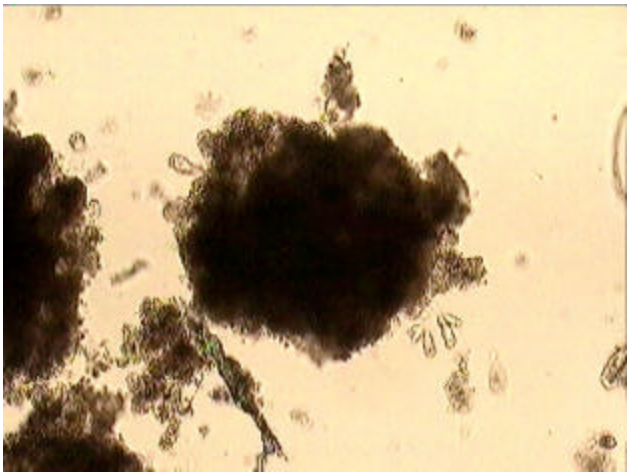
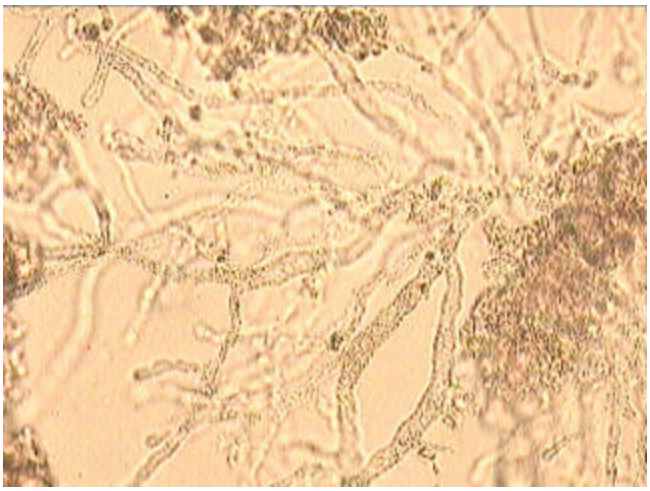
**Well, it all seems so easy when you look at them one at a time.** Unfortunately, that is not how they grow in the MLSS. Usually they are mixed together, the sample under the microscope while it appears flat to us, is actually many layers thick, especially where the floc is. One thing to do is use your stains for help, try to get the sample drop not so large when placing it on the slide. Let the slide dry out a bit. Oftentimes, I look at the higher life forms first, then the floc structures and by the time you are done fiddling around and taking photos and notes of all that, the slide is drier, which thins it out on the slide and the cover slip presses the filaments flatter into the slide, so it is a bit easier to see more of the details.

Let's pick a few different samples and look at them and see what we come up with. Remember, this is not a test and you do not get an A + for getting every single filament correct. The whole goal of this is to find out what is going wrong in the system and how you can change parts of the process to select for the right biomass and make the filaments less of a problem.

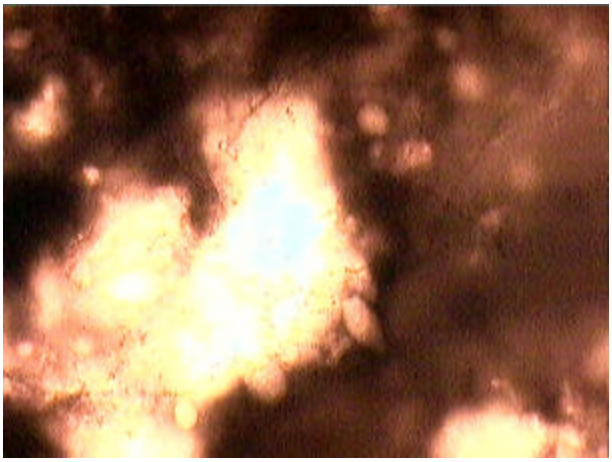
1.) Here is our first sample. This was from a **Pig farm.** There were some round, compact floc structures, some stalked ciliates present and some rotifers. They are having a hard time dewatering the sludge and it was very jelly like.
We looked closer, guess what we saw-



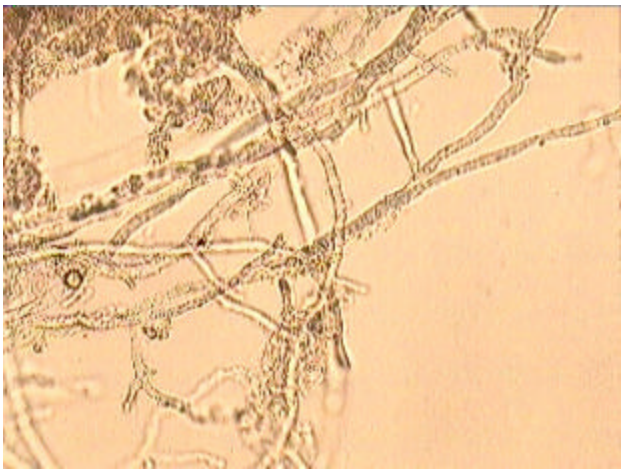
**Here is a photo of compact floc and stalks 100x Bright field**



**Here is fingered zooglea at 400x Bright field**

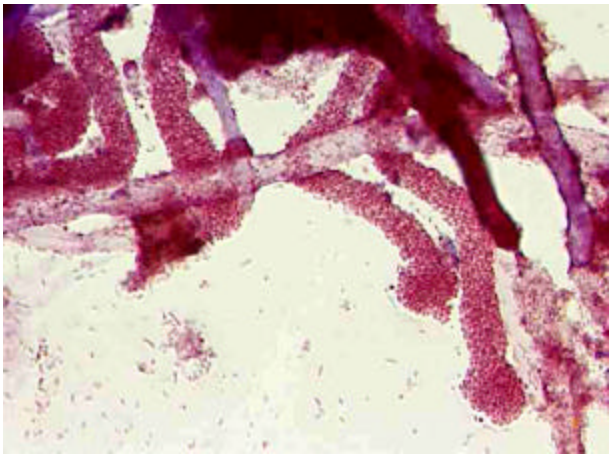


**The polysaccharide coating was very high here 100x India Ink**

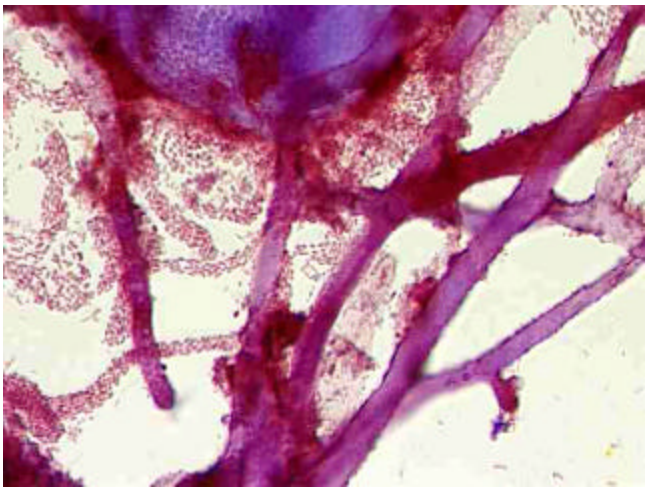


**There was a ton of Fungi 400x Bright field.**

We did perform Gram and Neisser stains.



**1000x Gram stains Fingered zooglea**

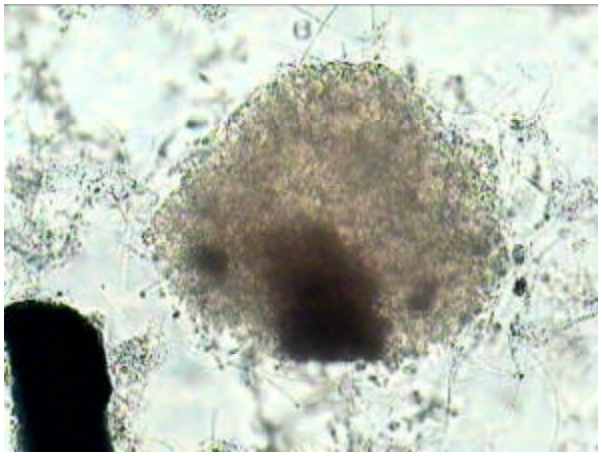


**1000x Gram stains Fingered zooglea and Fungi**

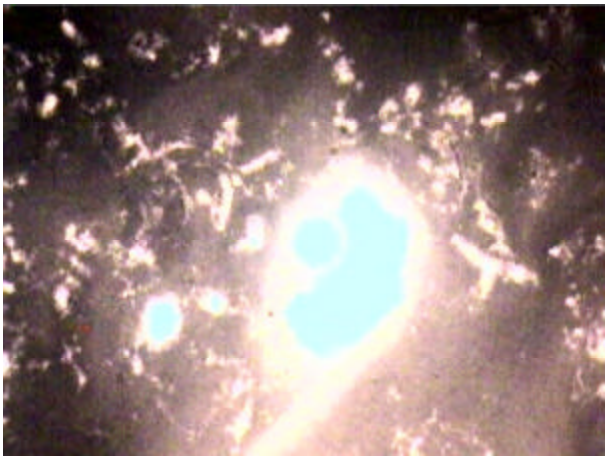
There was a ton of fingered zooglea and fungi present. We asked them if the pH was low at the plant. They adjusted pH at the plant and checked for nutrients since they were deficient at the plant slightly. The plant now has solved its problems with the help of the microscope!

2. ) Here is our next example-**a municipal plant** that had tons of foaming problems. NO this is not chocolate mousse, there were thick layers of foam on the oxidation ditch. The plant is brand new and was having problems.

We pulled a sample. There were large, dead spots in the floc structures and high spots w/ polysaccharide coating on the India ink staining also.

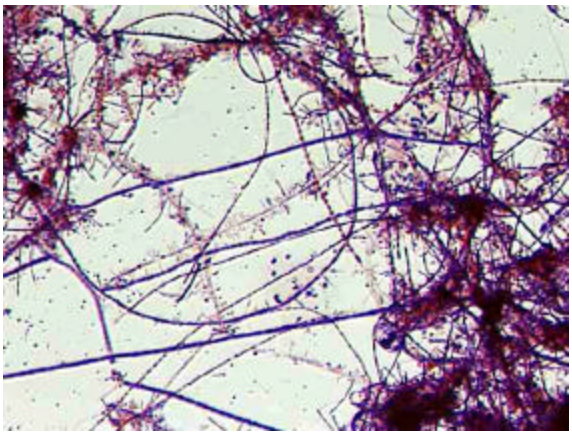


**400x Bright Field floc with black spots indication lack of $O_2$**

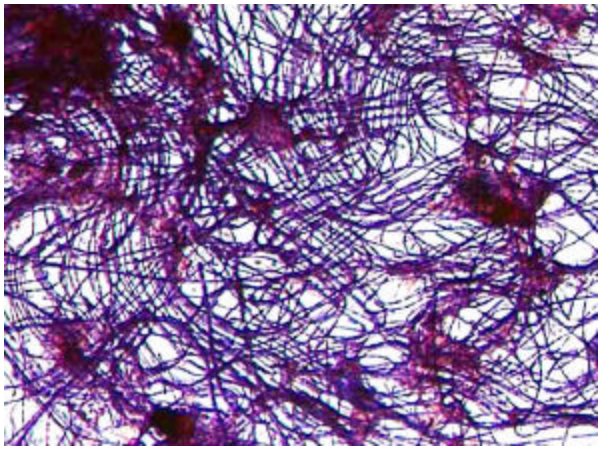


**100x India Ink high**

There are many of higher life forms, free swimmers, stalks and rotifers. There were excessive levels of filaments with bridging and bulking. Most of the floc structures were weak, open and lacy. There was some fungi also. Gram and Neisser stains were performed as well as Lactophenol Cotton Blue.



**1000x Gram Stains tons of filaments**



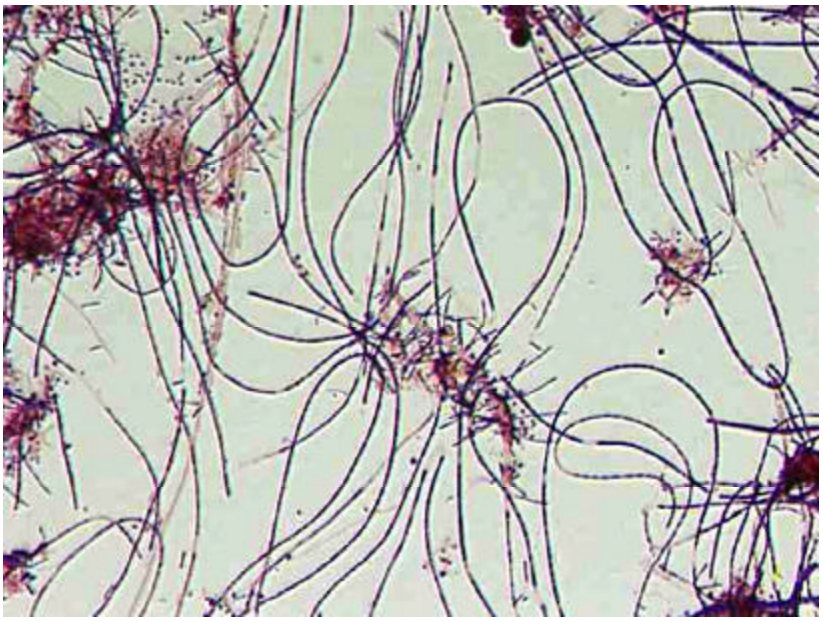
**1000x Gram Stains Excessive filaments**

It was a mess with a ton of different filaments. Lets start to look at some of them closer. Here on the right we see a ton of Type 0914. Many many bundles!

**1000x Gram Stains Type 0041 and 1851**

**1000x Gram Stains Type 0914**

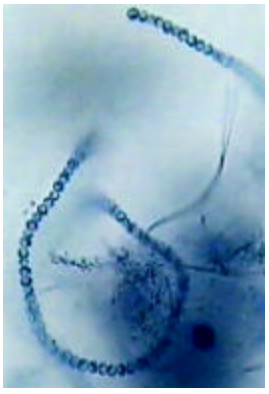
The first photo has Type 0041 with the thick heavy attached growth and the one on the left is Type 1851. Notice the sporadic growth and on opposite sides of Type 1851 and how much thinner the filament is.
The photo on the right is again small bundles of type 0914.



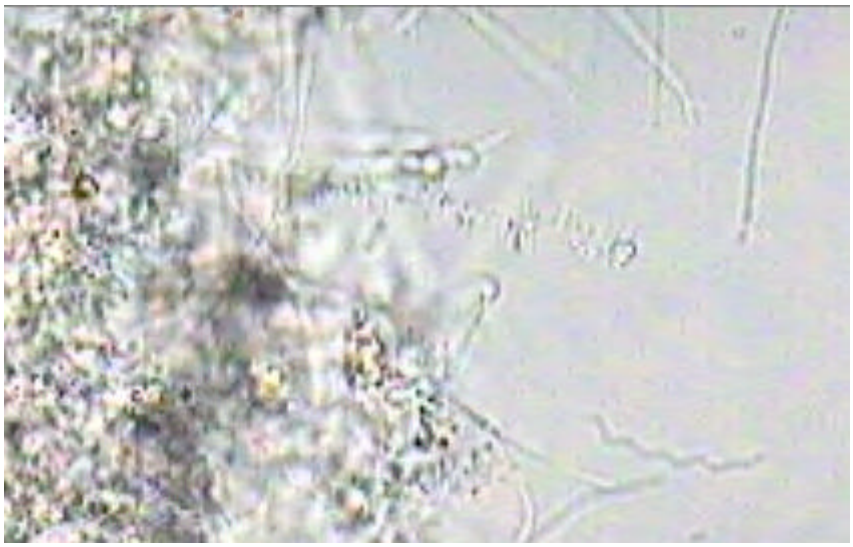
**1000x Gram Stains Microthrix parvicella**

This sample also had MicroThrix parvicella and you could see the sheath in small spots where chlorine had killed some of the cells.

1000x Lactophenol Cotton Blue N limicola III pearls

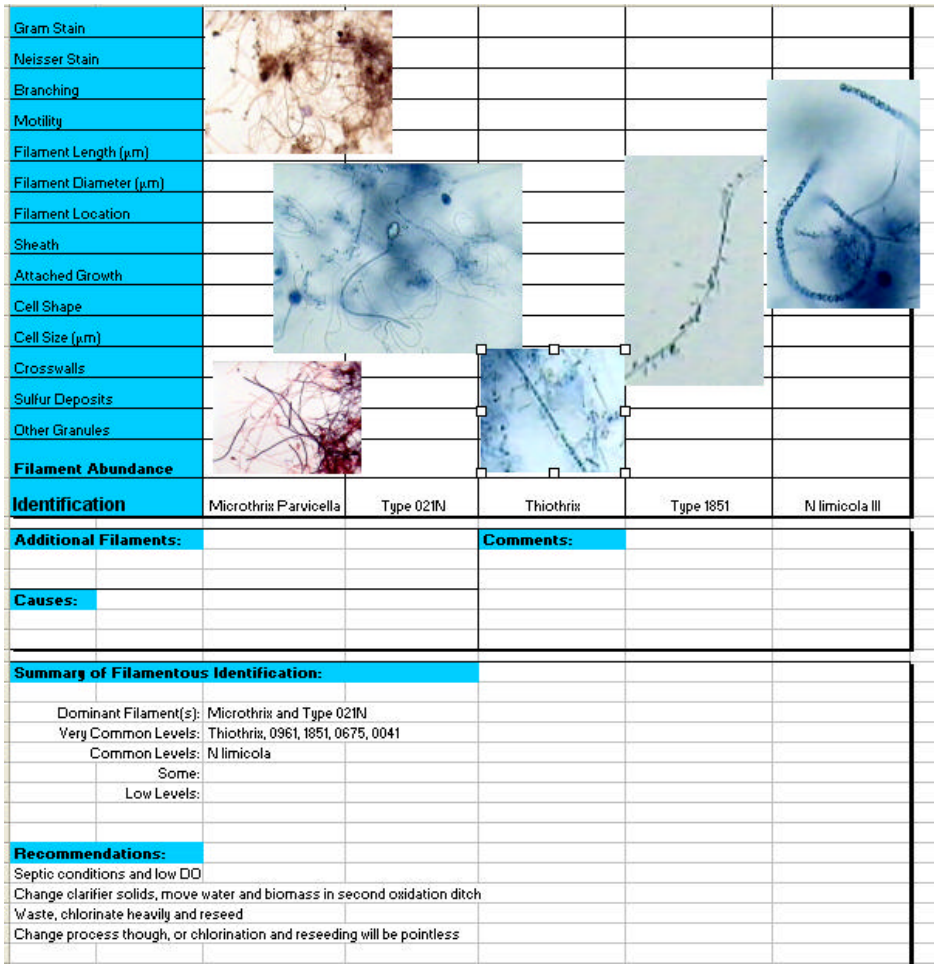


The sample also had some N. Limicola III- you can see it looks like a pearl necklace!



**1000x Gram Stains** **1000x Bright field spiral bacteria that typically indicate septicity**

Here you can see the spiral bacteria- spirillum 1000x on the left with a gram stain and on the right with the wet mount. Yes, they wiggle and spiral as they swim. If you see these in a sample, you automatically know you have septic conditions. We only see these usually in system that have some type of septicity problem, whether in an EQ, a primary, a digestor or in the sludge dewatering system where solids are held for a few days and then the supernatant comes back to the system. Usually septic conditions are not in the aeration basin!! Look elsewhere in the system. Remember, all the pieces are connected!



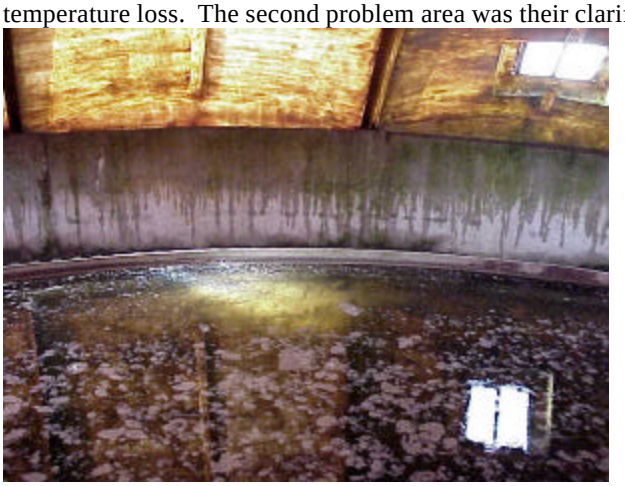
| | | | | | |
|---|---|---|---|---|---|
| Gram Stain | | | | | |
| Neisser Stain | | | | | |
| Branching | | | | | |
| Motility | | | | | |
| Filament Length (µm) | | | | | |
| Filament Diameter (µm) | | | | | |
| Filament Location | | | | | |
| Sheath | | | | | |
| Attached Growth | | | | | |
| Cell Shape | | | | | |
| Cell Size (µm) | | | | | |
| Crosswalls | | | | | |
| Sulfur Deposits | | | | | |
| Other Granules | | | | | |
| **Filament Abundance** | | | | | |
| **Identification** | Microthrix Parvicella | Type 021N | Thiothrix | Type 1851 | N limicola III |
| **Additional Filaments:** | | | **Comments:** | | |
| **Causes:** | | | | | |

| **Summary of Filamentous Identification:** | |
|---|---|
| Dominant Filament(s): | Microthrix and Type 021N |
| Very Common Levels: | Thiothrix, 0961, 1851, 0675, 0041 |
| Common Levels: | N limicola |
| Some: | |
| Low Levels: | |

| **Recommendations:** |
|---|
| Septic conditions and low DO |
| Change clarifier solids, move water and biomass in second oxidation ditch |
| Waste, chlorinate heavily and reseed |
| Change process though, or chlorination and reseeding will be pointless |

Here is our worksheet for this sample. As you can see there were tons of filaments. All leading to the same thing though, they had septic conditions and low DO conditions that were causing problems at the plant. They are a municipality though and have grease come in also. It was very cold, and you can see the top layers of the MLSS were forming a frozen blanket on the surface. Numerous problems and issues, so we walked through the plant and addressed each cause.

They needed better control on the flows through the ditches. Just because the engineers designed the plant very large with enough capacity for the plant to grow, does not mean all had to be used immediately. Believe it or not, it is harder to run an overdesigned plant than and underdesigned plant. Shut down some of the clarifiers and channels in the ditches and do not use them until needed or use them for high rains for now. This got things moving faster in the channels and more DO where needed, as well as better mixing. Also with less space, the bacteria had less surface area and that would reduce temperature loss. The second problem area was their clarifier.



They were holding solids too long in the clarifier and there was significant ashing and gassing on the clarifiers. Solids control was adjusted along with wasting and dewatering. One of the problems during dewatering was the digested solids. They would not settle in one of the side channels, so they would turn off the air for sometimes up to 2 days in order to try to get the solids to dewater and decant some of the liquids. This created a low DO and septic condition. All that supernatant went back to the head of the plant, thereby causing filaments with septicity problems. That was changed immediately and they just short term used more chlorine and polymer to decant the existing filaments and get them out of the system and then in the future, they did not have any problems because they adjusted all three areas. They do add biological products upstream a little in the lift stations for grease removal to help combat the Microthrix, but just a small amount.

What a difference, no more Mousse!

They installed sprayers in the clarifier to knock down any solids to help with the ashing.