FILAMENTIOUS BACTERIA IDENTIFICATION & PROCESS CONTROL
A Simple Approach

INTRODUCTION

Under normal conditions in activated sludge, bacteria occur singly, in small chains or clumps. Under adverse conditions however, bacteria that grow in filaments begin to form longer chains called filamentous bacteria or "filaments". Filaments can dominate in the wastewater treatment system under a variety of conditions. These conditions are usually less favorable for the floc-forming bacteria so, this allows the filaments to gain an advantage.

The presence of some filaments in the activated sludge is advantageous. They aid in settling by providing a "back-bone" for floc-forming bacteria to attach to. However, when filaments begin to grow in excess amounts, extending from the floc into the bulk fluid, they can interfere with settling and my cause foaming upon aeration. Different types of filaments dominate under different conditions. Identifying which filaments are dominating in the system will help the operator to understand the condition in the treatment system so that corrective changes can be made.

Microscopic evaluations to identify filamentous bacteria can be complicated and time consuming. This lesson will provide a simple approach to identifying filaments in the activated sludge as well as provide suggestions for corrections.

Microscope Requirements

Objectives

The objectives on the microscope are used to magnify the specimen. In order to begin observing filaments under the microscope, it would be helpful to have a 10X (magnifies the specimen 100 times) or 20X (magnifies the specimen 200 times) objective along with a 40X (400 times) and an oil immersion objective. The oil immersion objective must be used with a drop of immersion oil and magnifies the specimen 1000 times.

Phase contrast Microscope

The normal, compound microscope is equipped with a condenser that focuses the light beam onto the specimen. This produces a brightfield illumination. Most microorganisms are relatively clear and since the fluid is also clear, under brightfield illumination it is difficult to see 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 is especially useful when observing filamentous bacteria. There are a variety of characteristics that are unique to these bacteria and are important when

trying to identify them. These characteristics can be clearly seen using the phase contrast condenser.

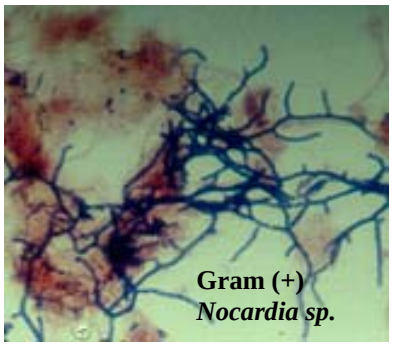
Slide Preparation & Staining

In order to observe the unique characteristics of the different types of filamentous bacteria, it is important to use a wet mount. Using a wet mount, a drop of the sample is placed on a clean dry slide and is covered with a cover slip. This allows the observer to view the microorganisms live in their environment. With a live sample, measurements can be taken and cell shape and size can be accurately determined.

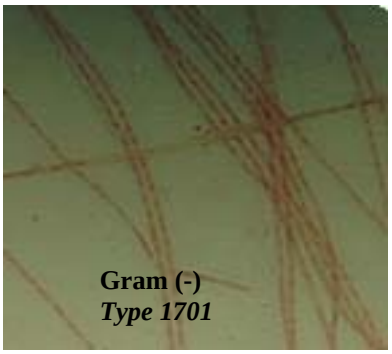
Smears on the other hand, are not useful for determining cell size and shape. To make a smear, a drop of sample is placed on a clean slide and is smeared across the slide and allowed to air dry. The drying process distorts the size and shape of many of the cells. Smears are useful for staining only and are particularly useful when identifying filamentous bacteria.

The most commonly used staining techniques are simple staining and differential staining. The simple stain uses only one stain, which dyes all the microorganisms, the same color. This stain is used to simply make the microorganism more visible for observation. Differential staining uses more than one dye which reacts differently with different types of microorganisms. It helps to distinguish one type of microorganism from another. The differential staining technique is the one more often used in the wastewater treatment lab.

There are several stains that are often used when identifying filamentous bacteria (See Appendix A for *Staining Procedures*). The most common are the Gram Stain and the Neisser Stain.



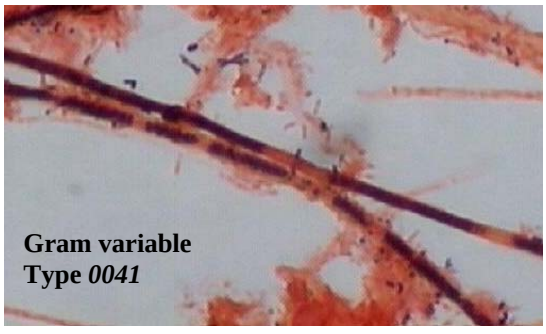
Gram (+)
Nocardia sp.



Gram (-)
Type 1701

Gram Stain

The Gram stain separates bacteria into two categories: gram (+), those that retain the purple color of the crystal violet and gram (-), those that do not retain the crystal violet when decolorized but is counter stained pink. The procedure uses four solutions; a crystal violet solution, an iodine solution, alcohol for decoloration and a counter stain safranin (Detailed staining procedures are listed in Appendix A).

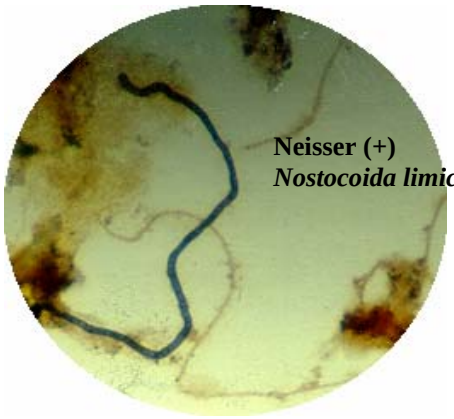


Gram variable
Type *0041*

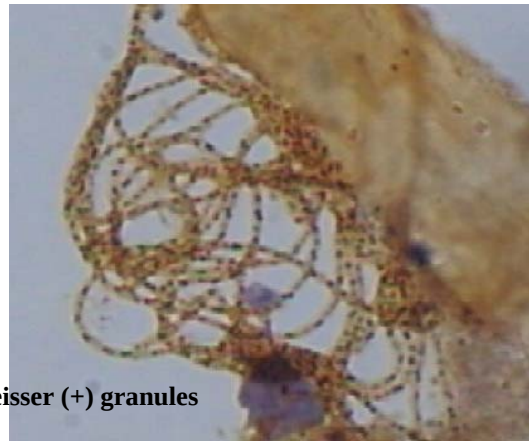
Only a few filaments stain gram (+), most of the filamentous bacteria found in activated sludge are gram (-). There are a few however, that stain "gram-variable". This means that a portion of the filament stains gram (+) while another portion of the same filament stains gram (-).

Neisser Stain

The Neisser stain also separates the bacteria into two categories. It distinguishes those bacteria that have the ability to accumulate polyphosphate. Those that accumulate polyphosphate are Neisser (+) and stain bluish purple. Neisser (-) bacteria stain brown. Some bacteria however do store granules that contain polyphosphate. In this case, Neisser (+) granules can be seen inside of the filament (Details staining procedures are listed in Appendix A).



Neisser (+)
Nostocoida limicola



Neisser (+) granules

As with the Gram stain, only a few filaments stain Neisser (+). The majority of the filaments in wastewater stain Neisser (-). There are a few however, that contain Neisser (+) polyphosphate granules.

TYPICAL OBSERVATION OF FILAMENTOUS BACTERIA

The typical process for identifying filamentous bacteria can be time consuming and difficult. Most wastewater treatment plant operators are charged with the responsibility of operating, maintaining, and cleaning the plant, along with performing all of the laboratory tests for process control and discharge permit compliance. Many are also responsible for drinking water, city lawn mowing and snow removal. It is not fair to add the typical, tedious task of identifying filamentous bacteria to their already overloaded work schedule.

In the past many keys or charts have been developed to help the operator to identify filamentous bacteria. In order to use the charts however, the operator must first gather information about the filament and then using this information, follow the chart to identify the probable filament. The time consuming part is gathering the information. To use the charts the operator would be required to measure the length and width of the filament, the length and width of the individual cells, record the shape, along with many other characteristics.

This course is an attempt to simplify the process, so that the operator or lab personnel with a little time to spare can identify these filaments with relative ease. Before discussing this simple approach, let's learn a little more about the different characteristics of filamentous bacteria.

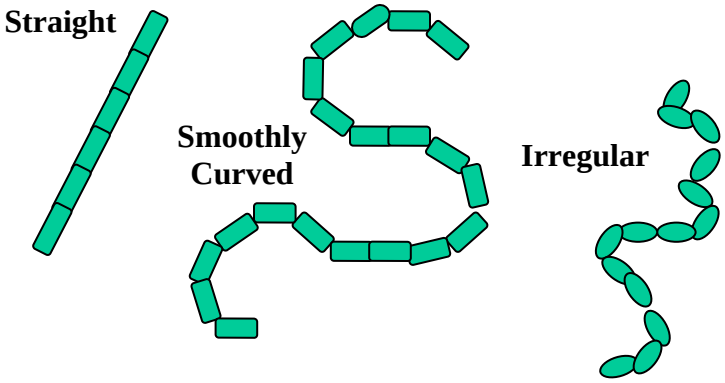
Filament Structure

In the activated sludge treatment system, bacteria may occur singly, or in small chains or clumps. Shifts in the activated sludge environment such as changes in pH, dissolved oxygen, nutrients etc. will often cause a change in the behavior of the bacteria. In stead of single cells, small chains or clumps, the bacteria will begin to form longer

"chains" which develop into filamentous bacteria. Round bacteria will form a chain with other round bacteria, square with square, rectangle with rectangle etc. I jokingly call these "chain gangs". These longer chains or "filaments" allow the bacteria to compete better in the changing environment.

Filament Shape & Size

Filament shape is one of the characteristics often used to help identify filamentous bacteria. Some filaments are smoothly curved, some are straight and others are simply irregularly shaped. Filaments can range in size from 0.8 to 5 μm in width and from 5 to > 500 μm in length.



Cell Shape & Size

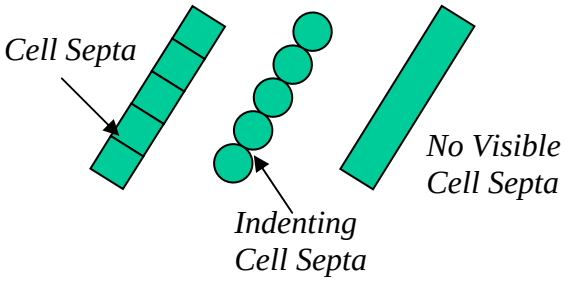
The individual cells that make up the filament can be either bacillus shaped which includes square, rectangle or barrel-shaped cells or coccus – shaped which includes round, oval, or rod-shaped cells. There is also a wide variation in the size of the individual cells that make up the different filaments.

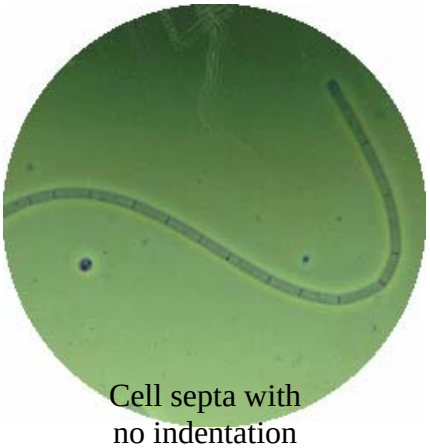
Another characteristic that is often listed on many filament identification charts is the presence of visible cell "septa". In most cases filaments are made up of individual cells stacked together (a few filaments are made up of one long cell). Cell septa can be defined as the visible line separating each individual cell. In some filaments the cell septa can be clearly seen while in others the septa is not visible at all.
These same charts will also ask if the cell septa are "indenting". This can be observed if there is an indentation at the septa as noted in the figure.

Other Characteristics

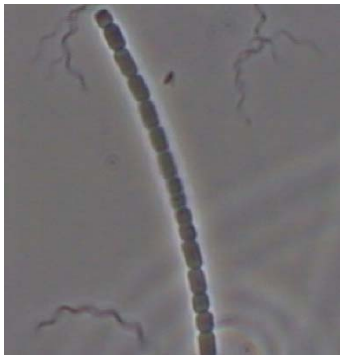
There are several other characteristics that when observed will help in the process of identifying the different types of filamentous bacteria.

- The presence or absence of a sheath
- The presence of epiphyte (attached growth)
- True or false branching
- Motility





Cell septa with
no indentation



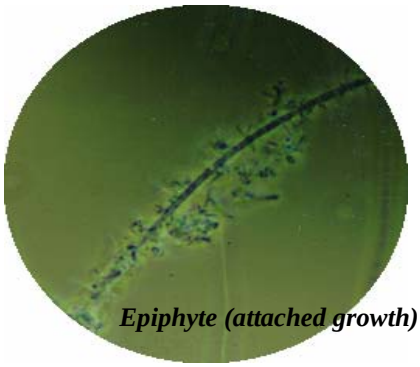
Cell septa with
indentation

Cell septa not
clearly seen

Individual cells within a sheath

The Presence or Absence of a Sheath

Some filaments are made up of cells stacked together and contained within a tight fitting sheath, much like a clear soda straw with cells stacked inside. Not all filaments have a sheath. Those that do may vary in size and shape and may have other characteristics that are not similar at all.



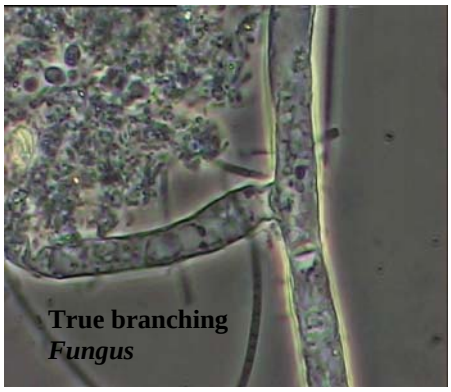
Epiphyte (attached growth)

The Presence of Epiphyte (Attached Growth)

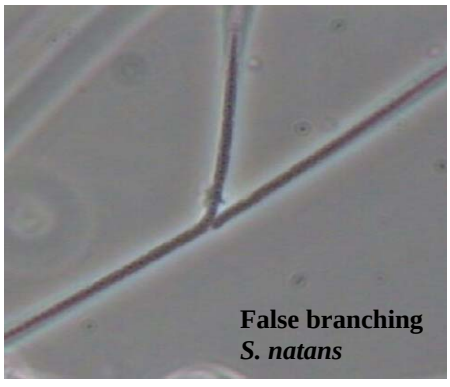
Epiphyte is a term used to describe bacteria that are attached to the sheath and growing perpendicular along a filament. This only occurs with filaments that have a sheath. However, not all sheathed filaments have attached growth.

True or False Branching

Branching is another characteristic that is unique to some filaments. Some exhibit true branching, where there is a continuum between branches while others may simply form the appearance of branching when two filaments attach to one another.



True branching
Fungus



False branching
S. natans

Motility

Motility refers to the organisms' ability to swim or move about in the water. There is only one filament commonly found in wastewater treatment systems that is motile. This characteristic makes it very easy to identify. Beggiatoa sp. is the only motile filament. It can be seen gliding slowing through the fluid.

A SIMPLE APPROACH TO IDENTIFYING FILAMENTOUS BACTERIA

Filamentous bacteria are generally always present in the activated sludge system. As a matter of fact filaments can aid in the settling of the sludge by creating a "backbone" for floc-forming bacteria to attach to. It is only when they are present in excessive amounts that they can cause operational problems. Excessive amounts of filaments extending from the floc into the bulk fluid can cause sludge to "bulk". Bulking sludge will not settle well and in severe cases sludge will flow over the clarifier weirs and into the final effluent. This can lead to BOD and suspended solids violations. Some filaments can also cause foaming upon aeration. The foam caused by filamentous bacteria can be very stable and can measure from inches to several feet thick. Many times this foam will freeze in the winter time and can even float over the weirs. Although foaming rarely cause effluent violations, it can lead to a considerable loss of solids and poor aesthetics.

The operator must first determine if the bulking or foaming is due to filamentous bacteria. Bulking and foaming will also occur under nutrient deficient conditions. To determine if the bulking is due to filamentous bacteria, take a representative sample of the mixed liquor and examine it using a wet mount. If filaments can be seen in the floc and extending into the bulk fluid, then most likely the filaments are contributing to the bulking condition. To determine if filamentous bacteria are causing the foam, take a sample of the foam and examine it using a wet mount. If filaments are causing the foaming, then they will be abundantly present in the foam.

A particular filament will dominate in the treatment system only when conditions favor their growth over another filament. For instance, some filaments are favored in low DO conditions, another in low nutrient conditions etc. In some cases, several filaments will dominate at the same time. This usually happens when the treatment plant has varying conditions or loadings that may cause the activated sludge environment to change from day to day. It is normal for some filaments to be present in the treatment system. It is only when conditions change that favor their growth over floc-formers and cause them to dominate, that they become a problem.

The simple approach involves identifying the more visible characteristics of filamentous bacteria to help in determining which filaments are causing problems in the treatment plant. The first step is to categorize the filament as either a "bulker" or a "foamer". Generally, the bulkers do not cause foaming and the foamers do not cause bulking.

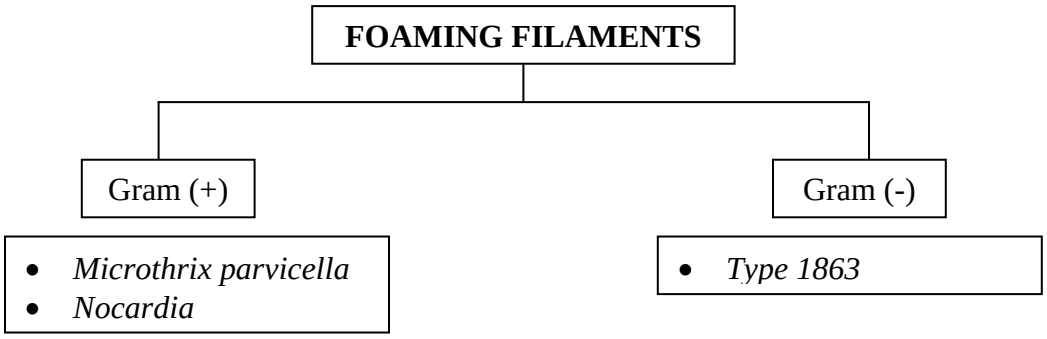
"Foamers"

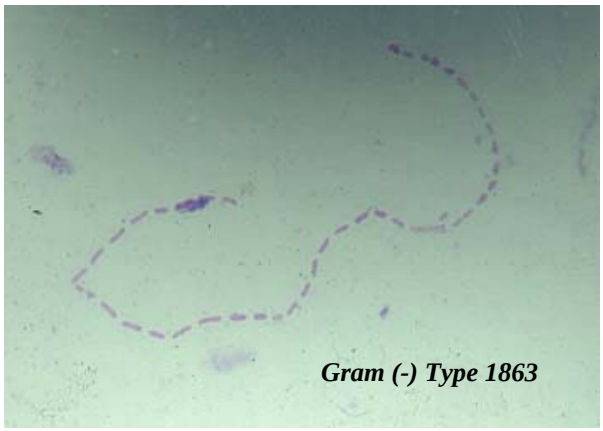
There are only three filaments that are responsible for the majority of the foaming in activated sludge treatment systems. This makes the identification process relatively simple. A simple Gram stain is all that is needed to identify these filaments.

- Type 1863
- Microthrix parvicella
- Nocardia

Identifying Foamers

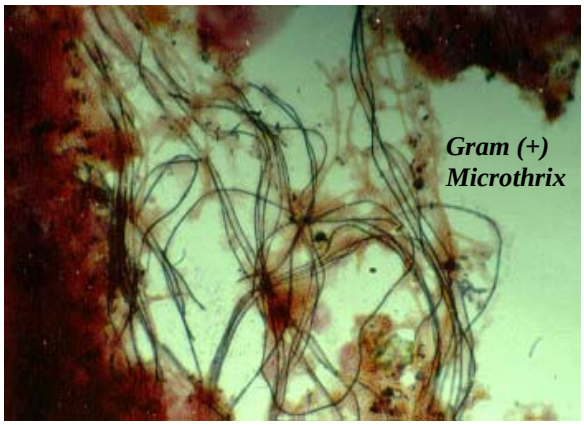
1) Grab a sample of the foam, make a smear and let it air dry.
2) Stain the smear with the Gram stain.

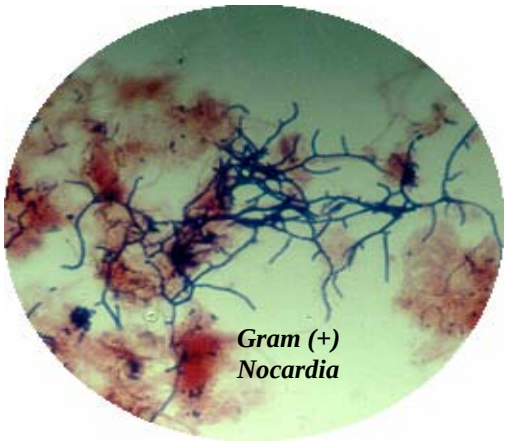




Remember, Gram (-) bacteria stain pink and Gram (+) bacteria stain purple. Type 1863 is the only foaming filament that is Gram (-). It is very easy to identify because it simply looks like a pink dashed line. So, if the treatment system is foaming, grab a sample, make a smear and do a Gram stain. If you see a pink dashed line, its type 1863.

Microthrix parvicella and Nocardia are both Gram (+) but are structurally very different from one another. This makes it very easy to distinguish one from the other. Microthrix is very thin and smoothly curved. When stained, it looks like purple spaghetti. Nocardia on the other hand, is a short-branched filament and resembles a purple patch of branches.





All foaming filaments have "hydrophobic" or water resistant cell walls. This gives them the ability to float upon aeration. They have a few other things in common. Each thrives in wastewater high in greases, oils and fats under low F/M conditions. In other words, greases, oils, fats and longer sludge ages give these filaments the advantage over floc-forming bacteria. Although they have a lot in common, there are conditions that will give one the advantage over the other.

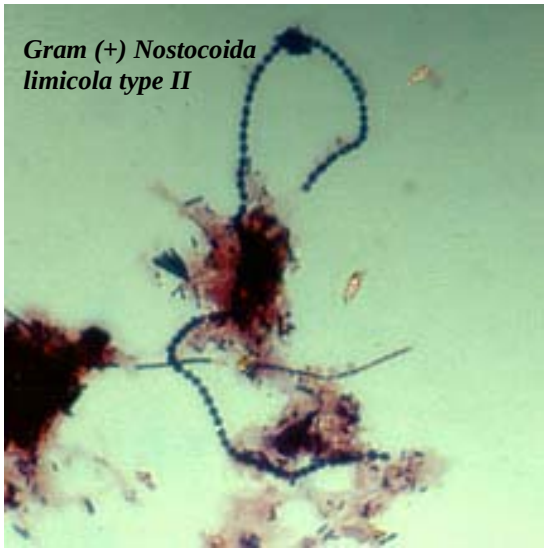
Filament type 1863 is favored when there is a decline in the aeration basin pH. Microthrix is favored in colder temperatures and generally prefers animal and vegetable greases, oils and fats. Nocardia on the other hand is favored in warmer temperatures and longer sludge ages.

Filament Type	***Common Conditions***	***Unique Conditions***
Type 1863	Greases, oils & fats Low F/M	A decline in aeration basin pH
Microthrix parvicella	Greases, oils & fats Low F/M	Colder temperatures & animal & vegetables greases oils and fats
Nocardia	Greases, oils & fats Low F/M	Warmer temperatures Longer sludge age

Identifying filaments that cause foam is fairly simple. There are only three and they have very different structural characteristics (a pink dashed line, purple spaghetti or purple branches).

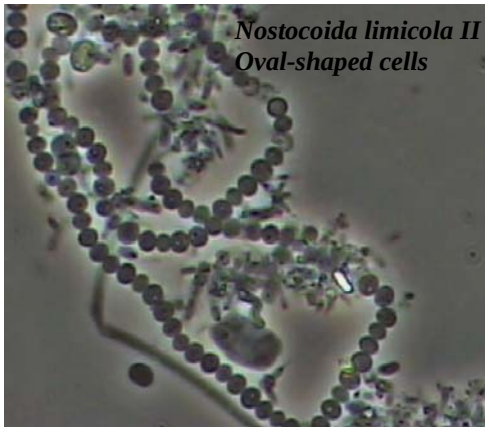
"Bulkers"

Identifying bulking filaments is not as simple. The majority of filaments causing problems in the activated sludge system cause bulking. Using stains to identify bulking filaments is not as helpful as it is with foaming filaments unless you are lucky and you happen to have the only Gram (+) bulking filament, Nostocoida limicola or one of the only two Neisser (+) filaments, Nostocoida limicola or type 0092. The majority of bulking filaments are Gram (-) and Neisser (-). Just in case you are lucky, let's look at the few Gram (+) and Neisser (+) bulking filaments first.



Gram (+) Nostocoida limicola type II

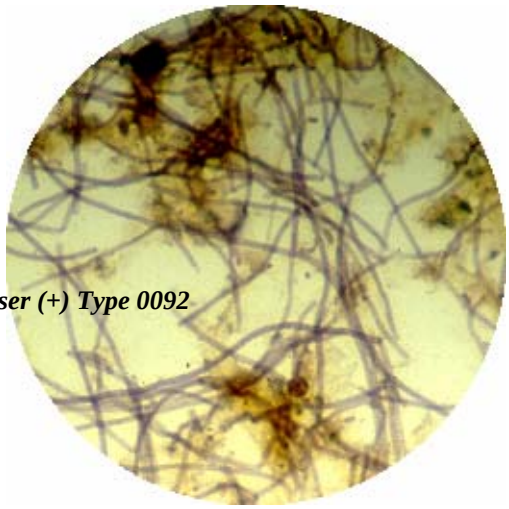
There is only one bulking filament that stains strongly Gram (+). ***Nostocoida limicola*** is very easy to identify. When Gram stained it resembles a "purple beaded necklace". It is also very easy to identify without staining using a wet mount because of it unique oval cells. There are three Nostocoida filament types; type I, type II and type III. Type II and III are the most common in wastewater systems. They both have oval cells. However type III is larger than type II. Distinguishing between the three of them



really does not matter because they generally thrive under the same conditions.

This filament is more often seen in industrial treatment systems. However it is still commonly seen in municipal systems. This filament is favored under low F/M and low nutrient conditions and also in the presence of wastes that are heavy in starch such as potato processing industries.

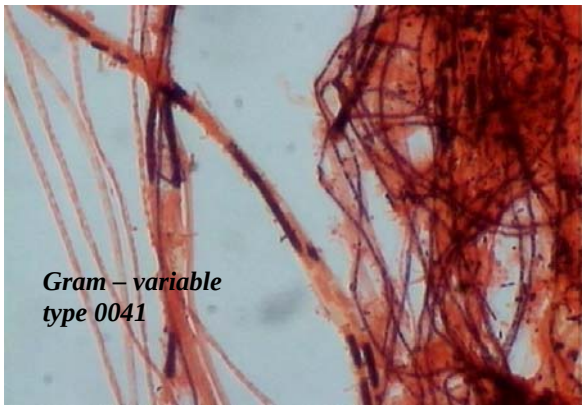
Nostocoida limicola is also Neisser (+) as well as filament type 0092. They are very easy to distinguish from one another. Type 0092 resembles short slightly curved sticks and can be difficult to identify without the Neisser stain. Filament type 0092 thrives in conditions very similar to Microthrix parvicella; colder temperatures, low F/M and oils and greases and is often present when Microthrix is present. It is often mistaken for a foamer because can become trapped in the foam caused by Microthrix.



Neisser (+) Type 0092

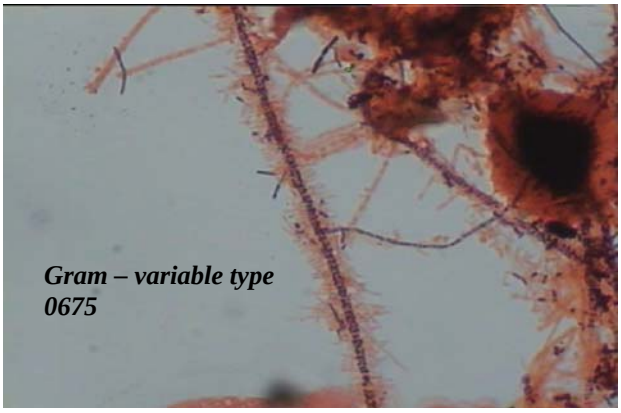
Neisser (+) *Nostocoida limicola* and Neisser (-) *Thiothrix*

Identifying filaments would be very easy if we only had to use the Gram and Neisser stain. Unfortunately, as I stated before the majority of the filaments causing bulking are Gram and Neisser (-). However, there are three filaments that are not considered to be Gram (+) or Gram (-), but that exhibit characteristics of both. These filaments stain Gram – variable.



Gram – variable type 0041

Gram – variable type 1851



Gram – variable type 0675

Filament type 0041, type 0675 and type 1851 all stain Gram – variable. We will discuss the conditions that cause them to dominate in the treatment system a little later in the lesson as we discuss some of the other unique characteristics of these filaments.

In most cases we will not be able to rely on the Gram or Neisser stain reaction to help us identify which filament is causing problems in the treatment system. Let's look at some of the other unique characteristics that can help simplify the identification process.

- The Presence of a Sheath
- The Presence of a Sheath with attached growth
- The Presence of Sulfur granules
- Branching
- Motility

The Presence of a Sheath

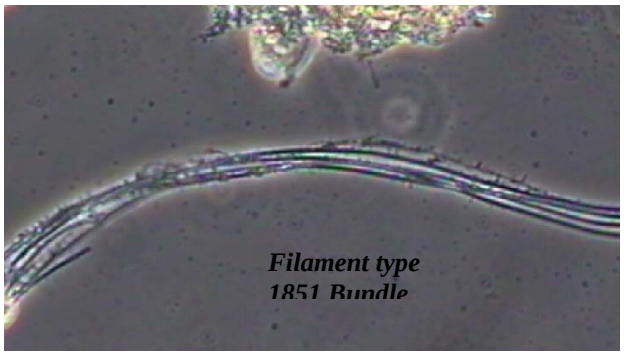
As mentioned earlier, sometimes cells are stacked inside a sheath like a chain and sometimes there is one continuous cell inside the sheath. Cells may escape leaving an empty space in the sheath. As you examine the sample, look up and down the length of the filaments looking for "missing cells" or empty spaces between cells. Of all the filaments that are commonly found in activated sludge, 7 of them have a sheath. The following filaments have a sheath:

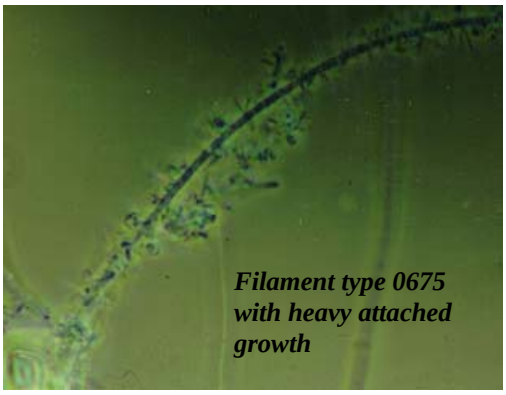
- Sphaerotilus natans
- Thiothrix
- Haliscomenobacter hydrossis
- Type 0041
- Type 0675
- Type 1701
- Type 1851

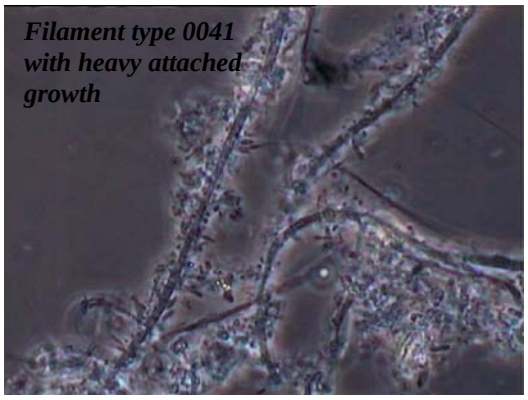
The Presence of a Sheath with Attached Growth

Some but not all sheathed filaments have attached growth. Filaments with attached growth resemble a "bottle brush". Of the most common sheathed filaments 4 of them are often seen with attached growth. The sheathed filaments that have a "type and number" name are the ones that are commonly seen with attached growth. This makes it easier to remember. It is important to note that these filaments can also grow in the treatment system without the attached growth. This usually means that conditions are such that they are able to grow quite rapidly. This also makes it a little more difficult to identify them.

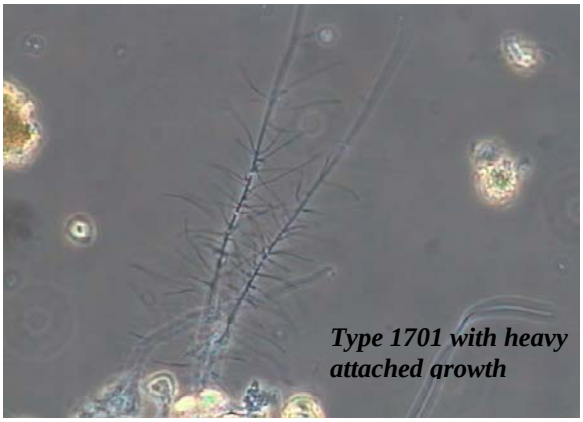
Identifying the sheath will help to narrow your options to seven but it will take additional observations to determine which of these is dominating in the treatment system. Type 1701, type 0041 and type 0675 are often seen with heavy attached growth. However, the growth is only sparsely attached to type 1851. Remember that type 1851 is Gram variable and it grows in bundles. So even if you did not Gram stain the sample, if you see a filament with sparse attached growth, growing in bundles it is probably type 1851. Types 0041 and type 0675 are also Gram variable and as described earlier, type 0041 is larger than type 0675 and they both have square-shaped cells.


Filament type 1851 Bundle


Filament type 0675 with heavy attached growth

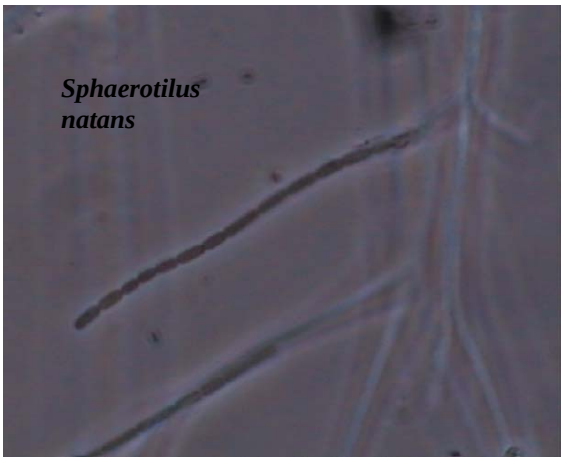

Filament type 0041 with heavy attached growth

Looking at the size and shape of the individual cells will also help to identify the filaments. Type 1701 has cells that look like round-ended rods or "link sausages". The "sausage-shaped cells are stacked in a tight fitting sheath. Type 1701 is a very thin filament. Even if it has heavy attached growth, it is easy to identify.

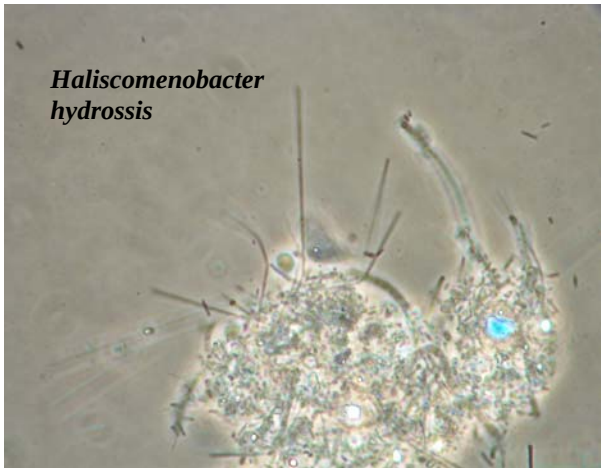

Type 1701 with heavy attached growth


Type 1701 without attached growth

Sphaerotilus natans, *Thiothrix spp.*, and *H. Hydrossis* are sheathed filaments but are generally not seen with attached growth. Nevertheless, they are very different from each other and easy to identify. S. natans also has "sausage-shaped" cells like type 1701 but it is more than twice the width of type 1701. It is also the only filament the exhibits false-branching. *Thiothrix* both type I & type II, have barrel-shaped" cells. However, type I is about twice the width of type II. Another characteristic that distinguishes *Thiothrix* from *S. natans* and *H. hydrossis* is the storage of sulfur granules within the cells.



H. hydrossis, on the other hand is a very straight, thin filament. So thin that it is very difficult to detect a sheath. *H. hydrossis* is most commonly seen protruding from the flock like thin pins in a cushion. The sheath is difficult to detect. This filament is associated with low DO, low F/M and nutrient deficient conditions.



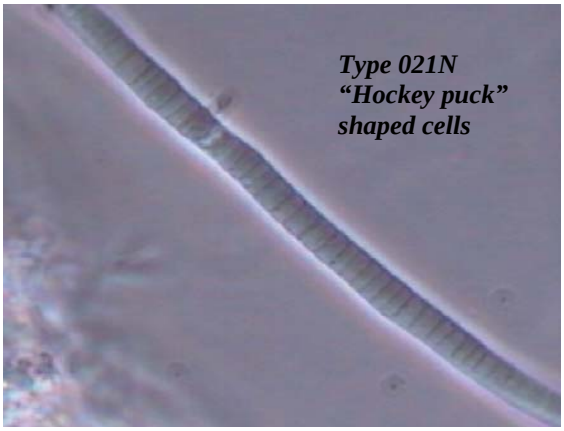
The Presence of Sulfur Granules

If the filament causing bulking at the treatment plant is Gram (-), Neisser (-) or does not have a sheath with or without attached growth, there are other unique characteristics that we can look for. Under phase contrast, look for sulfur granules within the cell. This can often be observed in systems where reduced sulfur compounds such as H_2S are present. Reduced sulfur compounds are found in septic wastewater.

There are four filament types that are commonly seen with intracellular sulfur granules.

- Type 021N
- *Thiothrix* spp.
- Type 0914
- *Beggiatoa*

Type 021N is a very unique filament. The cells are discoid shaped. They look like short fat barrels or "hockey pucks" stacked on top of each other. Sulfur granules can be seen within the cells. The cells of type 021N stain Gram (-) but display a slight reaction to the Neisser stain by turning a soft gray color.



Type 021N "Hockey puck" shaped cells

Type 021N with sulfur granules

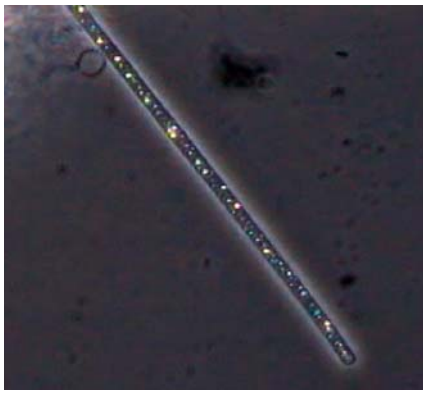
The cells of *Thiothrix* type II and I are barrel-shaped. Sulfur granules are more readily seen in the smaller type II. *Thiothrix* I is relatively large and the sulfur granules appear much smaller.

Thiothrix I with sulfur granules

Thiothrix II with sulfur granules

Type 0914 is smaller but can be easily distinguished by its rectangular shaped cells and rectangular shaped sulfur granules. *Beggiatoa* on the other hand is motile. In other words it slowly glides along in the surrounding fluid.

If the wastewater treatment system is being impacted by septic wastewater it really doesn't matter which one of the sulfur filaments are present. If a



filament is present that has sulfur granules you can contribute the cause to septic wastes. Septic wastes can enter the treatment plant when sewerage sits stagnant in long pipe lines for a long period of time. It can also occur when the system has little to no oxygen. The common causes for the growth of these filaments are:

- The presence of reduced sulfur compounds (septic wastes)
- Wastes deficient in nitrogen
- The presence excess amounts of organic acids

It is not always necessary to specifically identify the filament that is causing the problem. If you see sulfur granules, it doesn't matter which one. The same is true for some of the other filaments. I like to call these filament "couples".

Filament Couples

Filament couples are those that have similar structures and thrive under the very similar conditions. In this case it is not important to distinguish between the two. Filament type 1701 and Sphaerotilus natans are very similar in structure although there are some very unique differences. Type 1701 is often seen with attached growth and Sphaerotilus natans exhibits false branching and is much larger. Nevertheless they both have a sheath and the same shaped cells. Both have "sausage-link" shaped cells in a tight fitting sheath and thrive in low DO conditions (mainly not enough DO for the applied organic load).

Filament type 0041 and type 0675 are also very similar in structure. Both have square shaped cells in a tight fitting sheath and are often seen with heavy attached growth. The only difference is that type 0041 is slightly larger then type 0675. No need to take out the measuring grid to see which filament it is because they are often seen together and thrive under the same conditions. They are both associated with low F/M and nutrient deficient conditions.

OPERATIONAL CONSIDERATIONS

FOAMING FILAMENTS

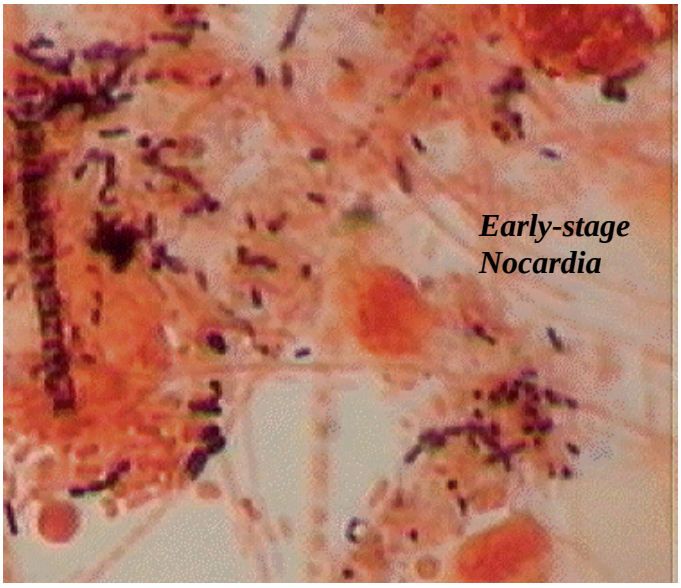
Filament Name	**Characteristics**	**Cause**
Type 1863	Gram (-), pink dashed line	Excess oils & grease; decline in aeration basin pH
Microthrix parvicella	Gram (+); looks like purple spaghetti	Animal & vegetable oils and grease; colder temperatures; low F/M
Nocardia	Gram (+) Branched	Excess oils & grease; longer sludge ages; low F/M

All foaming filaments generally thrive in wastewater that contains excess amounts of oils and greases. Microthrix is favored in colder temperatures and low F/M, type 1863 is favored at a lower than normal pH and Nocardia is favored in warmer temperatures and longer sludge ages. Influent oils and grease must be controlled. High density restaurants or any industry discharging oils and grease must be required to install grease traps and then have the collected grease removed (instead of rinsing it down with hot water and

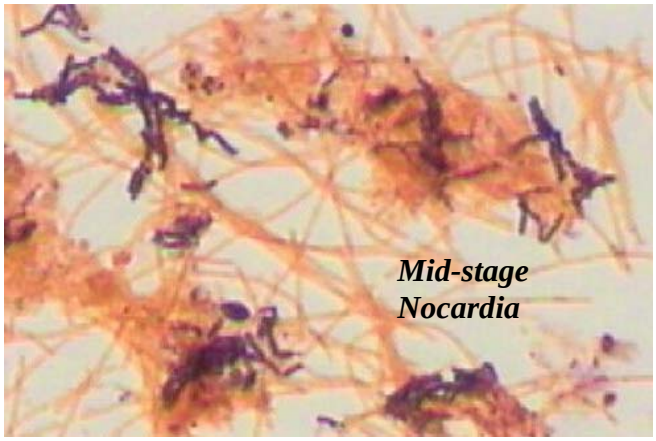
sending it to the treatment plant). To eliminate foaming from type 1863, aeration basin pH must be adjusted as well.

Control of Microthrix can be affected by raising the F/M ratio. The F/M ratio is a measure of how much "food" is available compared to the amount of microorganisms present. If there is a little bit of food and a lot of microorganisms, the F/M ration will be low. This ratio can be increased through wasting.

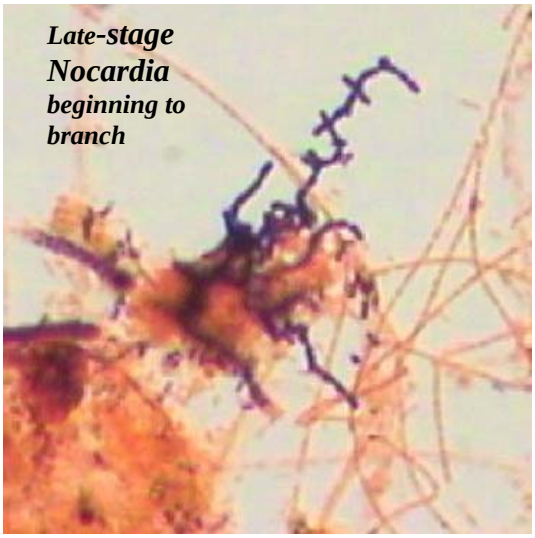
Nocardia on the other hand is more difficult to eliminate. Nocardia foam is very stable foam. This filament can continue to live and multiply in the foam. Since it is a soil bacterium, it is not affected by the UV light from the sun, nor is it affected by dry conditions. While chlorination can help control foaming from type 1863 and Microthrix it is not useful in controlling Nocardia foaming. In fact, it will make it worse. Many times operators will spray chlorine on the foam to break it up. Spraying chlorine on Nocardia foam will cause it to multiply. How?



Nocardia, in its early growth stage is harmless in the treatment system does not cause foaming. In fact in this stage, it is unrecognizable as *Nocardia*. Instead of the "classic" branched filament, it exists as small cylindrical, short, Gram-positive (purple) cells. However, as the temperature of the wastewater begins to rise, the small cells begin to form small "nodes" as the branches begin to form. In this stage, *Nocardia* still does not cause any significant foaming. Once the wastewater temperature rises above 16 C, the branches will begin to elongate at a rapid pace. It is not until *Nocardia*, fully branched, reaches this advanced growth stage that it causes foaming upon aeration. A unique characteristic of *Nocardia* is that as a part of its natural growth cycle, the branches break up into the cylindrical, short cells and the foam will begin to dissipate. The short cells will begin to grow and branch once again and the growth cycle starts all over again. The increased number of branches will create a greater amount of foam than before. The growth cycles continue with each generating more foam than before.

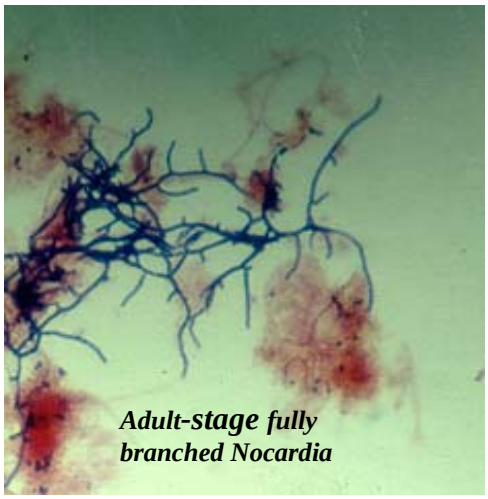


The addition of chlorine works well for most filamentous bacteria. The reason it works so well is that the filaments have a lot more surface area than the floc-forming



bacteria or other protozoa. The chlorine can have an affect on the filaments without adversely affecting the other microorganisms. But this is not so with *Nocardia*. Applying chlorine sprays to the foam will cause the *Nocardia* branches to prematurely break up into tiny cells, decreasing the surface area. So, in essence the operator will be serving as the "midwife" delivering premature babies that will eventually turn into branched filaments causing more foam. So, what should the operator do?

Floc-forming bacteria grow rapidly in the activated sludge system, peaking in number early in the process. *Nocardia* however, require a much longer time to grow from its early growth stage to fully branched filaments. In other words, the sludge age determines to a great degree whether or not *Nocardia* will remain in the early growth stage and cause no foam or if it will mature to fully branched filaments and consequently, cause foaming in the system. As long as the sludge age remains short enough, *Nocardia* will continue to exist as harmless, small cells. What about all the foam on the tanks, in the wet wells and on the clarifiers?



As long as the foam remains on the surface of the aeration basin, clarifiers and wet wells, *Nocardia* will continue its growth cycle. The foam, once accumulated, also provides a source of seed for Nocardia growth. So, the foam must be removed; sucked off or skimmed off, to prevent *Nocardia* from multiplying. Most importantly, don't put the skimmed off foam back into the head of the treatment system. It is better to land apply it or digest it. Eliminating foaming problems caused by *Nocardia* will require time, patience and work. But it can be done. By decreasing the amount of time *Nocardia* remains in the aeration basin, you prevent it from maturing into branching filaments and removing the foam from the system will prevent it from multiplying. After one or two summers, your system should be foam free!

Foaming can give the appearance that the treatment plant is doing a poor job of treating the wastewater. Although, aesthetically it looks bad, foaming generally does not interfere with good treatment unless the foaming is very severe.

BULKING FILAMENTS

Filament Name	**Characteristics**	**Cause**
Sphaerotilus natans	Sheath; round-ended rod (sausage-shaped) cells, false branching; Gram (-)	Insufficient DO for the applied organic loading
Halicomenobacter hydrossis	Sheath difficult to detect; thin straight (like pins in a pin cushion); Gram (-)	Low DO, Low F/M; nutrient deficient conditions
Thiothrix I & II	Sheath; "Barrel-shaped" cells; stores sulfur granules; Thiothrix Type I & II; Type I is twice the size as Type II; Gram (-)	Septic wastes; waste deficient in nitrogen; excess organic acids
Type 0041	Sheath; square-shaped cells; Gram-variable; attached growth	Low F/M; nutrient deficient conditions
Type 0675	Sheath; square-shaped cells; Gram-variable; attached growth; slightly smaller than type 0041	Low F/M; nutrient deficient conditions
Type1701	Sheath; thin; round-ended rod (sausage-shaped) cells, attached growth; Gram (-)	Low DO
Type 1851	Sheath; sparse attached growth; Rectangular-shaped cells; Grows in bundles	Low organic loading
Type 021N	Discoid-shaped cells; "stacked hockey pucks"; round sulfur granules; slight reaction to Neisser stain (gray color)	Septic wastes; waste deficient in nitrogen; excess organic acids
Beggiatoa	Motile; slowly gliding; stores sulfur granules	Septic wastes; waste deficient in nitrogen; excess organic acids; organic overload
Type 0914	Rectangular cells with rectangular shaped sulfur granules	Septic wastes; waste deficient in nitrogen; excess organic acids

Most Importantly

There are so many different variables that can contribute to the dominance of filamentous bacteria in the treatment system. It is important for the operator to keep a process chart to record the plant conditions when things are operating well. For instance, the operator should record the following:

- Influent load (BOD or COD)
- pH

- DO
- Sludge age
- Aeration basin F/M
- Mixed liquor suspended solids
- Temperature

If the operator sees that filamentous bacteria is beginning to dominate in the treatment system, he can look back over his data and see what change may have contributed to their growth. For instance he may notice that the DO dropped for a period of time. Every treatment plant is different so it is important to know the parameters that make the plant operate well.

APPENDIX

STAINING PROCEDURES

Gram Stain

1. Completely flood the smear with crystal violet and let stand for one minute. Gently wash the slide with water. Both Gram-negative and Gram-positive bacteria become directly stained and appear purple.
2. Completely flood the smear with Gram's iodine solution and let stand for one minute. Gently wash the slide with water and blot with absorbent paper. A crystal violet-iodine complex is formed that helps the bacteria to retain the purple color.
3. Decolorize the smear by holding the slide at an angle and applying Gram's alcohol solution, drop by drop until the violet color washes off of the slide. This generally takes about 25 seconds. Be careful not to over decolorize. Quickly rinse off the slide with water and blot with absorbent paper. The Gram-positive cells remain purple and the Gram-negative cells become colorless.
4. Flood with the counterstain safranin and let stand for one minute. Rinse well with water and blot dry. The colorless Gram-negative bacteria are stained pink by the safranin.
5. Examine under oil immersion using the 100X objective with brightfield illumination (do not use phase contrast).

Neisser Stain

Preparation.

Solution 1: Separately prepare and store the following:

A:	Methylene Blue	0.1 g
	Ethanol, 95%	5 mL
	Acetic acid, glacial	5 mL
	Distilled water	100 mL
B:	Crystal Violet	
	(10% w/v in 95% ethanol)	3.3 mL
	Ethanol, 95%	6.7 mL
	Distilled water	100 mL

Mix 2 parts by volume of A with 1 part by volume of B; prepare fresh monthly.

Solution 2:

Bismark Brown (1% w/v aqueous)	33.3 mL
Distilled water	66.7 mL

Procedure.

1. Prepare a smear and thoroughly air dry.
2. Completely cover the smear with Solution 1 for 30 seconds and rinse for 1 second with water.
3. Completely cover the smear with Solution 2 for 1 minute, rinse well with water and blot dry.
4. Examine under oil immersion using the 100X objective with brightfield illumination (do not use phase contrast).