INTRODUCTION

Volumes of wastewater termed sewage are generated from many different sources. This sewage represents thousands of tons of organic matter. Why microbiology? Wastewater treatment is a biological process! A wastewater treatment plant is a microbiological zoo that houses bacteria, protozoa, metazoa and other microlife. The microorganisms do the actual breakdown and removal of nutrients and organic material in the wastewater. Like you and I, they perform best when all their needs are met (food, pleasant environment etc.).

Wastewater treatment facilities are designed to allow the natural process of the breakdown of pollution to occur under controlled conditions. These systems include physical and chemical processes to remove solids and heavier materials. However, left behind is the liquid containing soluble and insoluble organic material. The one process all sewage facilities have in common is the biological treatment of this organic material or "nutrients". That is, they rely on the use of certain microorganisms to convert these organic nutrients into materials that are beneficial for the environment. Sewage contains nutrients of every type; phosphorus, nitrogen, sodium, potassium, iron, calcium and compounds such as fats, sugars and proteins. Microorganisms use these substances as a "food" source for energy, for the synthesis of cell components and to maintain life processes.

It is the task of the operator to provide a favorable environment for the microorganisms. The operator provides the "food" and the favorable environment and the microorganisms do the work of removing nutrients and providing wastewater treatment. The health and well being of these microorganisms are critical to the adequate treatment of sewage.

Many types of microorganisms can be found in the wastewater treatment system. However, the types of organisms that will dominate will be the ones that are best suited to the "environment" or conditions in the system. Wastewater treatment systems are designed to foster an "environment" that suits a certain type of microorganism. These microorganisms not only remove organic wastes from the water, but they also "settle out" as solid material for easy removal. Wastewater treatment operators are required to maintain the right conditions in the treatment system for the right type of microorganisms. If the right conditions are not present, the wrong microorganisms will dominate. These "wrong" microorganisms not only interfere with the successful removal of wastes from the water, but they themselves may be difficult to remove from the system.

MICROSCOPY

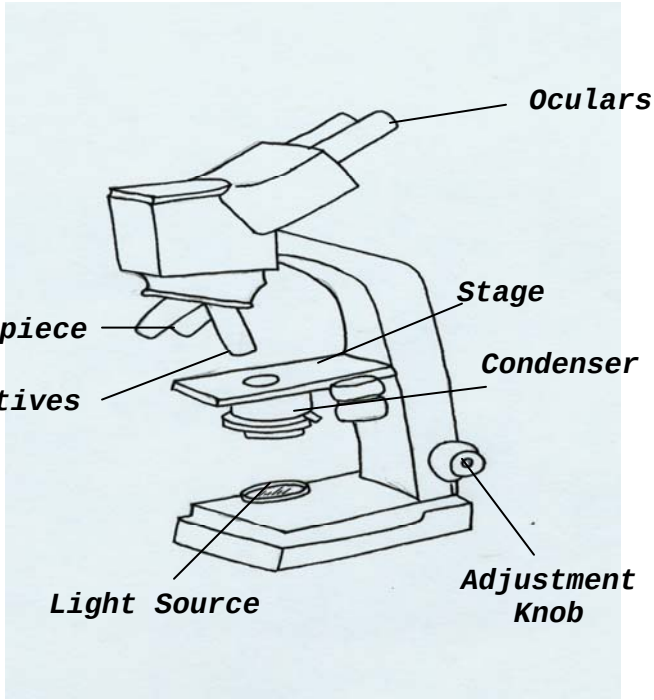
The wastewater treatment process is a biological process, therefore in order to properly evaluate this process you need to use biological tools

The Tool: *Microscope*

The human eye is not capable of distinguishing objects with a diameter less than 0.1 mm. Bacteria have a diameter of approximately 0.001 mm. In order to view their activity we must use a microscope.

An ordinary light microscope consists of:

–A microscope stand
–A specimen stage (where the slides lies)
–A nosepiece (holds the objectives)
–Oculars (eyepieces)
–A condenser
–A light source

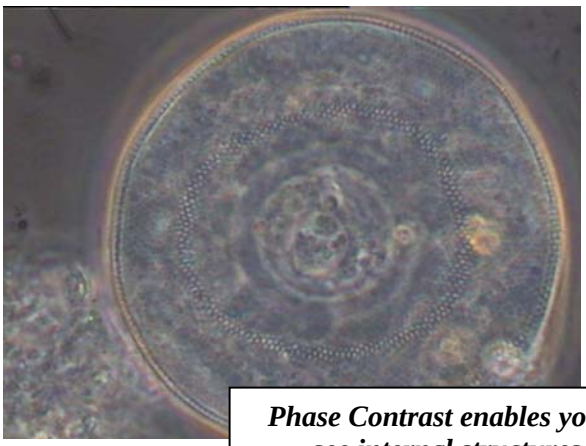


The Objectives:

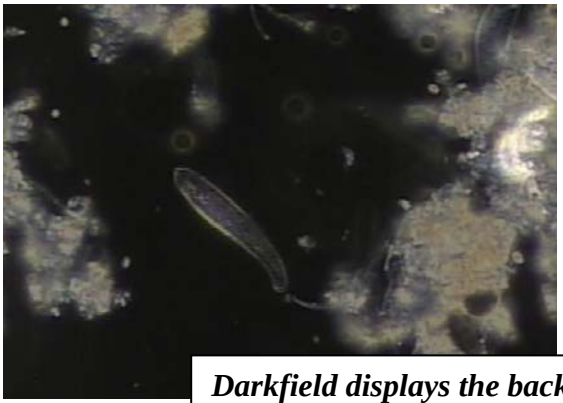
–10 X objective (magnifies 100 times)
–20 X objective (magnifies 200 times)
–40 X objective (magnifies 400 times)
–100 X objective (magnifies 1000 times) this is the oil immersion lens and must be used with immersion oil.

Under normal operation, light rays from a light source are passed through the condenser, which directs the rays through the specimen. This produces a brightfield illumination. However, most of the microorganisms found in wastewater are transparent. This makes it difficult to distinguish cell structures since the water is also transparent. There are several microscopy techniques that enable us to see clear and distinct cell structures.

Phase contrast illumination uses a special condenser, which slows down the light as it enters the denser parts of the microorganisms. This allows certain structures to stand out from other less dense parts of the cell and from the surrounding fluid. This technique allows you to observe the organisms while they are alive. Cell shape and structure are more visible than with brightfield illumination. Phase contrast is more suitable for observing live organisms. This lens translates small differences into clearly observable differences.



Phase Contrast enables you to see internal structures.

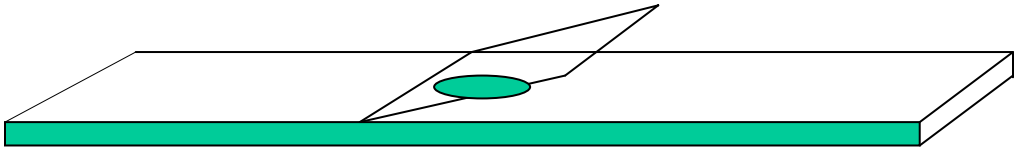


Darkfield displays the background as black and the organisms white

Another technique called darkfield microscopy makes the microorganisms appear white while the background appears black. The light does not pass through the specimen but hits only the sides of the specimen. There are several other microscopy techniques but those I mentioned are the most common used in the wastewater laboratory.

Slide Preparation

Wet Mount
A wet mount is used to examine live microorganisms. Cell measurements and cell shape can be determined.
–Place a drop of a well-mixed representative sample on a clean grease-free slide.
–Place a clean cover glass is on top. Avoid entrapment of air as much as possible.
–The size of the drop is critical. If the drop is too big the cover glass will float. If the drop is too small the sample will dry up too quickly. Use a pipette with a small opening.



Fixed Smears & Staining

Wet mounts are used to examine live cell structure and motility. Stains reveal different properties. In order to stain a sample it must be fixed to the slide so that it will not wash off during the staining process.
–Place a drop of sample on a clean, wax-free slide and smear across the slide.
–Let the slide air-dry. DO NOT HEAT FIX!
Once the sample is dried completely, it is ready for staining.

The two most common sample techniques are simple staining and differential staining. Simple staining uses only one stain. The differential stain uses more than one stain and dyes different types of microorganisms different colors. The different colors may be based on inclusions within the cells or differences in how the cell wall is structured. These stains help to distinguish between organisms, which would otherwise be difficult to tell apart. The most common differential stain is the Gram stain.

The Gram stain divides bacteria into two groups, 1) Gram-positive, those bacteria that retain the crystal violet and stain purple and 2) Gram-negative, those bacteria that lose the crystal violet color when rinsed with alcohol and stain pink with the counter stain safranin. Gram stain solutions can be purchased in kits already made up. The Gram stain procedure is done in 4 steps using 4 different solutions. Make sure that when you stain, you are staining the side of the slide with the smear on it.

Gram Stain Procedure:

1. Completely cover the smear with crystal violet solution and let stand for 1 minute. Gently wash with water. At this point both the gram-positive and gram-negative bacteria are stained purple.

2. Completely cover the smear with iodine solution and let stand for 1 minute and gently wash with water. A crystal violet-iodine complex is formed that helps the gram-positive bacteria to hold on to the purple dye.
3. Hold the slide at an anger and gently decolorize the smear by adding alcohol drop-wise for about 25 seconds or until purple dye is no longer running off the slide. Gently blot. At this point, the gram-positive bacteria are purple and the gram-negative bacteria are colorless.
4. Completely cover the smear with safranin solution and let stand for 1 minute and wash with water. Blot dry. This counter stain colors the gram-negative bacteria pink.

Using the Microscope

1. Rotate the revolving nosepiece and place the low power objective above the microscope stage. Adjust the objective until it is at least 3 cm above the stage.
2. Place the slide on the stage. Make sure the smear or wet mount is facing upwards and centered directly under the objective.
3. Peering through the eyepiece, use the coarse adjustment knob and move the objective towards the slide until the specimen begins to come into focus.
4. Use the fine adjustment knob to bring the specimen into sharp focus.
5. Once the specimen is focused using the low power objective, simply rotate the nosepiece to place the higher power objective over the specimen. The specimen should be nearly in focus. Make adjustment using the fine adjustment knob.
6. To move to the oil immersion lens, rotate the nosepiece until it is between objectives. Place a drop of oil directly on top of the stained smear or on top of the cover glass on the wet mount. Slowly rotate the 100X objective into the oil. The image should be nearly in focus. Bring the image into sharp focus using the fine adjustment knob.

Sample Collection

When examining mixed liquor, the sample should be collected from the discharge end of the aeration basin. At this point in the process most of the organic nutrients should be removed from the system. Slides should be made from a fresh sample of mixed liquor If the sample is collected and cannot be examined right away, the sample should be aerated.

MICROBIOLOGY OF ACTIVATED SLUDGE

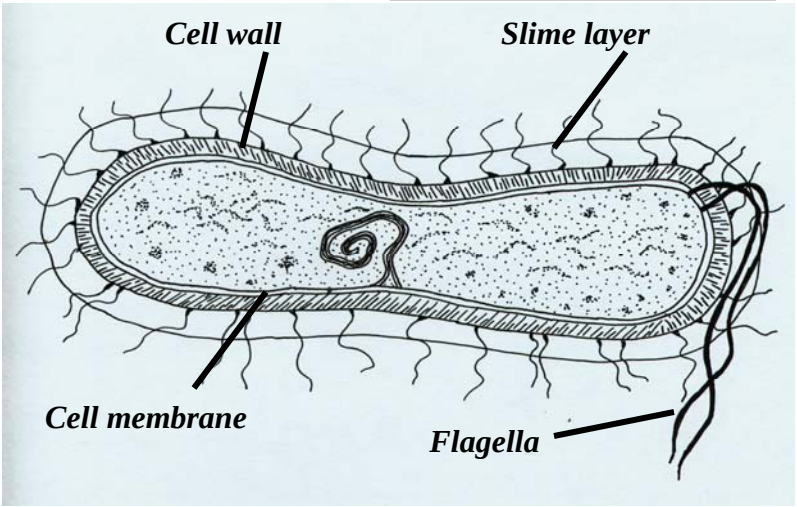
Activated sludge is a mixture of microorganisms that come in contact with and digest biodegradable materials (food) from wastewater. Once most of the material is removed from the wastewater, microorganisms form floc and settle out as sludge. Some type of microorganism will always grow in the system. The organisms that will dominate will be the ones that are best suited to the environment. So, it is important that the operator create an environment that will foster the type of microorganisms that we want – floc-forming bacteria.

For the purpose of studying activated sludge we will look at 3 different types of microorganisms:

•Bacteria (95%)
•Protozoa (4%)
•Metazoa (1%)

Bacteria

Typical Bacteria Cell



Bacteria are single-celled microorganisms that come in three basic shapes: 1) bacillus, which is rod-shaped, square or rectangular, 2) coccus, which is round or oval shaped, and spirillum, which is spiral or cork-screw shaped. Bacteria are classified based on how they respond to oxygen. Aerobic bacteria require oxygen to live; facultative bacteria prefer oxygen but can survive for some time without it and anaerobic bacteria cannot live in the presence of oxygen. The most important microorganisms in the activated sludge system are the aerobic bacteria. They consume the biodegradable material found in wastewater. They consume proteins, carbohydrates, fats and many other compounds.

Bacteria can only consume soluble organic material. Solid particles of "food" must be eaten by a two-step process.
1) Adsorption
2) Absorption

During adsorption, food particles that are too big to pass through the cell membrane and bacteria stick to each other. The bacteria secrete enzymes, which dissolve food particles into very small units. These small units of food can now pass through the bacteria's cell wall. Absorption is the process by which smaller dissolved units of food pass into the cell membrane.

Most modern treatment plant designs have eliminated primary treatment. Primary treatment played a principal role in removing most of the particulates or "settleable solids" and floating off most of the grease. Primary effluent entering the aeration basin contains mostly dissolved nutrients. This allows the bacteria to work more efficiently by eliminating the need to break down nutrients before they can be absorbed in the cell body. Without primary treatment, they have to work twice as hard. For example

compare how long it would take you to eat a bag of pecans that are in the shell compared to how much faster you could eat a bag of pecans if they were already out of the shell?

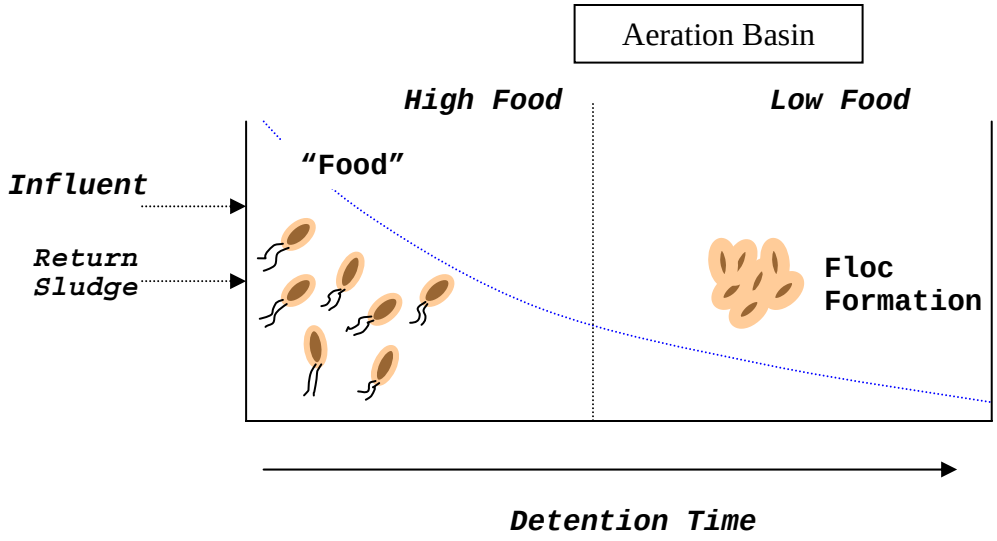
Enzymes
Enzymes are compounds that are made by living organisms for the purpose of helping biochemical reactions to occur. All biochemical reactions require enzymes. Bacteria need enzymes to breakdown nutrients. Enzymes are strange compounds that only work when the conditions are right. If the enzymes do not work, the bacteria will not function properly and will not survive.

Growth Characteristics

In a typical activated sludge system. Influent wastewater or wastewater leaving primary treatment enters the aeration basin. Microorganisms in the settled sludge are returned to the aeration basin. Here the microorganisms mix with the wastewater. The mixture is called "mixed liquor". When the influent wastewater first enters the aeration basin it contains a high level of nutrients or food. Here at the head end of the basin there is plenty of food available and bacteria use the food mostly for growth and some for energy. A growing bacterium has flagella (hair-like structures on the outside of the cell). The flagella make it motile and able to move in search of food. They are multiplying rapidly and do not settle to form floc.

When food is limited however, most of the available food is used for energy and cell maintenance. There is less food left for growth, thus less reproduction occurs. The bacterium takes steps to conserve energy by losing its flagella. The waste products start to form a thick slime layer outside the cell wall causing the bacteria to stick together to form floc.

The detention time must be sufficient in the aeration basin to allow an area to develop where the amount of food is low. If the microorganisms are in the basin for too short a time, they will still be actively swimming and multiplying and will not form floc. This will yield a turbid effluent.



Bacteria Growth Curve

Lag-phase
During the lag-phase, bacteria are becoming acclimated to their new environment. They are digesting food and are developing the enzymes need to break down the types of nutrients that the bacteria have detected. Growth does not occur during this phase.

Accelerated Growth-phase
Bacteria begin to grow at a rapid rate because of the excess amount of food that is available. The cells are mostly dispersed and active. They are not sticking together to form floc.

Declining Growth-phase
Reproduction slows down at this phase because there is no long an excess amount of food. There are a large number of bacteria that have to compete for the remaining food. The bacteria begin to lose their flagella.

Stationary-phase
Because of the lack of food, some bacteria are reproducing but an equal number are also dying. So, the number of bacteria remains relatively constant. They have not lost their flagella and have formed a stick substance covering the outside of the cell wall which allows them to agglomerate into floc.

Death-phase
In this phase the death rate increased with little or no growth occurring. The total number of bacteria keeps reducing.

Bacteria in the activated sludge system must be allowed to hang out in the aeration basin until they reach the stationary-phase. If they flow out of the basin too early, they will be active and motile and will not settle out as floc.

Food: Microorganism Ratio

The food to microorganism (F/M) ratio measures the amount of food that is available for the amount of microorganisms present in the aeration basin. The amount of food is determined by the biochemical oxygen demand (BOD) or chemical oxygen demand (COD) test. If there is too much food and not enough microorganism (high F/M ratio), settling problems may occur because in the presence of excess food bacteria are active and multiplying and will not develop into floc.

$$\frac{F \text{ (Determined by the BOD or COD test)}}{M \text{ (Determined by the MLVSS)}}$$

On the other hand a low F/M ratio indicates that there is a little bit of food and a lot of microorganisms. This means that food is limited, the bacteria lose their flagella, are no longer multiply but is forming the slime layer needed to develop floc. However, we must be careful not to operate with a F/M ratio that is too low. When food is severely limited, nutrient deficient conditions may occur which can cause other problems we will discuss later.

Factors Affecting Bacteria Growth

It is the responsibility of the operator to provide the best possible environment for the floc-forming bacteria to grow. The operator can control some of the conditions they require and there are some conditions they cannot control. For instance, the operator has no control of the weather and very little control over the types and amount of nutrients entering the treatment plant. So, it is important that the operator understand how the following factors affect the growth of the bacteria.

•Oxygen Utilization
•Sludge Age
•Dissolved Oxygen
•Mixing
•pH
•Temperature
•Nutrients

Oxygen Utilization

Actively growing bacteria eat food at a rapid rate therefore using oxygen at a rapid rate. The rate of oxygen use is normally termed the Oxygen Uptake Rate and is measured in mg O_2/hr/gm of MLSS. Generally a higher Uptake Rate is associated with a higher F:M ratio and younger sludge ages. A lower Uptake Rate is associated with a lower F:M ratio and older sludge ages.

Sludge Age

As bacteria first begin to develop in the system they grow singularly, in small clumps and chains. They are very active with flagella and do not have a well-developed slime layer. The bacteria are disperse and do not settle well. As the sludge is allowed to age, bacteria lose their flagella and accumulate more slime. The small clumps and chains begin to stick together and form floc large enough to settle.

Dissolved Oxygen

Aerobic bacteria require at least 0.1 - 0.3 mg/L of oxygen to survive. At least 2 mg/L of oxygen must be maintained in the bulk fluid in order for the bacteria in the center of the floc to get 0.1- 0.3 mg/L of oxygen. If not, the bacteria in the center will die and the floc will begin to break up.

Mixing

Mixing is required to bring the bacteria, oxygen and nutrients in contact with each other. Remember, once food is limited the bacteria lose their flagella and can no longer

swim. Without sufficient mixing, the bacteria will not bump into each other to form flow and proper treatment will not take place.

pH

It is the bacterial enzymes that are very pH dependent. Their optimal pH is between 7.0 and 7.5. Rapid pH changes should be avoided.

Temperature

Biochemical reactions are temperature dependent. Reactions are slower in colder temperatures so the system will require more organisms to do the work. Reactions are faster in warmer temperatures therefore fewer bacteria are required to do the same job during the summer months.

Nutrients

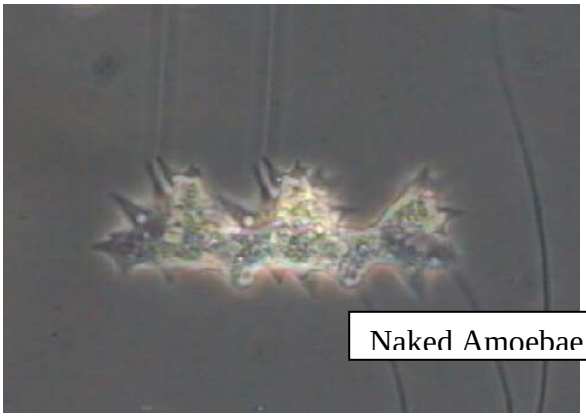
Bacteria require basic nutrients for growth (carbon, nitrogen, phosphorus as well as trace amounts of sodium, potassium, magnesium and iron. All these are present in normal domestic sewage. Generally, industrial wastes do not contain sufficient nutrients and must be supplemented. We will deal with this in more detail in a later chapter.

Protozoa

While 95% of the microorganisms in activated sludge are bacteria on 4% are Protozoa. Protozoa contribute very little to the removal of organic nutrients however their presence greatly enhances the clarity of the water. While bacteria are difficult to see under the microscope, Protozoa are not. Although they contribute little to the removal of organics, their behavior and the numbers of the different types of protozoa will give a an indication of treatment system performance.

Protozoa are single-celled microorganisms that come in a large variety of sizes and shapes. Their main function in the treatment process is to remove non-flocculent bacteria and very small floc that would not settle. They can be classified based on the way they "eat". Holozoic protozoa are capable of ingesting food such as bacteria through special mouths. Holophytic protozoa absorb dissolved nutrients directly into their cells just like bacteria. For the sake of studying their behavior in activated sludge we will classify them in the following five categories:

•Amoeba
•Flagellates
•Free-swimming ciliates
•Crawling ciliates
•Stalked ciliates



Naked Amoebae

Amoeba

Amoebae are the most primitive single celled protozoa. They feed mostly on solid particles in the water and will slowly extend lobe-like projections called pseudopodia until it has completely

surrounded its food. After the food is surrounded it secrets enzymes that will break the food particle into smaller unit that will absorb into the cell. There are two types of amoeba; the naked amoebae and the testate amoebae. The naked amoeba is the one most are accustomed to seeing in the science book. It looks like a blob. The testate amoebae has a shell or "test". Some secrete substances to form the shell while others form a shell from debris it collects as it travels in the water.

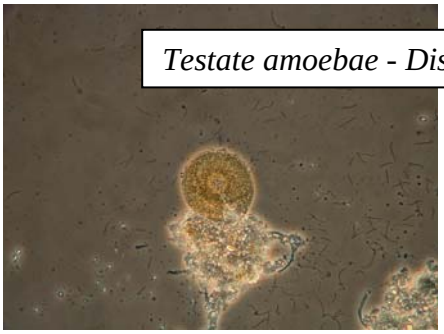
Testate amoebae - Arcella

Amoebae can only multiply when the nutrient level in the aeration basin is quite high or if there is very little competition for the food. Therefore they can only dominate early in the process.

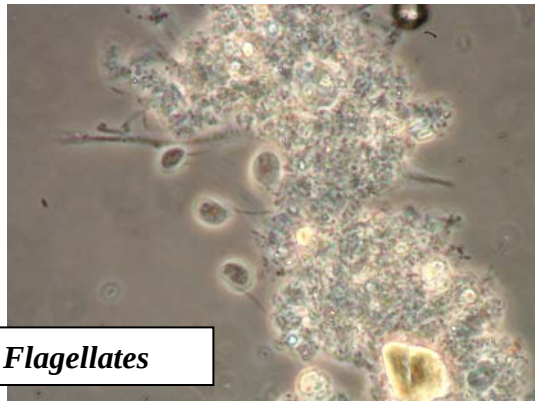
If large numbers of amoeba are found in a sample collected from the discharge end of the aeration basin (where most of the nutrients should be removed), this may indicate one of the following:

- A Shock load of BOD. This would make extra food available that will allow them to compete.
- The presence of large amounts of particulate matter. Amoeba favor particulates.
- Lack of oxygen. Amoebae move very slowly and require less oxygen then other protozoa.

Testate amoebae - Discoides

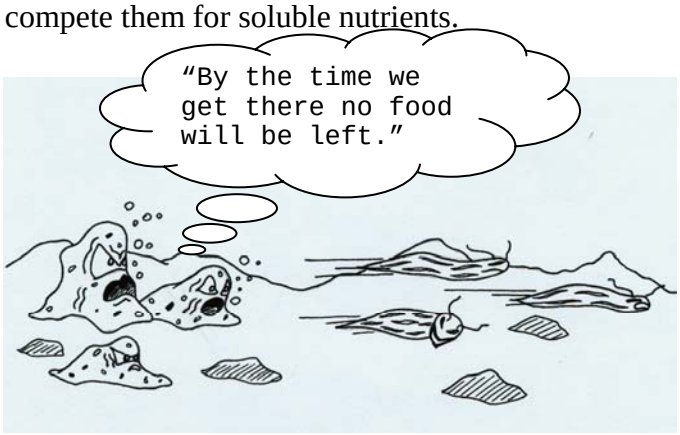


Flagellates



Flagellates

Most flagellates absorb nutrients just like bacteria so they compete with bacteria for dissolved nutrients. Flagellates peak in number while the soluble food concentration is high and the number of bacteria is still quite low. This, just like with amoeba is usually early in the process. However, once the bacteria become acclimated to the environment, they multiply much faster than flagellates and will eventually out compete them for soluble nutrients.



Ciliates

Ciliates also contribute very little to the removal of organic material from the wastewater. They feed on bacteria not on dissolved organics. Bacteria and

flagellates compete for dissolved nutrients but ciliates compete with other ciliates and rotifers for bacteria. The presence of ciliates is usually an indication of good treatment. They dominate after the formation of floc and when most of the organic nutrients have been removed. They are necessary for removing excess bacteria and algae from the fluid and clarifying the effluent.

Ciliates can be classified into three categories.

•Free-swimming ciliates
•Crawling (grazing) ciliates
•Stalked (sessile) ciliates

Free-swimming Ciliates

Free-swimming ciliates swim freely in the fluid. They are usually covered with "cilia" which are hair-like projection that they used for locomotion and for sweeping food into their mouths. They appear as the flagellates begin to disappear. As the bacteria population increases much of the organic nutrients have been removed and there is a lot of disperse bacteria available for feeding. Free-swimming ciliates begin to dominate as they feed on the increased number of bacteria.

Free-swimming ciliate - Paramecium

Crawling Ciliates

Crawling ciliate - Aspidisca

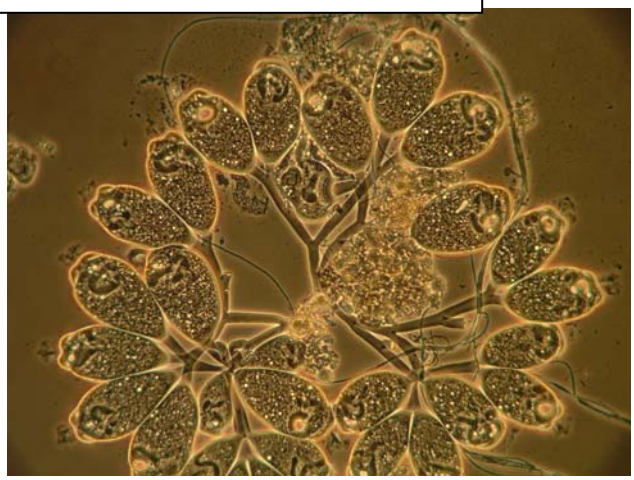
As the amount of nutrients decrease, food is limited and the bacteria begin to lose the flagella and form a sticky slime layer that allows them to stick together to form floc. As floc particles enlarge, crawling ciliates begin to dominate. Crawling ciliates have cilia on the under side of the body. The cilia are twisted together to form "tufts" or legs that are used for crawling along the floc. Crawling ciliates graze on floc particles and feed on the straggling bacteria on the edges of the floc. As the population of disperse bacteria decreases and floc increases crawling ciliates out compete free-swimming ciliates because they can find food within the floc and the free-swimming ciliates cannot.

Stalked Ciliates

Stalked ciliates only have cilia surrounding the oral groove or mouth and are used to create a current that will bring food into the mouth. Stalked ciliates appear in mature

Single stalked ciliates - Vorticella

Colonial stalked ciliate - Carchesium



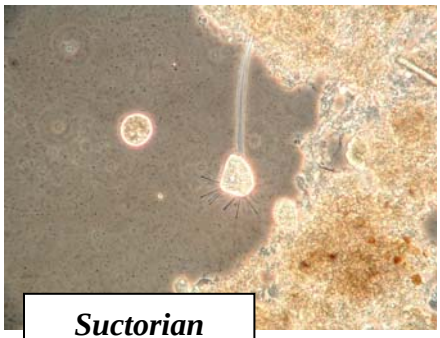
sludge. They dominate when most of the dissolved nutrients have been removed. In Mature sludge, crawling ciliates and stalked ciliates compete for dominance. While crawling ciliates must crawl around to find food, stalked ciliate can anchor themselves on the stalk and bring the food to them. Stalked ciliates grow singly or in colonies. Single stalked ciliates have one "zooid" or head per stalk. Colonial stalked ciliates can have up to 300 heads branching from one or more stalks. As the sludge ages and less and less food becomes available, the colonial stalked ciliates begin out compete the single stalked ciliates for dominance. A good way to remember this is, "the more head on the stalk, the older the sludge."

As the sludge continues to age other types of stalked ciliates begin to compete for dominance. Stentors are large bell shaped protozoa that also uses cilia to sweep food into the mouth but while others can only hold enough food t fill the mouth, the Stentor can fill its entire body with food. Vaginocola is much like the Stentor except it is enclosed in a sheath and is often seen in pairs.

Stentor

Vaginocola

If the sludge continues to age (usually too old), and with no food or bacteria left to feed on, protozoa-eating protozoa begin to dominate. The suctorian is so named because it has "suckers" or tentacles extending from its head. The suctorian will wait for an unsuspecting protozoan and will suck it into its tentacles, secrete a toxin to paralyze it and will begin to suck the body juices out.



Suctorian

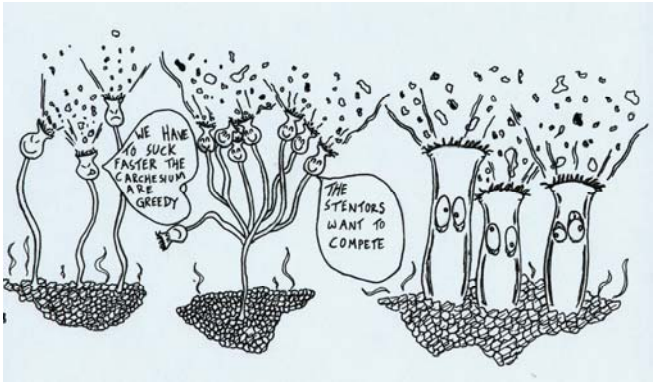
Factors Affecting Protozoa Growth

Like bacteria, there are several factors that influence how the protozoa will grow in the treatment system.

- Temperature
- pH
- Dissolved Oxygen
- Nutrients

Temperature

Most protozoa can survive and reproduce in the temperature range of most activated sludge systems. However, hey grow best in ambient temperatures of (15 - 25 degrees C).



pH

Protozoa are more sensitive to pH than floc-forming bacteria are. They have an optimum range of 7.2 - 7.4 but can tolerate 6.0 - 6.8.

Dissolved Oxygen

Like bacteria, protozoa must have oxygen to survive. Lack of oxygen will severely limit the kind and number of protozoa present.

Nutrients

Most municipal wastewater systems contain sufficient nutrients to support most protozoa. Industrial wastes are more likely to be deficient in nutrients.

Metazoa

Metazoa are multi-cellular microorganisms that feed on bacteria, algae and protozoa. They can have very simple to highly complicated physical structures. Following are metazoa that are commonly found in the wastewater treatment system.

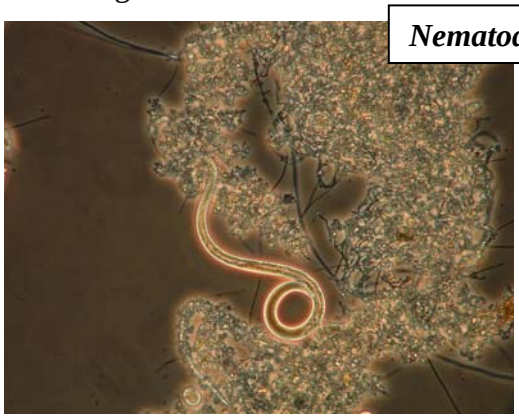
Rotifer

- Rotifers
- Nematodes
- Tartigrades (water bear)
- Annelids
- Ostracods (Daphnia)
- Copepods (water flies and mites)

Rotifers

Rotifers are commonly found in wastewater treatment systems play a principle role in the activated sludge treatment but they should never be dominant in the system. They do an excellent job of polishing off and removing any remaining material in the water. They also secrete a sticky substance that helps the floc to remain firm and clumped together. Rotifers come in a variety of sizes and shapes and as male and female. Most rotifers in the treatment system are female since the male's only purpose for

existence is to fertilize the female and die. Rotifers are good indicators of wastewater toxicity. They are usually the first to be impacted by a toxic load.

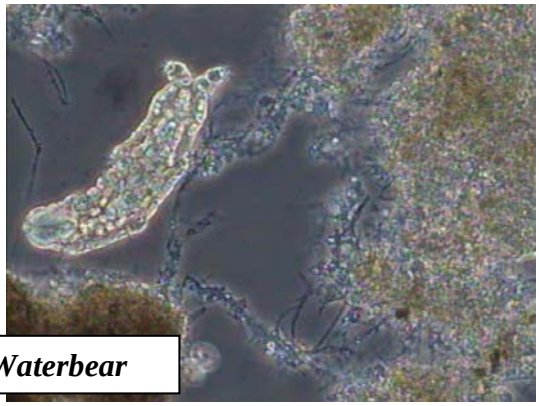

Nematode

Nematodes

Nematodes may be seen in significant amounts in longer aged systems or older sludges. They do not contribute to the overall treatment and feed on bacteria, protozoa, fungus and sometimes they eat other nematodes. Some nematodes have teeth and some have a spear they can stick into their prey and then use the spear to suck in food like a straw.

Tartigrade (Water Bear)

Water bears are commonly found in the same environment with rotifers and nematodes. They feed on algae and small protozoa. The interesting thing about them is they have developed ways to survive extreme environmental swings. If there is not enough oxygen, they swell up like a balloon and float around for a few days. When the environment dries up, they shrivel up like a raisin. They are however, very sensitive to toxic conditions. Their presence usually indicates that there is little to no ammonia present.


Waterbear

Water bears have five body segments and four pairs of short stumpy legs with claws and can be seen in different colors; red, orange or even green. They have a head with eyes and a mouth that they use to pierce their food before sucking out the inner parts.

PROCESS CONTROL

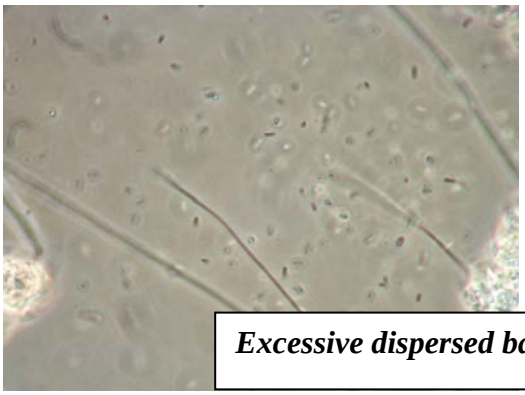
The majority of wastewater treatment operators have multiple responsibilities, many of which do not pertain to the treatment plant. Many are responsible for drinking water, mowing the grass, plowing the snow and you name it. There is not a lot of time available to perform complicated microbiological assessments of the activated sludge. The good news is that there are some quick and simple tests that the operator can do with the microscope that will give him an indication of the condition of the treatment system.

Dispersed Growth

Dispersed growth is a population of suspended, growing, non-flocculated bacteria, algae or fungi (most is bacteria). In a properly operating system little dispersed growth should be present in the liquid around the floc. Dispersed bacteria are removed from the liquid as bacteria develop the “slime” layer and clump together to form floc. They are also removed by ciliates and rotifers. The presence of significant dispersed bacteria is

due to improper floc formation. There are several reasons why floc does not form properly.

- Young sludge – In younger sludges there is a lot of food still available. Bacteria are swimming and multiplying and are not forming floc.
- Toxicity/Lack of ciliates – Low levels of toxicity my kill most of the ciliates whose primary role is to remove the free-swimming dispersed bacteria.
- Slug discharge – A slug discharge brings in a load of food, which in turn causes bacteria to swim around and multiply
- Excessive shearing and /or surfactants – Excessive shearing will break up the floc and surfactants prevents the "stickiness" of the bacteria and prevents them from clumping together to form floc.

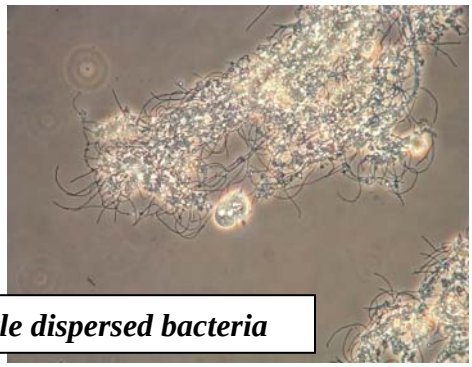


Excessive dispersed bacteria

<u>How Do You Rate Dispersed Growth?</u>

Place a drop of mixed liquor on a slide and cover with a cover slip. Observe the sample under oil using the 100X ocular lens and phase contrast if you have it. Look for small moving, swimming or wiggling bacteria in the surrounding fluid.

There is an *excessive* amount of dispersed cells in the sample if you can estimate 100s of dispersed cells per field of view at 100x magnification. There is a s***ignificant*** amount of dispersed cells if you can estimate - 10s of dispersed cells per field of view at 100x magnification. It is i***nsignificant*** if there are less than 20 dispersed cells per field of view.



Very little dispersed bacteria

Under normal operating conditions the fluid surrounding the floc should be relatively clear.

Protozoa Count

As the environmental in the aeration basin changes one type of microorganism is replaced by another. The microorganism best suited for the environment will emerge until the environment changes again. Changes in pH, dissolved oxygen, temperature, nutrients, competition etc., all determine which species will dominate. The protozoan species that are most dominant in the treatment system indicate which conditions are most dominant. Although, protozoan species dominance should not be relied on solely, to troubleshoot wastewater treatment conditions, this information is very helpful in assessing the conditions of the activated sludge process.

This protozoan count procedure is not designed to determine the total number of each type of protozoan that is present in the system. Instead, what is important is the

relative numbers of one type in comparison to another type. In other words, the purpose it to determine which species seems to be dominating. The count will examine protozoa in the following categories:

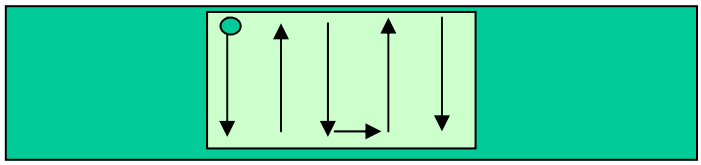
- Amoebae
- Flagellates
- Free-swimming ciliates
- Crawling ciliates
- Stalked ciliates
- Metazoa (rotifers, nematodes, water bear etc.)

In a well-operating system, the ciliates will most likely be the dominant species. Ciliates dominate when most of the nutrients have been removed from the wastewater. In a poorly operated system, amoebae and flagellates will probably dominate. Amoebae and flagellates can only compete for dominance when there are still plenty of nutrients remaining. On the other hand stalked ciliates and metazoa will be predominant in longer age systems mainly because of their ability to compete when very little nutrients are left and their ability to feed on other protozoa.

<u>The Procedure</u>

Collect a fresh, well-mixed, representative sample of mixed liquor from the discharge end of the aeration basin. If the concentration of the mixed liquor is quite high, the sample should be diluted. If you choose to dilute the sample, always use the same dilution if you plan to do routine counts. Ideally, the sample should be observed under the 20X objective using the phase contrast condenser. Using a smaller objective (10X or below) will make it difficult to see the smaller flagellates and using a larger objective (40X or higher) will narrow your field of view.

1. Place one drop of mixed liquor on a clean grease-free slide and cover with a cover slip.
2. Scan the slide using 5 passes. Focus the objective at the top left corner of the cover slip. Moving down the cover slip, count and record the number of each type of protozoa that you see. This completes one pass. Move over to the right slightly and this time move up the cover slip keeping a running total the number of each type of protozoa. This will complete the second pass. Move up and down the slide until you have completed 5 passes.



Record the running total number of each type of. Remember, if you encounter colonies of stalked ciliates you must count (or estimate) the total number of heads. The motto is, “The more heads, the older the sludge”. For the best results, scan 3 or more slides and average the number of each protozoan type.

3. Calculate relative dominance
    a. Count and record the total number of protozoa in each category. Record the counts from all three slides (See example Count Worksheet).
    b. Next, calculate the average number of each organism from all three slides.
    c. Calculate the percentage by dividing the average number of each organism by the total number of microorganisms counted and multiply by 100.
    d. Group amoebae and flagellates together and combine the percentage. Group all the ciliates together and combine the percentage and calculate the percentage of Metazoa. (See Appendix A – for a blank Protozoan Count Worksheet)

Example Count Worksheet

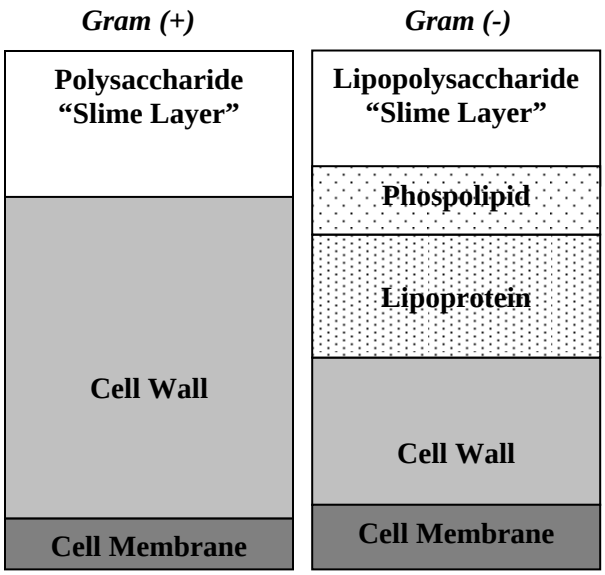
ORGANISM	**Slide #1**	**Slide #2**	**Slide #3**	**Average**	**Percent**	
Amoebae	6	3	1	3	4%	***14.5%***
Flagellate	13	6	6	8	10.5%	
Free-swimming Ciliates	15	12	7	11	14.5%	***81.6%***
Crawling ciliates	22	15	20	19	25%	
Stalked ciliates	40	32	23	32	42%	
Metazoa (Rotifers, nematodes etc.)	5	1	2	3	4%	***3.9%***
Total	**101**	**69**	**59**	**76**		

Amoebae and flagellates are grouped together because they are both indicators of young sludge or incomplete treatment. In a well-operated system, ciliates should dominate. Higher numbers of metazoa are associated with longer sludge ages.

Protozoan counts are not exact science and should not be relied on solely, for determining treatment system conditions. They are helpful however, in helping the operator to assess conditions within the aeration basin.

Slime Bulking

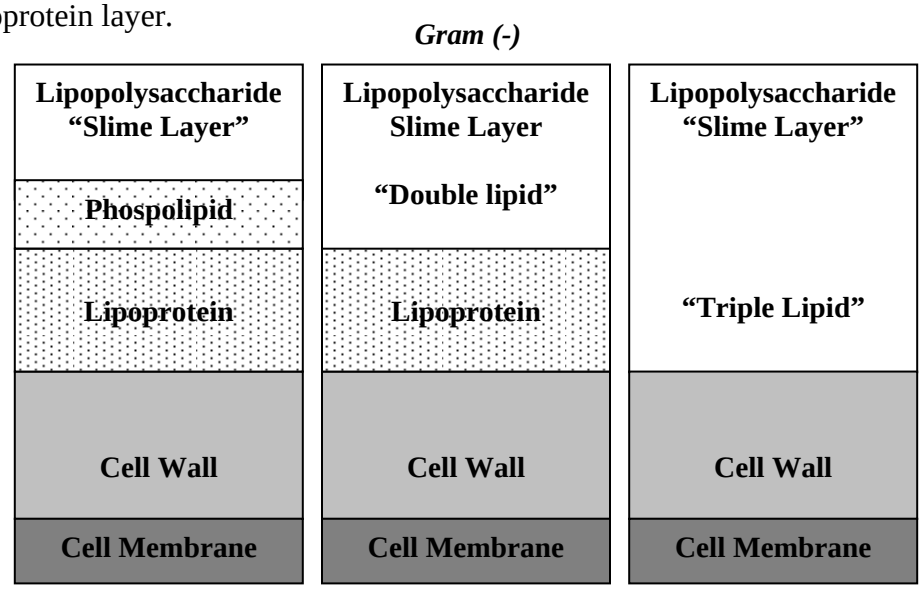
Slime bulking occurs when bacteria produce an excess of a substance called exocellular lipopolysaccharide, better known as “slime”. This occurs primarily in floc-forming, Gram-negative bacteria and is a function of the structure of the cell wall.



The cell wall on Gram-positive bacteria is made up of three main components: A lipopolysaccharide layer, the cell wall and the cell membrane. But, Gram-negative bacteria have five primary components: A lipopolysaccharide layer, a phospholipid layer, a lipoprotein layer, the cell wall, and the cell membrane. The lipopolysaccharide layer on Gram-negative bacteria is made up of a gelatinous, polymer-like substance that resists the purple dye of the Gram stain, hence the reason why it is Gram-negative.

Three main elements are required in order for the Gram-negative bacteria to develop its cell wall properly: 1) nitrogen, 2) phosphorus and 3) sulfur. If any of these elements are lacking the cell wall does not develop properly. For example, phosphorus is required in the development of the phospholipid layer, and nitrogen is required in the development of the lipoprotein layer.

Let's say for instance, there is a deficiency of phosphorus. The phospholipid layer will not develop properly. Instead of a phospholipid layer you will have a layer that is made up of predominantly lipid or "fat". So, now the cell wall has its lipopolysaccharide layer (which is already fat and slimy), along with an additional layer of fat, which I call "double lipid". If you add to that a deficiency of nitrogen, the cell will end up with a significant addition to the layers of fat (I call it "triple-lipo". This extra thick layer of fat and slime interferes with the development of floc. Instead of the bacteria cells compacting closely together by a thin layer of slime, they are now spaced farther apart by a much thicker layer of fat. This extracellular lipopolysaccharide is resistant to water and cause sludge bulking and foaming. The extra slime layer may also interfere with the cell's ability to remove nutrients from the water because it becomes difficult for them to penetrate through the slime layer to enter the cell wall.



Often in industrial treatment systems and sometimes in municipal systems nutrient deficiency may occur. This occurs when influent wastewater is deficient in nitrogen or

phosphorus. Bacteria generally require 10 mg/L of nitrogen and 1 mg/L of phosphorus for every 100 mg/L of BOD that it consumes; a nutrient ratio 100:10:1 (BOD: N: P). Slime bulking can result when there is a lack of sufficient nutrients for the bacteria. Bacteria will also produce an excess amount of slime in the presence of significant amounts of organic acids. These organic acids, usually produced under anaerobic conditions, can be recycled into the plant when anaerobic digester supernatant is slug dosed into the recycle stream. If this is the case, supernatant should be introduced into the system in smaller doses over a longer period of time. In some cases, when sludge is held too long in the primary clarifier, organic acids produced in the sludge can enter the aeration basin through the primary effluent.

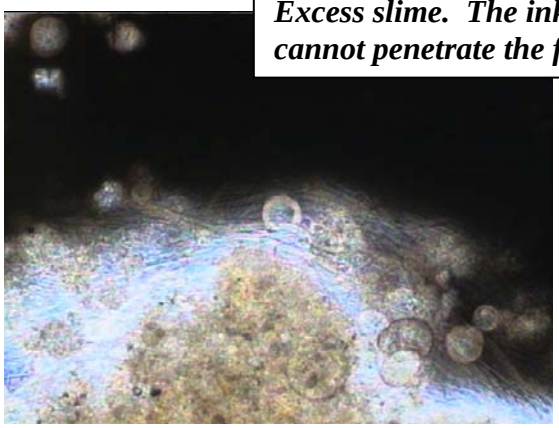
If a significant amount of this "slime" is present in the mixed liquor, you can detect its presence even without a microscope. The sludge will have a slimy consistency, as if it had been mixed with polymer. Otherwise, a simple procedure called the India Ink Test can be used to detect the presence of excess slime in the mixed liquor.

Very little slime. The ink penetrates the floc.

India Ink Test

1. Place a small drop of mixed liquor on a clean, grease-free slide.
2. Add a small drop of water-soluble India ink. This can be purchased at an art supply store. Cover with a cover slip. Be sure to remove excess fluid from around the slide by touching a piece of paper towel to the edge of the slide to draw off the excess.
3. Place a drop of immersion oil directly on top of the cover slip and view the slide using the 100X oil immersion lens.
4. Observe the sample using brightfield with the maximum light possible. Do not use the phase contrast condenser.

When India ink is added to a drop of mixed liquor, the carbon black particles from the ink penetrate the floc from outside to the inside. The slime surrounding the bacterial cells prevents the particles from penetrating the floc. The carbon black particles cannot penetrate through this slimy, polymer-like substance. If the slime is not present, the floc will appear black because the ink has penetrated it. If the slime is present the floc will appear white.



Excess slime. The ink cannot penetrate the floc.

<u>Operational Considerations</u>

The solution involves adding the deficient nutrient. If nitrogen is deficient, ammonia can be added prior to the aeration basin. Phosphoric acid can be added to

provide phosphorus. The excess lipopolysaccharide can be wasted out of the system or eaten by the bacteria but you have to stop the bacteria from producing the excess amounts by making sure they have sufficient nutrients. The BOD;N;P ration should be measured in the influent to the aeration basin. If you have a primary clarifier prior to aeration measure the ratio in the primary effluent.

Toxicity

The protozoa and metazoa are the first to be impacted whenever a toxic substance enters the treatment plant. If you examine a fresh sample of mixed liquor and you see a bunch of dead rotifers and inactive stalked ciliates, more than likely something toxic has entered the plant. Loss of BOD removal and an unusually low oxygen use are also indications of toxicity (dead microorganisms do not remove BOD). Often time you will see a bloom of flagellates. They love to feed on the fluids of dead microorganisms. Another indication is filamentous bulking upon recovery. Filamentous bacteria recover quicker that floc forming bacteria.

In the world of wastewater treatment process control, there are no absolutes. Wastewater is not homogeneous. There is no way to predict the amount of each substance entering the treatment system. The system is impacted by temperature, the availability of oxygen, pH, microbial competition and a myriad of other influences. There is no way to predict that if a F/M ratio of 0.3 is good for one treatment plant, it will work well in another. Therefore, it is important for the operator to get to know what works well for his own treatment system. The operator should keep a process chart and record information on plant processes when the system is running well. Once the operator is familiar with the process control parameters that are best suited for his treatment plant, he will have a baseline upon which to base any changes to his system performance. As he begins to notice changes in his plant performance, he will be able to review the information to see where any significant changes occurred. Changes in one parameter can affect others. The important thing is to get to know your own treatment plant.