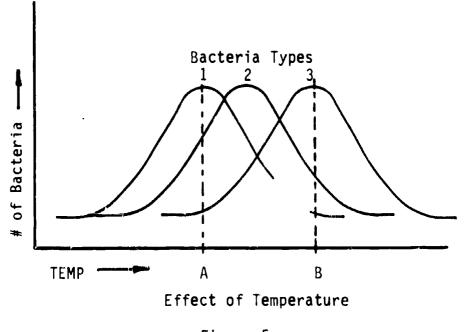




Figure 5 illustrates how the optimum ranges for three types of organisms might relate. From this illustration it can be seen how an increase in temperature from point A to point B would result in type 1 being outside its optimum while type 3 becomes more efficient. Such a temperature change would result in a change in predominance from type 1 to type 3. Decreases in temperature could also result in a similar situation.



Organisms that favor high temperatures are called thermophiles. Those favoring temperatures in the middle ranges  $(20-30^{\circ}C)$  are called mesophiles and those favoring lower temperatures are called psychrophiles. Most organisms in biological treatment systems are in the lower mesophilic range. However, heated anaerobic digesters may be considered thermophilic.

#### Effect of pH

Another growth pressure is pH. pH is a measure of the acidic or basic nature of the environment. As with temperature most organisms have an optimum pH at which they respire most efficiently. For most organisms the optimum pH range may be fairly narrow and near neutral pH (pH 7).

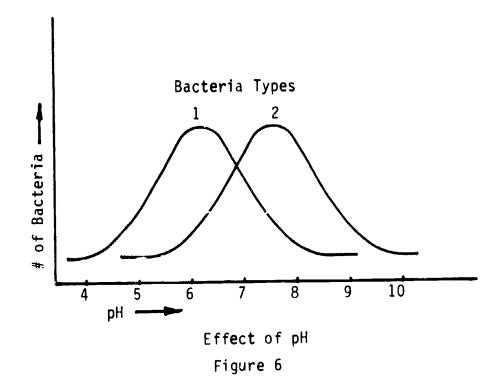


Figure 6 illustrates a pattern similar to the ranges for temperature. Again, it can be seen how a change in pH would result in a move from the optimum range for one type of organism into the optimum range for another type of organism. A shift in predominance would result. Most bacteria have optimums between pH 5 and pH 9. Fungi prefer lower pH's and thrive well at pH's of 4 or 5. Therefore, fungi will tend to predominate at low pH's.

#### Effect of D.O.

The presence or absence of dissolved oxygen (D.O.) is also a growth pressure. Recall that in the presence of dissolved oxygen strict aerobes and facultative microorganisms will be able to utilize substrate. Some aerobes can carry out respiration on less dissolved oxygen than others. If the dissolved oxygen concentration decreases, the predominance will shift to those capable of existing with low levels. The facultative organisms can also survive because they can shift to aerobic respiration. As dissolved oxygen disappears altogether, anaerobic organisms, as well as the facultative organisms, will predominate. In systems where that fluctuates from aerobic to anaerobic and back again, facultative organisms will predominate because they can tolerate both conditions.

#### Other Factors

The presence of toxic materials may be the most severe type of growth pressure. Some chemicals may outright kill some types of organisms while others may be only moderately affected, or perhaps not affected at all by the same material. The tolerance to chemicals differs from one organism to another. A chemical toxic to one type may be tolerated by another type. In the mixed population of most biological systems there is usually at least one type of microorganism that can tolerate almost any type of chemical material except the severe toxic substances. Predominance would tend to shift toward tolerant types in the presence of toxic materials.

Other growth pressures include salinity and sunlight. The salinity of the environment is the amount of salts dissolved in the water. Salinity can come from salt water intrusion from the ocean or from industrial discharges. Certain types of microorganisms can tolerate higher salinities than other organisms. Predominance would tend to shift toward those organisms that can tolerate high salinity if environmental conditions change in that direction.

Sunlight allows those microorganisms that carry out photosynthesis to grow. Photosynthetic organisms might predominate over non-photosynthetic organisms in the presence of sunlight. Even the amount of sunlight can be



 $\mathbf{30}$ 

a factor. Some organisms can survive with less sunlight than others and therefore might predominate beneath the surface of a pond during periods of less sunlight.

It is the growth pressures that define the makeup of the microbial population. The sum total of all of these environmental conditions combine to define a fragile balance of species that can be shifted when any one of the conditions is changed. In reality the environmental conditions in a treatment plant are continually changing because the organisms are consuming organics and the influent load is changing. As a result the makeup of the microbial population is continually changing. The challenge to the operator is to determine under what conditions the system operates most effectively. The operator must do everything in his/her power to keep the system near that optimum by reacting to the changing environmental conditions and applying pressure to force the system back toward the optimum condition. This challenge holds true no matter what type of treatment system is involved. Fixed biomass and dispersed biomass systems are dynamic populations under the influence of these environmental factors or growth pressures.

#### The Food Chain

A number of interesting and important relationships exist in the biological treatment system. One of these is the food chain. The food chain concept describes the prey-predator relationship universal in biological systems. One organism will prey upon a certain type of organism for food and at the same time be the prey of a third type of organism. Thus the predominance of certain populations is dependent upon the well-being of other organisms. Organisms at the "bottom" of the food chain are the ultimate prey and those at the "top" are the ultimate predator. The autotrophic bacteria and algae are typically at the bottom be use they require only inorganics. The heterotrophic bacteria and fungi not only require inorganics but also organics to survive. Both of these groups are at the bottom of the food chain.

The protozoa are one step up because they feed on bacteria, algae, and fungi as well as suspended and dissolved organics. Rotifers and worms are



another step higher because, along with bacteria, algae, fungi, and suspended organics, they feed on protozoa. The fly larvae and crustaceans feed on the rotifers, protozoa, and worms and in turn are food for minnows. Minnows are prey for small fish and ultimately the larger fish. Fish, of course, are not normally present in treatment systems, but would be at the top in a natural environment.

Organisms at the bottom are typically smaller, but in total have a larger mass than the organisms at the top. Ultimately those at the top are dependent upon those at the bottom.

#### Protozoa Population Dynamics in Activated Sludge

The principles of the food chain and predominance can be applied to the evaluation of an activated sludge system. By observing the types and numbers of protozoa present in the system the condition or "health" of the bacteria can be determined. Since the protozoa feed on bacteria, the condition of the protozoa is directly related to the conditions of the bacteria. An analogy would be to premium beef grazing on lush pasture compared to skinny cows on sparse pasture. The condition of the pasture can be determined by the conditions of the cattle feeding on the pasture.

But within the protozoa feeding on the bacteria there is competition for available food. This results in a predictable pattern of types and numbers of protozoa present under varying loading conditions.

Consider a system in which there are very few bacteria present and to which is suddenly added a large amount of organic food. When lots of food is available, but few bacteria, the amoeba and flagellates can successfully compete. As the bacterial population increases the free-swimming ciliates predominate because of the availability of abundant food. As the food supply and thus the bacterial population starts to decrease the stalked ciliates begin to predominate because they are more efficient at capturing the diminishing food supply. As the food as bacteria become even more limited the rotifers are present because they too are quite efficient under low food availability conditions



Therefore, by determining the types and counting the number of the different types of protozoa present it is possible to estimate the amount of organic food present and the number of bacteria present.

#### Algae-Bacteria Inter-relationship

In aquatic environments exposed to sunlight and organic materials, algae and bacteria live together in a mutually beneficial relationship called a symbiotic relationship. They do not compete for food, but instead help each other out.

Algae require sunlight, dissolved inorganics, and carbon dioxide to produce new algae cells and to survive. In so doing, they release oxygen into the water. Bacteria utilize the oxygen to metabolize the organics to produce new bacteria cells and survive. In so doing they release carbon dioxide and dissolved inorganics as end-products of metabolism. So the algae and bacteria each give to and receive from each other essential materials. This relationship is the basis of facultative lagoon operation.

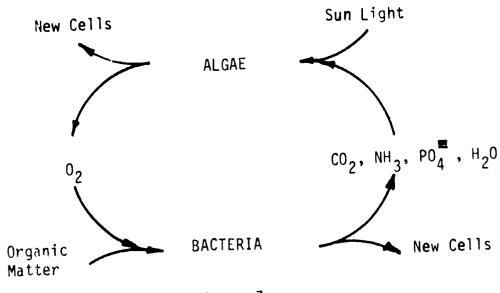


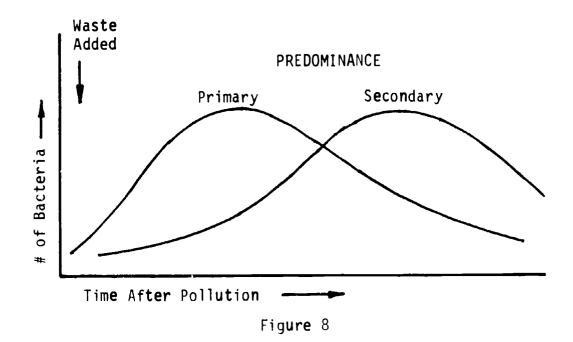
Figure 7



#### Primary/Secondary Predominance.

The type of bacteria which can most efficiently utilize the specific food in a wastewater will predominate as long as that food supply remains constant and other conditions are satisfactory and do not change. But when the food supply becomes limited the bacteria will die releasing cellular components into the water. These cellular components are mostly protein and are used efficiently by several somewhat specialized types of bacteria. Thus as the first type begins to die, the second group becomes predominate for a time. The first phase is called primary predominance because it acts first and is the most effective against the wastewater to begin with. The second phase is called secondary predominance because it acts second.

A healthy population of bacteria can be developed to act as primary predominance on almost any type of waste material. Many industries utilize primary predominance on their wastes in plants at the industrial site before releasing it into the municipal system. The municipal plant then acts as a secondary predominance to complete the stabilization.





#### Dynamic Equilibrium

A biological wastewater treatment system is a system in dynamic equilibrium. That is to say that the system is in balance (equilibrium) but the pieces are constantly in motion. In order for the system to perform efficiently the dynamic equilibrium must be maintained. This is the task of the operator: to maintain the balance within the system. It's kind of like keeping a teeter-totter balanced with people constantly getting on one end and others jumping off the other end. With the biological system energy is constantly entering the system, and new cells and energy in altered forms are leaving the system. The operator must be able to judge how much is entering the system and make the appropriate adjustments to keep the system balanced.



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## Worksheet 1 - The Microorganisms

- 1. We use the term "bugs" to mean all of the \_\_\_\_\_\_ in the biological treatment system.
- 2. The primary objective of wastewater treatment is to:
- 3. When organic material is allowed to enter a stream the natural biological activity in the stream depletes the \_\_\_\_\_.
- 4. In biological treatment processes, with the exception of anaerobic digestion, the bugs or microorganisms are supplied with \_\_\_\_\_\_ so they can consume the food in a controlled environment.
- 5. The only drawback to the bugs eating and eating is that they create excess
- 6. Name the five major groups of bugs typically found in biological treatment systems:

- 7. The microorganism which is responsible for the majority of the stabilization activity is the \_\_\_\_\_\_.
- 8. On the average bacteria are about wide.
- 9. The three structural shapes of bacteria are:

\_\_\_\_\_

\_\_\_\_\_



10.	The slimy, gummy coat around some bacteria is called the				
11.	The are a group of microscopic animals.				
12.	The four major groups of protozoa found in biological treatment systems are:				
13. 14. 15.	move about with the use of pseudopods or false feet. The two types of ciliates are the and the The <u>Mastigophora</u> move about with the use of				
16.	The resemble the ciliates except they have tentacles instead of cilia.				
17.	The are strictly parasitic because they have no enzyme systems.				
18.	The are true photosynthetic plants.				
19.	Four types of "higher" animal forms are frequently found in and around biological systems. These include:				



#### Worksheet 2 - Environmental Factors

- The main "objective" of the microorganisms in the treatment system is to obtain \_\_\_\_\_\_ and energy for maintenance and \_\_\_\_\_\_.
- 2. The environmental factors that cluse the microorganisms to react in some way are often called \_\_\_\_\_\_.
- 4. The phase of a bacterial growth curve in which the bacteria are increasing at a maximum rate is called the \_\_\_\_\_\_ phase.
- 5. The phase of the growth curve in which food is not available and the bacteria are utilizing energy stored within their own cells is called the \_\_\_\_\_\_ phase.
- 6. The amount of liquid entering the system is called the \_\_\_\_\_ load.
- 7. Most organisms in the biological treatment processes use free dissolved oxygen. These are called \_\_\_\_\_\_ organisms.
- 8. At times bacteria carry out \_\_\_\_\_\_ respiration in which they use chemically bound oxygen.
- 9. Many bacteria in biological systems are capable of using either aerobic or anaerobic respiration. These organisms are said to be \_\_\_\_\_.
- 10. Micrcorganisms that thrive at about 35 degrees Celcius are called organisms.
- 11. Microorganisms that prefer relatively high temperatures are called \_\_\_\_\_\_ organisms.
- 12. Most microorganisms in the biological treatment systems do well at a pH in the range of \_\_\_\_\_\_ to \_\_\_\_\_.
- 13. One group of microo. janisms tend to prefer lower pH and high carbohydrate conditions. These are the \_\_\_\_\_.



WOI	rksheet 3 - Population Dynamics
1.	The term is used to describe the
	continuous fluctuations that occur with the many types of microorganisms in the system.
2.	is established by the microorganisms that derive
	energy from the available food most efficiently.
3.	The process of converting energy in the seganic food to usable energy is called
4.	As a result of the energy derived aerobically from the organics, new are produced along with two by-products:
	and
5.	Anaerobic respiration produces different by-products depending on the source of bound oxygen used. These are:
	for nitrate bound oxygen =
	for sulfate bound oxygen =
	for carbon dioxide oxygen =
6.	Anaerobic respiration will derive only about percent as much energy from the same organics as from aerobic respiration.
7.	The food (organics) provides and also
	material needed to produce new cells.
8.	Efficiency of converting food to usable energy can be accomplished either by converting organics or
9.	Name three growth pressures other than oxygen and food that can affect population predominance.
10.	At times one organism will prey upon a certain type of organism and at the same time be preyed upon by a third type. This is an example of the

11. Organisms at the bottom of the food chain are typically smaller, but in total have a larger \_\_\_\_\_\_.



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- 12. \_\_\_\_\_ can be used to observe the conditions of the bacterial population breause the protozoa feed on the \_\_\_\_\_.
- 13. During photosynthesis the algae utilize \_\_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_\_ to produce new algae cells and oxygen.
- 14. The algae are provided with the inorganics and carbon dioxide by the \_\_\_\_\_\_\_\_ in the system.

