



Sludge Quality and Microscopic Examination

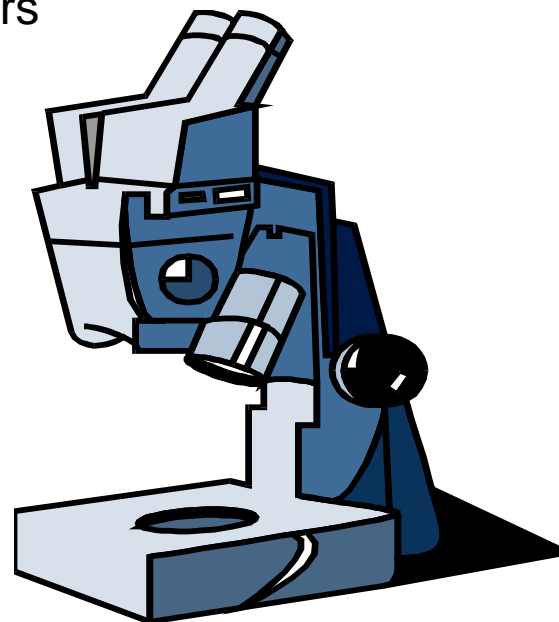
Western Chapter – Spring Wastewater Conference – March 23, 2016

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Sludge Quality and Microscopic Examination

Agenda

1. Microbiology of Activated Sludge
2. Higher Life Forms
 - Growth Pressures affecting population
 - Using higher life forms as stress indicators
3. Nonfilamentous Sludge Quality problems
4. Filamentous Growth Foaming
5. Staining
 - Gram and Neisser Staining
6. Sheaths
7. Inclusions
8. Causative Conditions
 - Low dissolved oxygen
 - Low F/M
 - Septic/High Organic Acids
 - Grease/fats/oils
 - Nutrient deficiency



Activated Sludge

Microscopic Examination

1. Photomicrographs are from a number of sources, courtesy of Iowa Rural Water, Tamdat Co., Turkey, Environmental Leverage, Plum Creek WRF and Toni Glymph.
2. Typically microscopic examination is used to identify problems with floc formation, settling, bulking and solids separation problems.
3. While the problem is generated upstream in aeration, solids separation in the clarifier is where the sludge quality problem becomes apparent.
4. Although watching the higher life forms (metazoa and protozoa), not a lot of troubleshooting information is discerned from the higher life forms – if you track the higher life forms daily, you can determine if problems are developing.

Microscopic Examination

Higher Life Forms

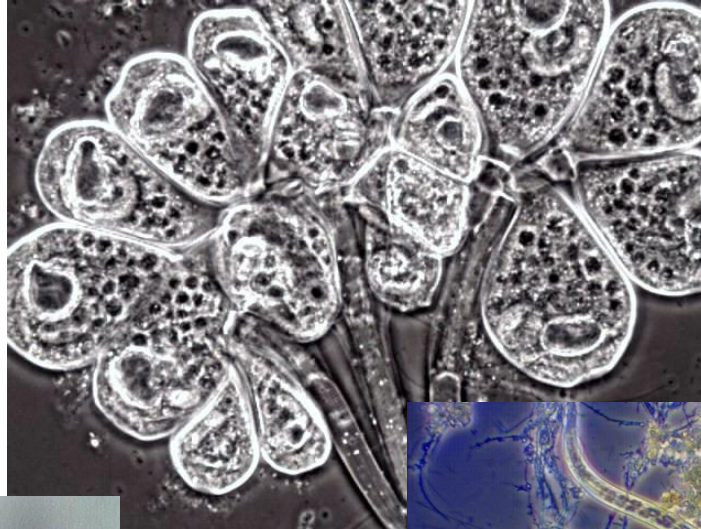
1. 6 basic groups of higher life forms are observed:
 - Flagellates
 - Amoeba
 - Free Swimming Ciliates
 - Attached or Stalked Ciliates
 - Rotifers (multicell or metazoan)
 - Invertebrates (worms and water bears)
2. Most have life cycles 1 day or higher and they develop according to their environment or food availability.
3. They account for a small population mass (less than 5%) and do not provide direct treatment of the wastewater. They can provide for effluent clean up by removing free swimming bacteria or turbidity.
4. They evolve as a function of the lower trophic level of bacteria.

Microscopic Examination Higher Life Forms

Amoeba



Stalked Ciliates



Worm

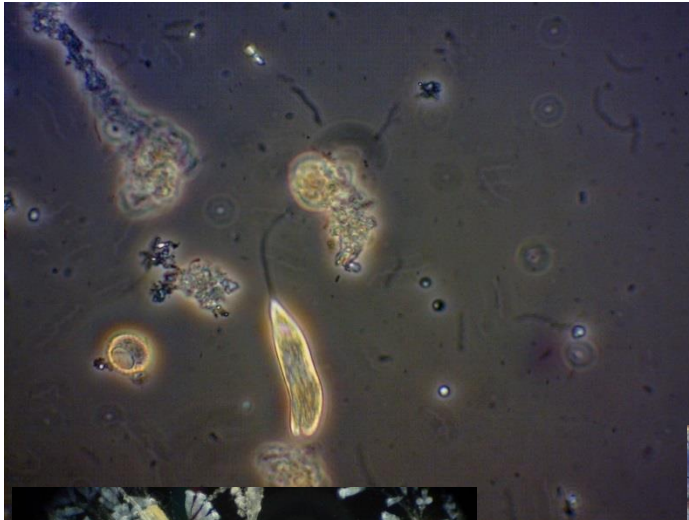


Rotifer

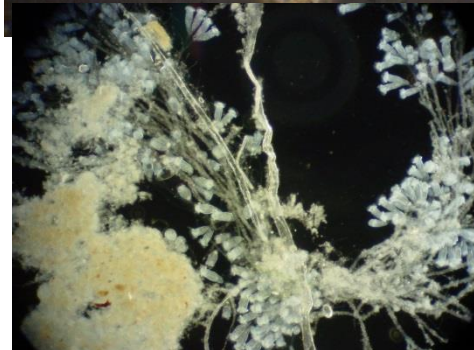


Microscopic Examination Higher Life Forms

Flagellates



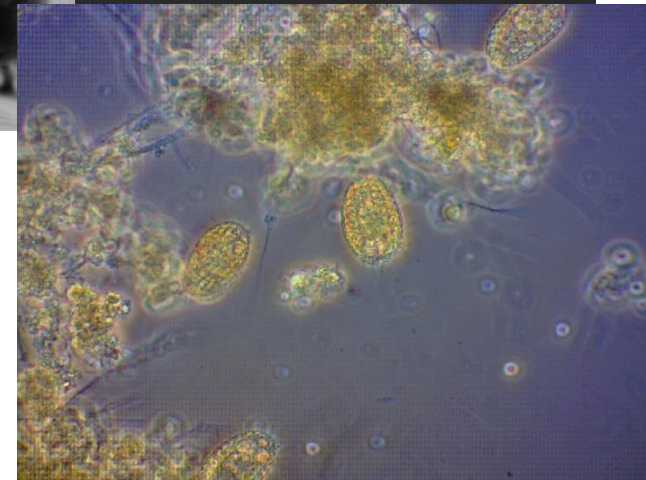
Water Bear



Stalked
Ciliates



Suctorian



Free Swimming
Ciliate

Microscopic Examination

Higher Life Forms

1. The protozoan and invertebrate groups develop based on available food source as the primary environmental condition (must be aerobic)
2. Some flagellates and amoebas can absorb some nutrients directly, but typically they require a high density of food (bacteria) as do small free swimming ciliates.
3. There is high amount of food dispersed bacteria during plant start up or low SRT operation. This is the time you will see amoebas, flagellates and small free swimmers increase in population.
4. Attached ciliates, rotifers and higher life forms of invertebrates develop with a lower food or prey density. These organisms expend less energy and are typically filter feeders.

Microscopic Examination

Higher Life Forms

5. Rotifers typically generate a dispersed food source by “grinding” into well developed floc and are in better controlled environment.
6. A balance of free swimming ciliates, stalks and rotifers is typically considered optimum.
7. Extreme conditions (high F/M or high SRT) will push the population to an overabundance of each associated life forms.
 - High F/M conditions: over wasting or an industrial discharger slug loading a treatable process wastewater that is very high in available BOD₅/COD.
 - High SRT conditions: not wasting enough or the loss of an industrial wastewater with pretreatment, holiday shutdown or other production related activity that lowers loading.

Microscopic Examination

Higher Life Forms

1. The most valuable use of watching higher life forms is they are early indicators of stress. You must scan each day to develop a general trend of the higher life forms as they are indirect indicator of biological health.
 - High loading and stressed dissolved oxygen levels
 - Toxicity stress is an early indication by the higher life forms before the bacteria show the loss of treatment stress.
 - A rapid shift towards flagellates indicates an over abundance of free bacteria and dispersed growth.
 - The loss of free swimming ciliates and limited higher life form counts indicates moving to a long SRT condition.
 - This type of microscopic exam is best done locally and can be beneficial at relatively low power (400x and less)

Microscopic Examination

Higher Life Forms

2. Determining the cause of chronic environmental and growth pressures that would provide insight into biological process control factors are beyond the gross or masking conditions of a high F/M/low SRT or a high SRT/low F/M condition of the higher life forms.
3. As stated earlier, higher life form monitoring is an indirect view of the system biology and you are looking for system response to larger changes in the growth pressures to the system.
4. More insight into subtle performance problems requires filamentous bacteria identification.
5. Sludge settling problems may not have much to do with growth pressure and little change in the higher life forms will give insight into filamentous development and predominance.

Microscopic Examination

Non Filamentous Performance Problems

Filamentous bacteria are to blame for the many plant performance problems (bulking and foaming). Estimates are 50-60% of the noncompliance problems in the US are due to overabundance of filaments.

But....there are non-filamentous performance issues that are not due to filamentous bacteria causing poor settling or liquid & sludge separation.

1. Denitrification: Floating sludge in the clarifier (if not caused by foaming filaments (*Nocardia* spp., *Microthrix* P. and type 1863)) is due to denitrification.
 - Denitrification is small nitrogen gas bubbles forming after settling, attaching to the sludge and causing solids to rise.
 - Denitrification can be due to higher organic loading, limited oxygen in aeration, step feeding and in general not stabilizing an active biology before settling – not settling's fault.
 - Denitrification can also be due to long sludge detention times, high blankets and mechanical sludge withdrawal problems that are not due to influent conditions or aeration operation - not aeration's fault.

Microscopic Examination

Non Filamentous Performance Problems

2. Nutrient Deficient Bulking/Foaming:

- Typically industrial wastewater that is high in carbon and nutrient deficient in nitrogen and/or phosphorus can develop a viscous or slime like bulking sludge (slime bulking). Paper mills, dairy plants and food service is typically high in soluble carbon and low in nitrogen/phosphorus.
- Nutrient deficiency can also select for certain filaments, but highly soluble wastewater will generate a viscous sludge.
- Municipal plants can also develop a nutrient deficient viscous slime bulking that do not have measurable nutrient deficiencies:
 - Industrial slug loadings of highly soluble wastewater
 - Long off cycles in aerobic digesters.
 - Lengthy off cycles where sludge is quickly provided with sufficient oxygen to have a high oxygen uptake (above 80 mg/L/hr) – a selector tank or BioP tank.

Microscopic Examination

Non Filamentous Performance Problems

2. Nutrient Deficient Bulking/Foaming:

- Rapid oxygen delivery to RAS/Influent after long selector or biological phosphorus reactor hydraulic retention time.
- Biological solids have rapid carbon assimilation and cannot uptake/utilize nitrogen at a similar rate and simulate a nutrient deficient condition. The biology generates excessive amounts of an exopolymer polysaccharide shunt byproduct.
- Nutrient testing can show adequate macro-nutrients (N & P), but it is the rate of carbon assimilation – too rapid.

3. Poor floc formation (Dispersed Growth – Pin Floc)

- Under steady state conditions, floc formers generate normal amount of polysaccharide slime to cement cells in floc/colonies under low growth conditions.
- Under high growth rates, bacteria maintain a dispersed non-settleable form as small flocs/bacteria. Typical of high organic loading from industrial wastes....

Microscopic Examination

Non Filamentous Performance Problems

3. Poor floc formation (Dispersed Growth – Pin Floc)

- Dispersed biology is also possible from toxic loadings. High concentrations of hydrogen sulfide can cause toxicity and dispersed floc/bacteria.
 - Hydrogen sulfide disassociates to a less toxic form at pH above 7 S.U. (HS^-). If pH is less than 7.0 S.U., H_2S predominates and is toxic (1 mg/L can lower OUR by 50%).
- Pin floc (<50 μm) can be due to long over aeration cycles. Small weak floc with little structure.
- Pin floc / turbidity will have very low BOD_5 compared to TSS in effluent. Pin floc turbidity is also a release of inerts and debris that had been held by healthy biological floc. Nondegradeable turbidity with TSS/ BOD_5 ratio as high as 5:1 is common.

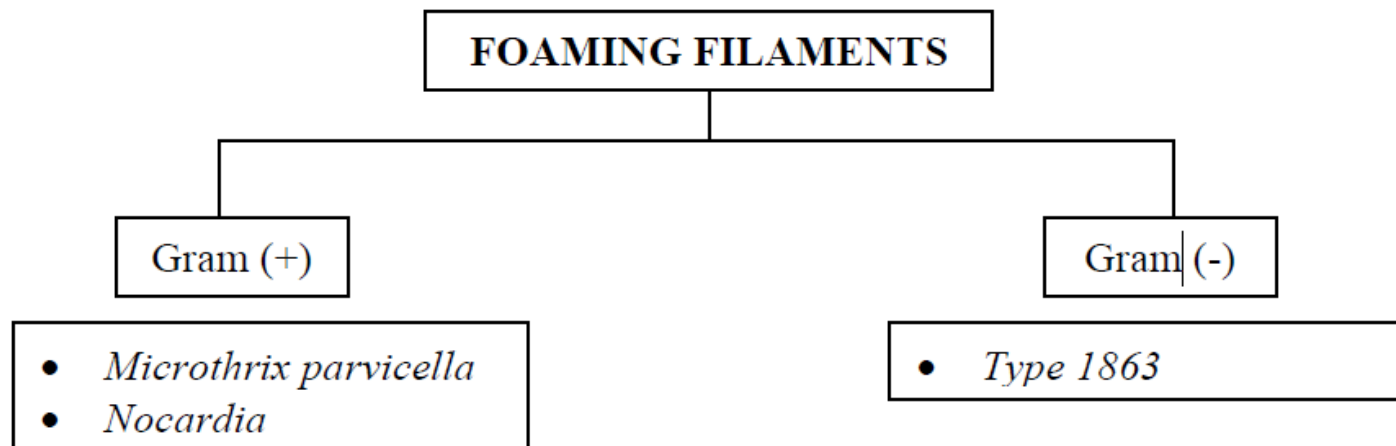
4. Zoogleal Bulking

- Fingered zoogeleal organisms extend to hinder settling. High F/M conditions that favor organic acids due to septicity or low D.O.

Microscopic Examination

Filamentous Performance Problems

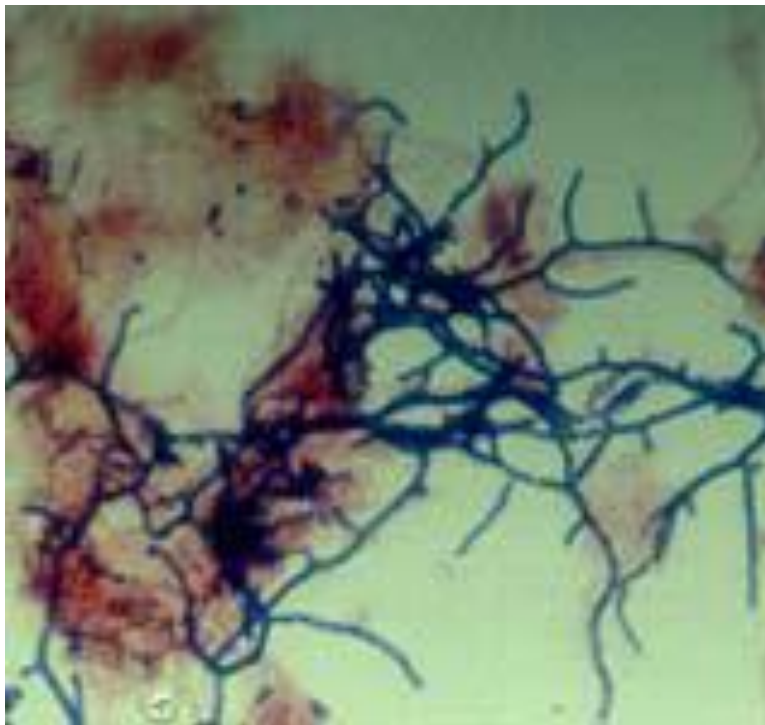
- Filamentous bacteria can predominate and cause poor settling (bulking).
- Bulking sludge is considered any SVI greater than 150 mls/gm.
- Many plants can operate and meet permit at SVIs as high as 200 mls/gm.
- Typically, filaments that cause foam and do not cause settling problems are *Nocardia* or 1863 if there also is low pH. A filament that does cause foam and settling problems is *Microthrix parvicella*.



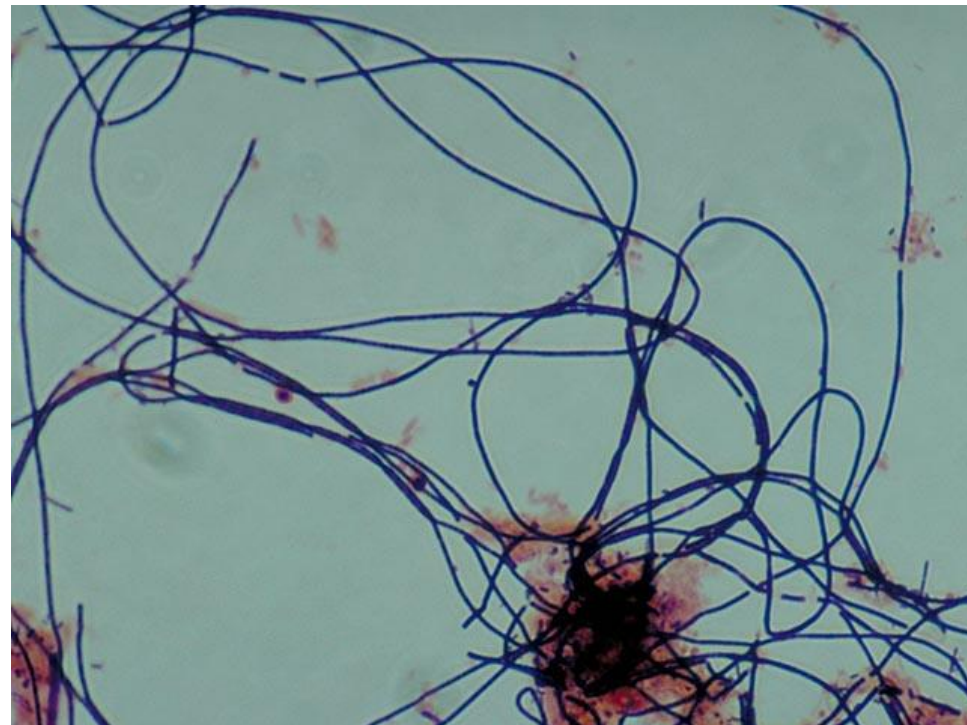
Microscopic Examination

Filamentous Performance Problems

- Both Nocardia and Microthrix are gram positive filaments (Gram+). Each are very distinctive in shape.



Nocardia



Microthrix parvicella

Microscopic Examination

Filamentous Performance Problems

- Filament 1863 is the only Gram⁻ filament. When stained with a Gram stain, the filament appears pink rather than the deep purple of the Gram⁺ filament.



1863

- Staining filaments is an identification technique where the slide is dried and retains the purple color of the crystal violet and those filaments that do not retain the deep purple color – but show as pink when decolorized with the staining are Gram⁻.

Microscopic Examination

Gram+/Gram- Cell Structure

- There are two basic types of bacterial cell wall structures that have been studied in detail:
 - Gram positive (G+): Following staining with the Gram stain. G+ cells are Purple.
 - Gram negative (G-). Following staining with the Gram stain. G- cells are red/pink.
- The cell wall is a critical structure in bacterial cells.
- Inside of the bacterial cell there is a high solute concentration and a great pressure on the membrane (75 lb/in²).
- Outside of the cell there is a low solute concentration. Mother nature will tend to flow water into a cell to equilibrate the amount of water inside and outside of the cell. Membranes prevent most other molecules from crossing them, but water can. Without something supporting the membrane the cell would swell and burst. A cell wall protects bacteria from bursting.

Microscopic Examination

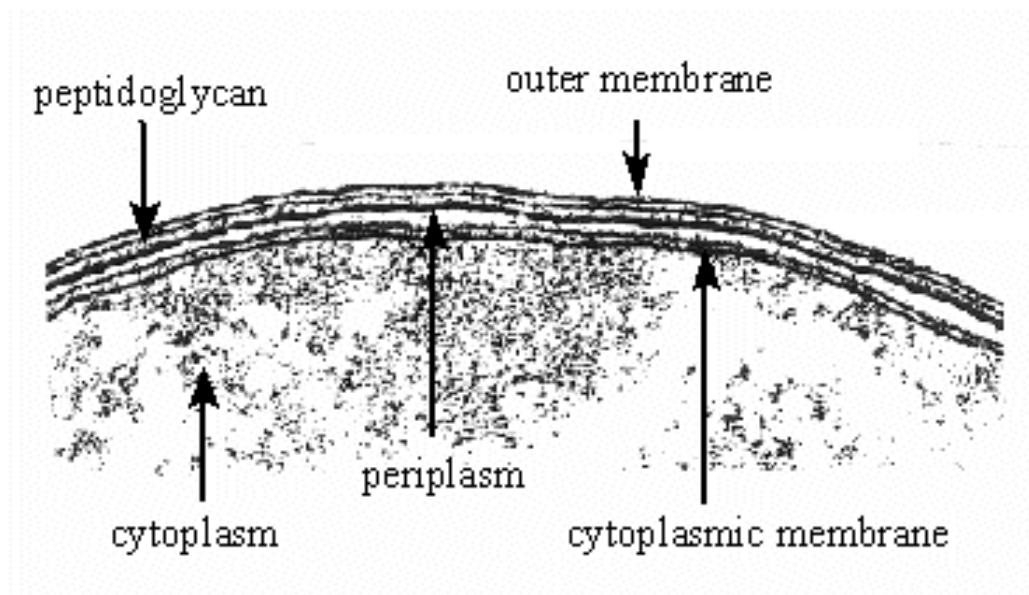
Gram+/Gram- Cell Wall Structure

- Peptidoglycan is a thick rigid layer that is found in both G+ and G- cells. The exact molecular makeup of these layers is species specific.
- The degree of cross-linking determines the degree of rigidity. In G+ cells the peptidoglycan is a heavily cross-linked woven structure that wraps around the cell. It is very thick with peptidoglycan accounting for 50% of weight of cell and 90% of the weight of the cell wall. Peptidoglycan to be 20-80 nm thick.
- In G- cells the peptidoglycan is much thinner with only 15-20% of the cell wall being made up of peptidoglycan and this is only intermittently cross-linked. It is not a barrier to solutes, the openings in the mesh are large and all types of molecules can pass through them.

Microscopic Examination

Gram+/Gram- Cell Wall Structure

- G⁻ cell walls have a more complicated structure but less robust to stress. There are 2 separate areas with an additional membrane besides the cellular membrane. Outside of the cytoplasmic membrane (CM) is a open area called the periplasmic space. Beyond this is a thin layer of peptidoglycan. Finally, external to the peptidoglycan is an additional membrane, the outer membrane (OM).



Microscopic Examination

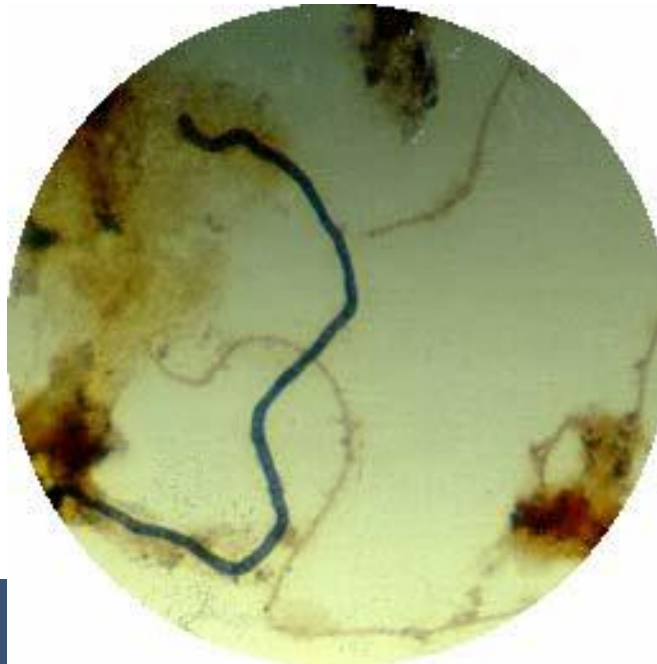
Gram+/Gram- Cell Wall Structure

- Gram positive filaments
 - Gram+ Filaments are very tough due to the amount of peptidoglycan cross linking for a cell wall.
 - A Gram+ filament can survive long off-cycles and are still active and intact after weeks anaerobic digestion.
- Gram Negative filaments
 - Off cycling and return of the biosolids from facultative digestion will not return viable gram negative filaments to the on-line process.
 - This type of filament can typically be controlled through lysis, and the population will be removed if long off cycles of the solids are provided.

Microscopic Examination

Neisser Stain – Polyphosphate Storage

- Neisser staining also separates the filaments into 2 categories, “Neisser +” that stain bluish and “Neisser –” for the those filaments that stain brown. Filaments that are accept Neisser stain are capable of storing polyphosphates. *Microthrix p.* for example will stain positive for polyphosphates. Anaerobic selectors will not remove *Microthrix P.*
- The most consistent filament for Neisser staining is *Nostocoida limicola*. It is consistently positive for Neisser staining while it can be variable for gram staining.



Nostocoida limicola

Microscopic Examination

Filamentous Performance Problems

- Neisser staining and Gram Staining (below) and the discoid shape are very distinctive for *Nostocoida limicola*. Phase contrast at 400x power will show the discoid filament.



Nostocoida limicola

Microscopic Examination

Outside Cell Wall Structures

- Structures outside the cell wall
 - Glycocalyx: many bacterial cells have an external coating excreted onto the outside of the cell. There are two types of glycocalyx are capsules and slime layers.
 - Glycocalyx is a general term for any network of polysaccharide or protein containing material extending outside of the cell. A difference in terms for a general term:
 - a capsule is closely associated with cells and does not wash off easily.
 - A slime layer is more diffuse and is easily washed away.

Microscopic Examination

Outside Cell Wall Structures

- The function of the slime layer:
 - Attachment as a colony
 - These structures are thought to help cells attach to their target environment.
 - Resistance to drying. Capsules and slime layers inhibit water from escaping into the environment.
 - Reservoir for certain nutrients. Glycocalyx will bind certain ions and molecules (slime layer)
 - Deposit waste products. Waste products of metabolism are excreted from the cell, and will accumulate in the capsule. This binds them up, and prevents the waste from interfering with cell metabolism.
- This layer can be highly evolved as a sheath and used in identification of sheath forming filaments.

Microscopic Examination

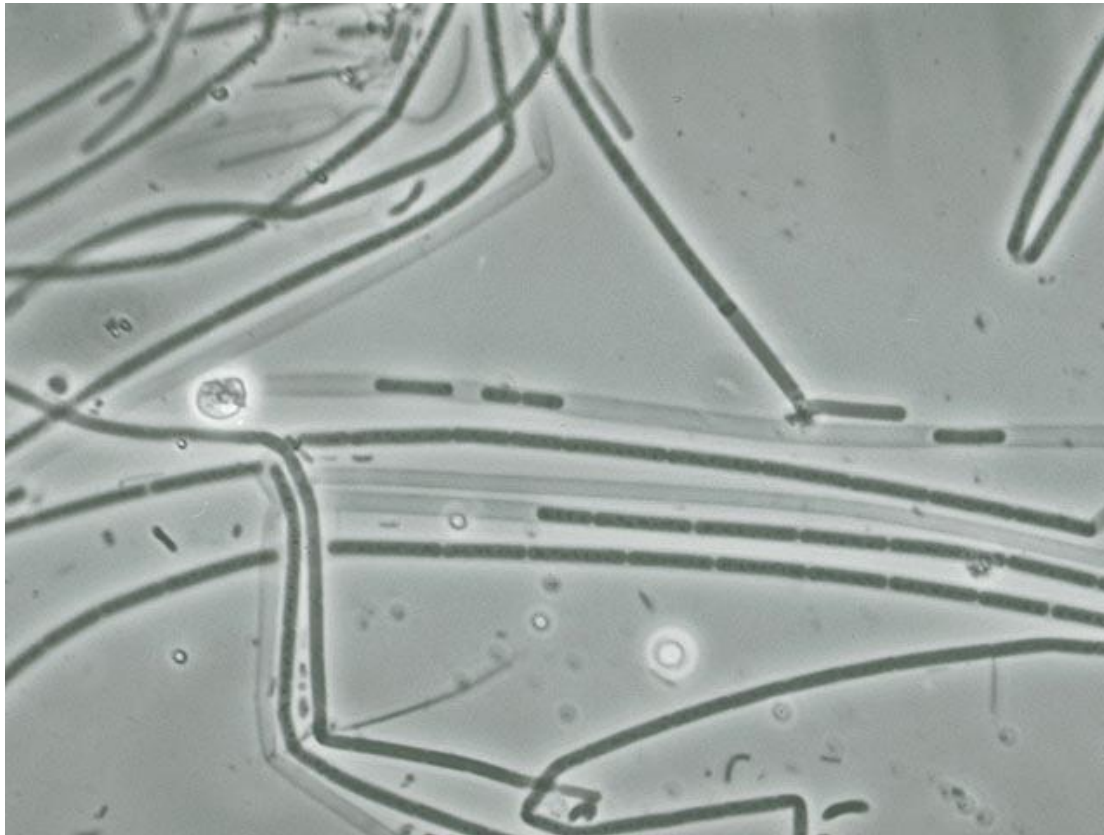
Filamentous Performance Problems - Sheaths

- Another distinguishing feature among filamentous bacteria is a sheath. The following 7 filaments have sheaths:
 1. Sphaerotilus natans
 2. Thiothrix
 3. Haliscomenobacter hydrossis
 4. Type 0041
 5. Type 0675
 6. Type 1701
 7. Type 1851
- When under stress or being chlorinated for control the sheath becomes more apparent as shown in the next slide for Sphaerotilus natans.

Microscopic Examination

Filamentous Performance Problems - Sheaths

- *Sphaerotilus natans* shows cell loss inside the sheath in this wet mount phase contrast photomicrograph.



Sphaerotilus natans

Microscopic Examination

Filamentous Performance Problems - Inclusions

- Inclusions are aggregates of various compounds that are normally involved in storing energy reserves or building blocks for the cell. Inclusions accumulate when a cell is grown in the presence of excess nutrients.
 - Poly-beta-hydroxyalkanoate (PHA): one of the more common storage inclusions is PHA. It is a long polymer of repeating hydrophobic units that can have various carbon chains attached to them. The function of PHA in bacteria is as a carbon storage product. We store fat, bacteria store PHA.
 - Glycogen is another common carbon and energy storage product. Humans also synthesize and utilize glycogen. Glycogen is a polymer of repeating glucose units.
 - Phosphate and sulfur globules: Many organisms will accumulate granules of polyphosphate, since this is a limiting nutrient in the environment. The globules are long chains of phosphate. Bacteria often use sulfides as their source of electrons (Thiothrix), some of them accumulate sulfur globules. These globules may later be further oxidized and disappear if the sulfide pool dries up.

Microscopic Examination

Filamentous Performance Problems - Inclusions

- Another distinguishing feature are those filaments that metabolize sulfur. The stored sulfur granules are visible with phase contrast or using the sulfur test.
- Sulfur depositors:
 - Type 021N
 - Thiothrix I & II
 - Type 0914
 - Beggiatoa



Thiothrix I with sulfur granules

Microscopic Examination

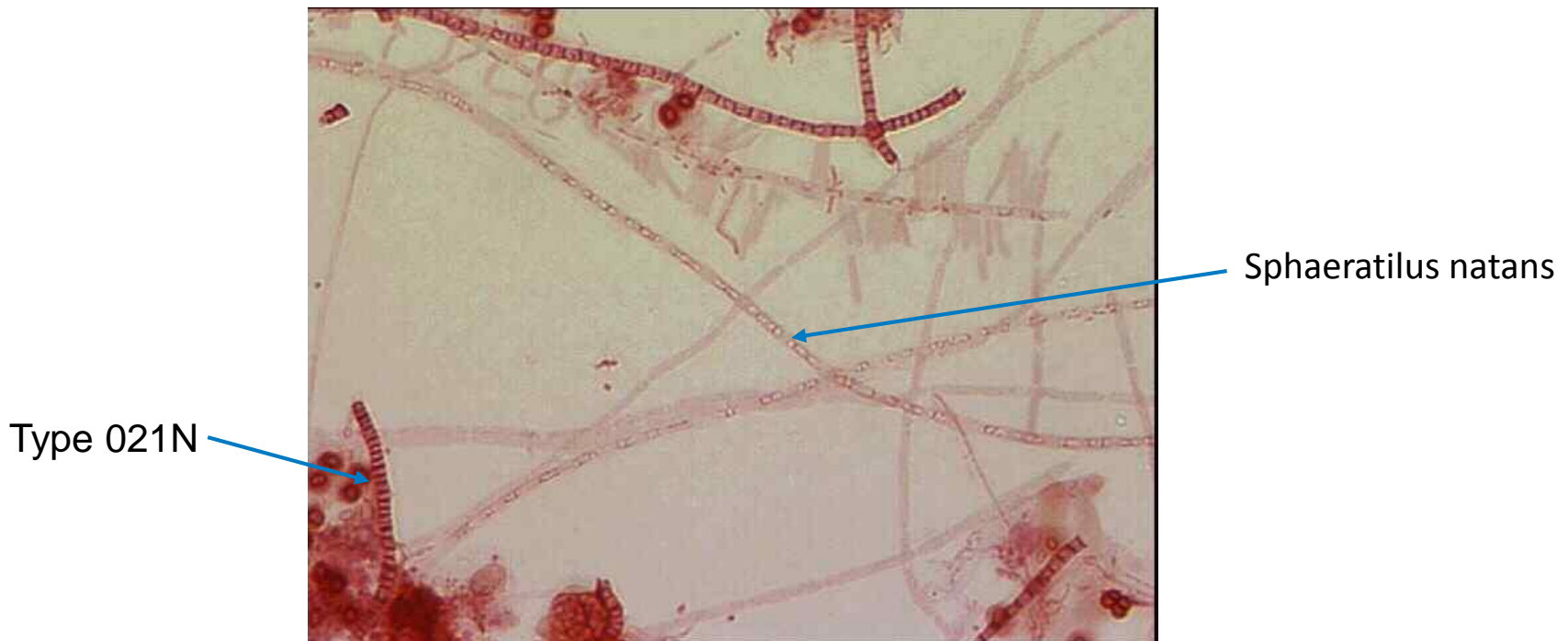
Filamentous Performance Problems

- Six (6) filaments (below) account for more than 90% of the bulking episodes in the US.
- Nocardia (most dominant but not causing bulking)
 1. 1701
 2. 021N
 3. 0041
 4. Thiothrix spp
 5. Sphaeratilus natans
 6. Microthrix parvicella
- Much has been learned about filaments, the operator exam in Ohio asked for a cause if there was a settling problem, Sphaeratilus natans was the correct answer as being only filament sludge quality problem in the late 70's. Not much research had been done at that time.

Microscopic Examination

Filamentous Performance Problems

- *Sphaerotilus natans* is caused by low dissolved oxygen. Early in the development of wastewater technology, coarse bubble diffusers installed as spiral roll aeration was the state of the art. Oxygen transfer was limited and limited oxygen was a typical problem. Many times, *Sphaerotilus natans* was the source of the bulking problems and became synonymous with bulking.



Microscopic Examination

Causative Conditions for Filaments

- Low Dissolved Oxygen – filaments are microaerophiles. Selector tanks (20-30 minutes) combine the biology (RAS) with raw influent under zero dissolved oxygen conditions. These anaerobic selectors allow the floc formers to gain a competitive advantage over the filaments.
 - Type 1701
 - Sphaerotilus natans
 - H. Hydrossis
- Low F/M conditions – Filaments that require tight wasting control are also typically controlled by high F/M selectors. Combining RAS and raw influent in a small mixing zone with a high F/M condition versus combining RAS and raw influent directly to the MLSS as done in oxidation ditches favor the following filaments:
 - type 0041
 - type 0675
 - type 1851
 - type 0803

Microscopic Examination

Causative Conditions for Filaments

- Low F/M conditions (con't)
 - Low F/M filaments if not provided with an anaerobic and/or high F/M selector will require very tight wasting control and result in high sludge yields.
 - The same plant can have much lower sludge yield and allow for variations in MLSS concentrations with little change in sludge quality.
 - It has been common for plant upgrades that have included filament control to allow operators to run MLSS concentrations up to 4,000 mg/L and higher and maintain excellent settling (SVIs under 80 mls/gm)
 - The same plant with poor RAS and raw influent blending may have only been able to vary MLSS concentrations no higher than 2,500 mg/L without having SVIs quickly go over 200 mls/gm.

Microscopic Examination

Causative Conditions for Filaments

- Septic Wastewater/Sulfides – high organic acids and septicity with sulfur loading.
- Septicity in collection systems has ranged from long force mains to septic loads discharging from grease traps / trash traps from a large development of commercial areas:
 - type 021N
 - *Thiothrix* I and II
 - *Nostocoida limicola* I,II,III
 - type 0914
 - type 0411
 - type 0961
 - type 0581
 - type 0092

Microscopic Examination

Filamentous Performance Problems



021N



0914 with sulfur granules

Microscopic Examination

Causative Conditions for Filaments

- Grease and Oily Waste:
 - Nocardia spp.
 - Microthrix parvicella
 - type 1863
- Nutrient Deficiency
 - Nitrogen: type 021N
Thiothrix I and II
 - Phosphorus: Nostocoida limicola III
Haliscomenobacter hydrossis
Sphaerotilus natans

Microscopic Examination

Filamentous Performance Problems

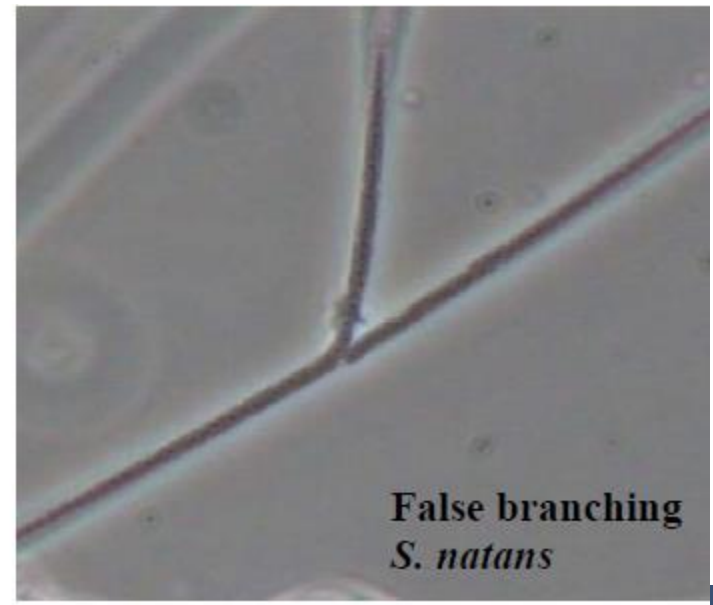


Very fine spike like filament that is easy to miss in a phase contrast wet mount scan. High quality microscope is typically required to not miss this filament under the microscope.

Microscopic Examination

Causative Conditions for Filaments

- Low pH: Fungus and type 1863. Fungus is extremely large true branching filament.
 - As phosphorus control and iron dosing becomes more common – loss of pH may be a factor in developing foaming or even fungus.
 - Egg cracking operation that separated yolks (acidic) from the egg white (basic). Total egg is neutral, but components are not.



Microscopic Examination

- Microscopic Examination can provide a daily complement to process control data.
- Higher life form indicators require frequent checking and provide more general support on loading, SRT, oxygenation and other major process control indicators.
- Filamentous indicators are useful for to determine causes for difficult settleability issues.
- Filamentous identification requires skills in drying, staining and a higher quality microscope.

Questions



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