



AQUAFIX

Wastewater Laboratories

University of Wisconsin Research Park



Date: 11/18/2022
To: [REDACTED]
Sample(s): Mixed Liquor: [REDACTED] Foam: [REDACTED]
Date Received: 11/16/2022
Date(s) Analyzed: 11/16/2022
Sample Analyzed By: Michael Lanphier (Research Scientist); AquaFix Inc.
Objective: Determine the cause of foaming in the [REDACTED]
[REDACTED] and recommend treatment.

Overview of Findings:

- The cause of foaming and bulking issues in the [REDACTED] [REDACTED] are due to Nocardia-like organisms. Nocardia-like organisms can cause foaming and/or bulking to occur. These filaments tend to reside in a higher sludge age with high level of incoming fats, oils, and greases (FOG) present.
- Nocardia-like organisms can also grow within floc, preventing proper floc formation from occurring. Foaming, bulking, and scum formation occurs when Nocardia-like organisms form a tangled mat-like structure and floats due to its low-density fatty cell walls. This same foam can also limit the transfer of oxygen promoting further growth of low D.O. filaments.
- High foaming/bulking filament presence and smaller lower-density floc appear to be the cause of high supernatant turbidity/ floating solids seen in the lab. These smaller particles can also reduce surface tension in an aeration basin and lead to increased foaming.
- If the chlorine tablets currently being used in the system are for effluent disinfection please continue use. If the chlorine tablets are being added somewhere in the return activated sludge, this can exacerbate foaming issues. This added chlorination is then likely harming the ability of floc to reform by damaging helpful filaments and providing stress on healthy bacteria. The chlorine will also damage/split the current Nocardia-like organisms and cause them to regrow even more quickly making foaming worse.

**** Details on microscopy and recommendations are available at the end of the report. ****

Microscopic Observations: Mixed Liquor: [REDACTED]

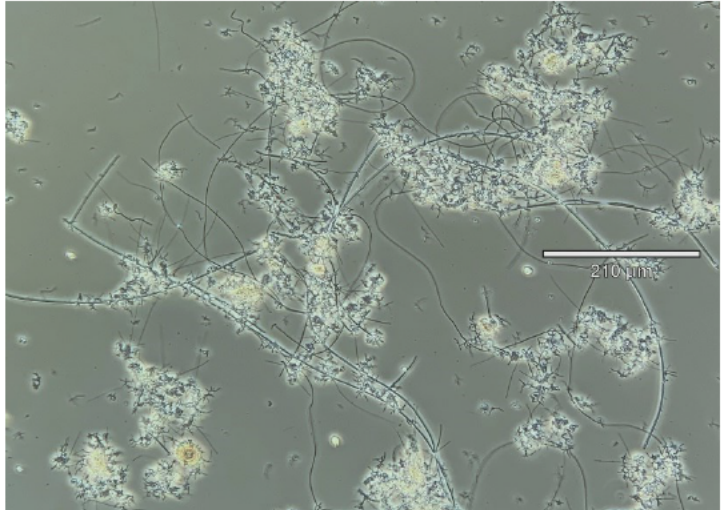
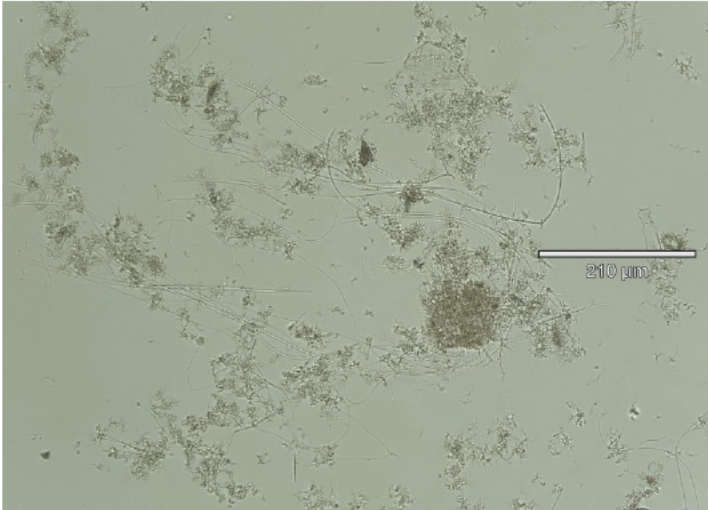


Figure 1-100x magnification (m) & Figure 2-100x (m) Phase Contrast: The floc in the "Mixed Liquor: [REDACTED]" sample appeared to be mostly small to medium in size with an average floc size of 267.21 µm. Determining the average floc size was difficult due to the high levels of filamentous bacteria creating interfloc bridging. Approximately 40% of the floc were small in size (<150µm), 40% of the floc were medium in size (150-500µm) and 20% of the floc were large in size (>500µm). Floc appeared primarily irregular in shape and open in structure. Moderate to high amounts of filamentous bacteria were observed within and extending from floc. Moderate to high amounts of filamentous bridging was occurring between floc.



Figure 3-400x (m): Moderate levels of stalked ciliates, with an occasional larger stalked ciliate colony (pictured, arrow) were observed in the "Mixed Liquor: [REDACTED]" sample. Stalked ciliates are typically observed in healthy sludge, however large colonies are indicative of an older sludge age.



Figure 4-400x (m): Low to moderate levels of rotifers (arrow) were observed in the "Mixed Liquor: [REDACTED]" sample. Rotifers consume dead and decomposing organic matter and are usually characteristic of a older sludge age.

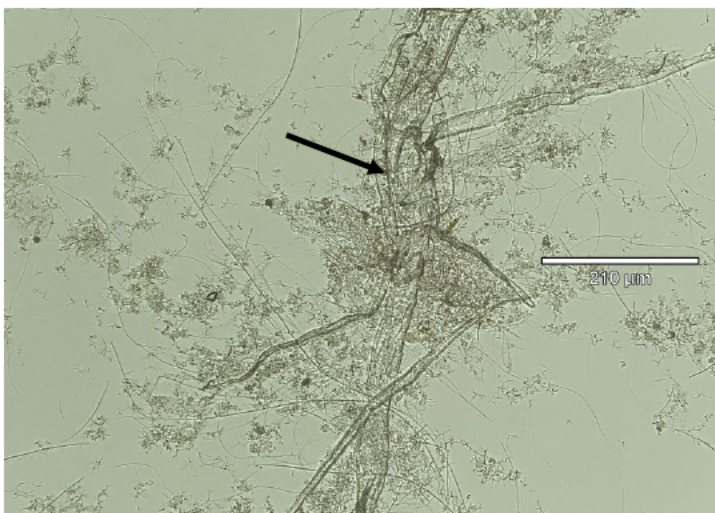


Figure 5-100x (m): Low to moderate amounts of plant/paper fibers (arrow) were also observed throughout the sample. Plant and paper fibers can add structure to sludge interfering with settling properties.



Figure 6-400x (m): Moderate levels of gastrotrichs (arrow), were observed in the sample. Gastrotrichs are a relatively rare organism that can be found in all wastewater plant conditions with older aged sludge.

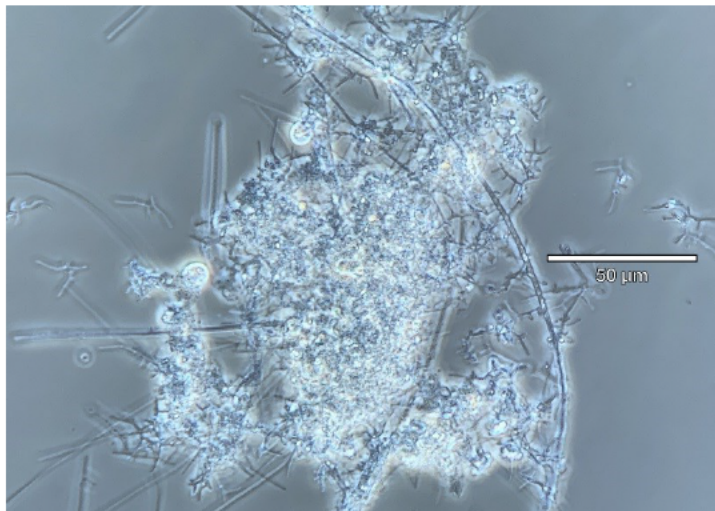


Figure 7-400x (m) Phase Contrast: When viewed under phase contrast the floc were mostly white in color. This is indicative of good oxygen penetration into the floc.

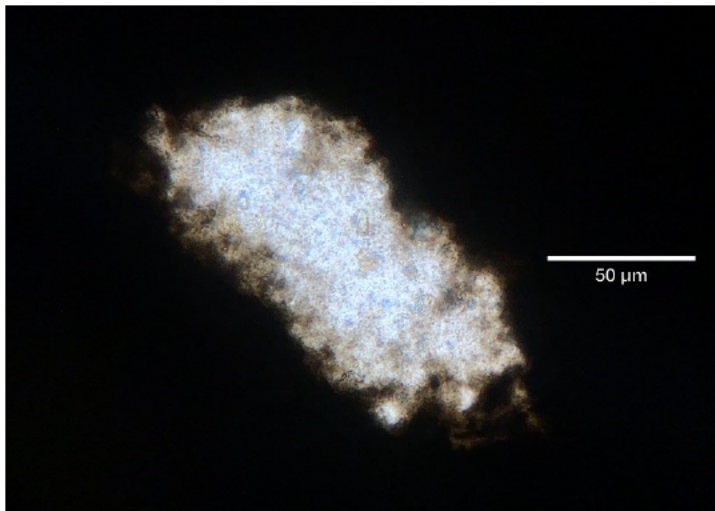


Figure 8-400x (m) India Ink Stain: Moderate levels of extracellular polymeric substances (EPS) were observed in the floc. EPS is a glue like substance that helps floc forming bacteria stick together and form strong floc.

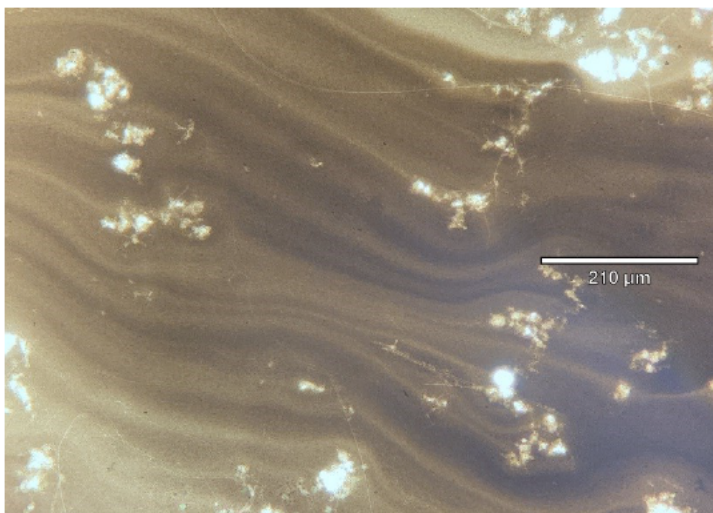


Figure 9-100x (m) India Ink Stain: Moderate levels of EPS were observed diffusing from floc. Diffused EPS tends to be a sign of stress in a wastewater system.

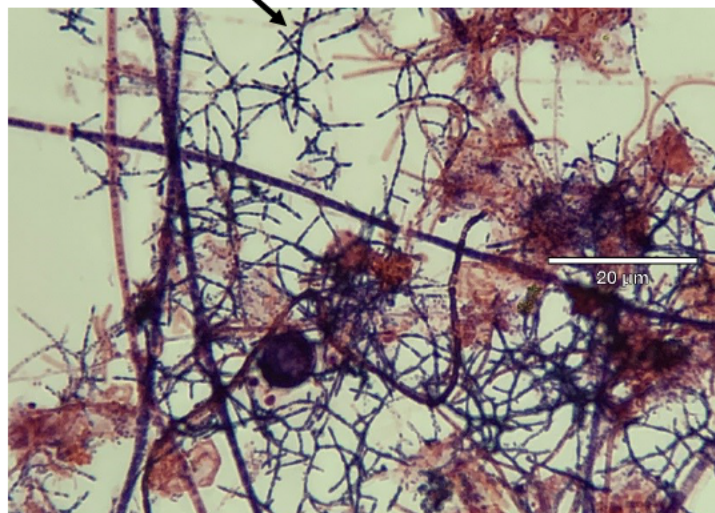


Figure 10-1000x (m) Gram Stain: Moderate to high levels of Nocardia-like organisms (arrow) were observed in the "Mixed Liquor: [redacted] sample. Nocardia-like organisms thrive in environments with high levels of incoming fats, oils, and greases (FOG) as well as in old sludge. If abundant enough these filaments can cause foaming to occur.

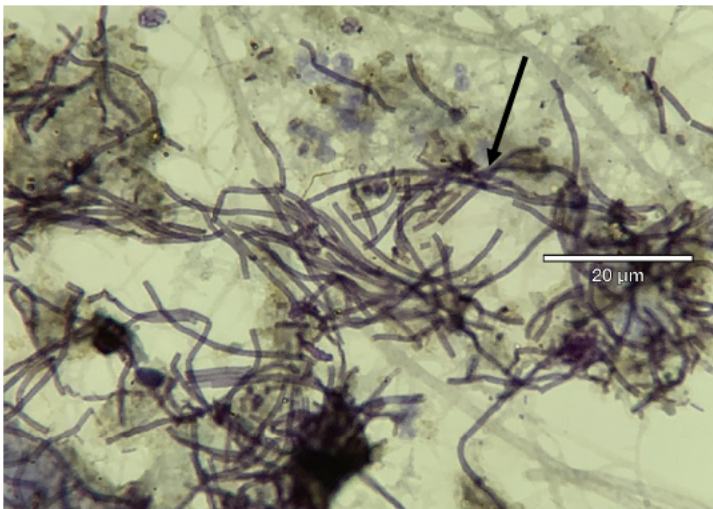


Figure 11-1000x (m) Neisser Stain: Moderate to high levels of Type 0092 (arrow) were observed. Type 0092 thrives in conditions with high levels of incoming fats, oils, and greases (FOG) as well as warm temperatures. This filament can cause bulking to occur at higher levels.

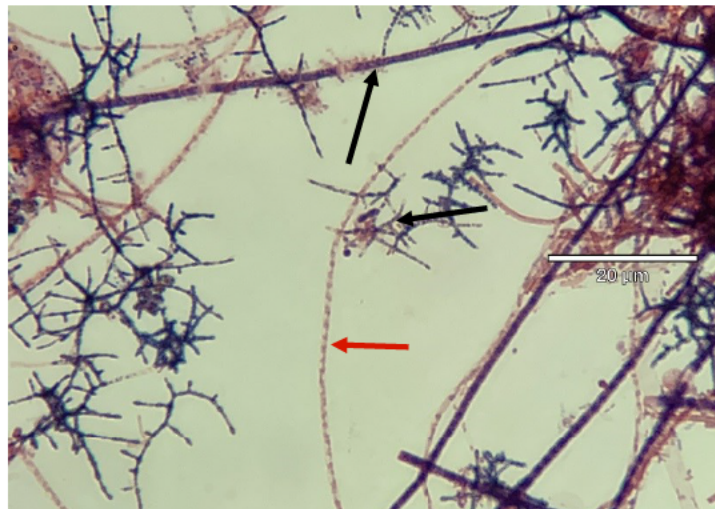


Figure 12-1000x (m) Gram Stain: Moderate levels of Type 0041/0675 (black arrow) were observed in the sample. Type 0041/0675 thrives in low F:M conditions. Low to moderate levels of *Thiothrix*/Type 021N (red arrow) were observed. *Thiothrix*/Type 021N prefers conditions with low levels of dissolved oxygen, incoming septic waste, nitrogen deficiency, and/or sulfur reducing conditions.

Microscopic Observations: Mixed Liquor: [REDACTED]

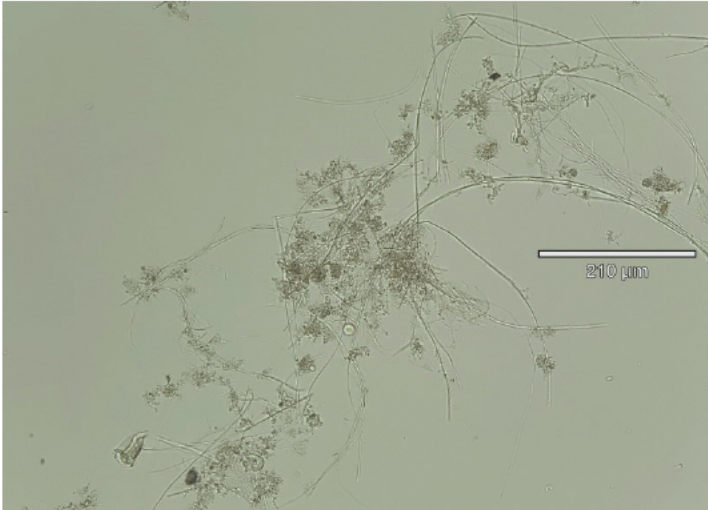


Figure 13-100x magnification (m) & Figure 14-100x (m) Phase Contrast: The floc in the "Mixed Liquor: [REDACTED]" sample appeared to be mostly small to medium in size with an average floc size of 178.12 µm. Determining the average floc size was difficult due to the high levels of filamentous bacteria creating interfloc bridging. Approximately 50% of the floc were small in size (<150µm) and 50% of the floc were medium in size (150-500µm). Floc appeared primarily irregular in shape and open in structure. Moderate to high amounts of filamentous bacteria were observed within and extending from floc. Moderate to high amounts of filamentous bridging was occurring between floc.



Figure 15-400x (m): Moderate levels of stalked ciliates, with an occasional larger stalked ciliate colony (pictured, arrow) were observed in the "Mixed Liquor: [REDACTED]" sample.



Figure 16-400x (m): Low to moderate levels of rotifers (arrow) were observed in the "Mixed Liquor: [REDACTED]" sample.

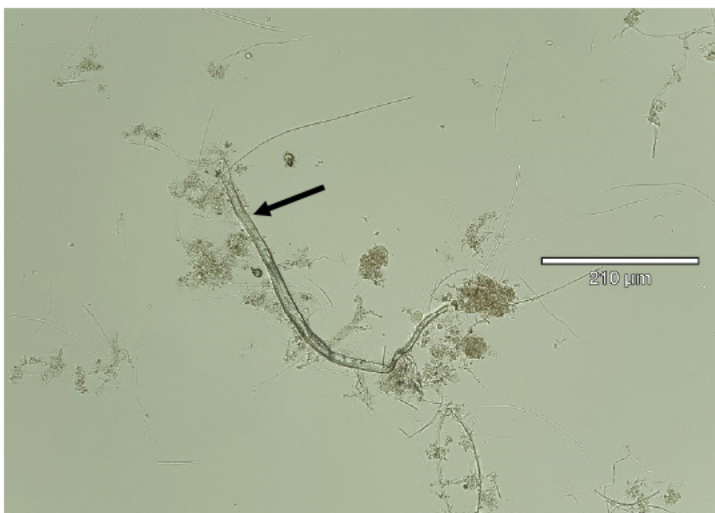


Figure 17-100x (m): Low to moderate amounts of plant/paper fibers (arrow) were also observed throughout the sample.



Figure 18-400x (m): Moderate levels of gastrotrichs (arrow) were observed in the sample.

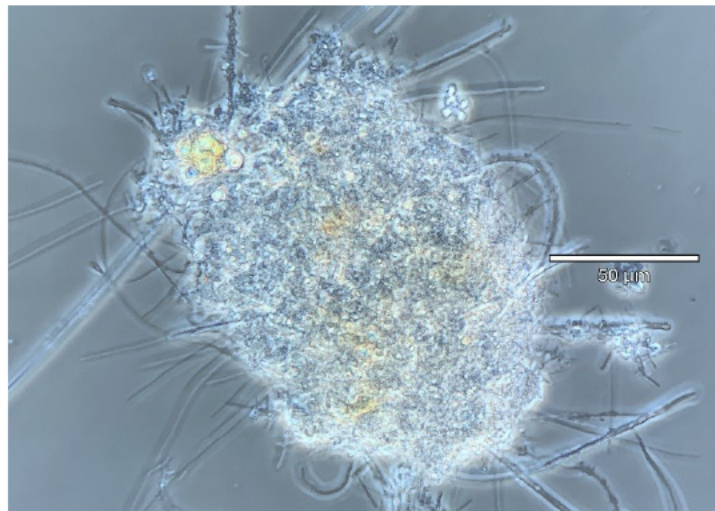


Figure 19-400x (m) Phase Contrast: When viewed under phase contrast the floc were mostly white in color with some light tan colored areas. This is indicative of good oxygen penetration into the floc.



Figure 20-400x (m) India Ink Stain: Moderate levels of extracellular polymeric substances (EPS) were observed in the floc. EPS is a glue like substance that helps floc forming bacteria stick together and form strong floc.

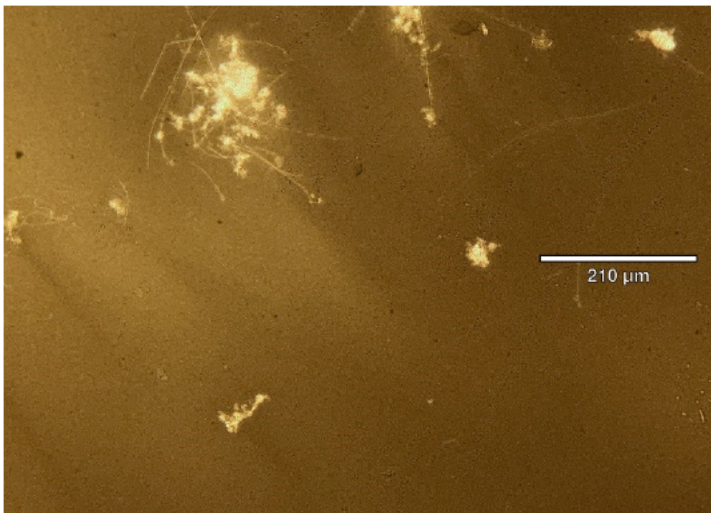


Figure 21-100x (m) India Ink Stain: Moderate levels of EPS were observed diffusing from floc. Diffused EPS tends to be a sign of stress in a wastewater system.

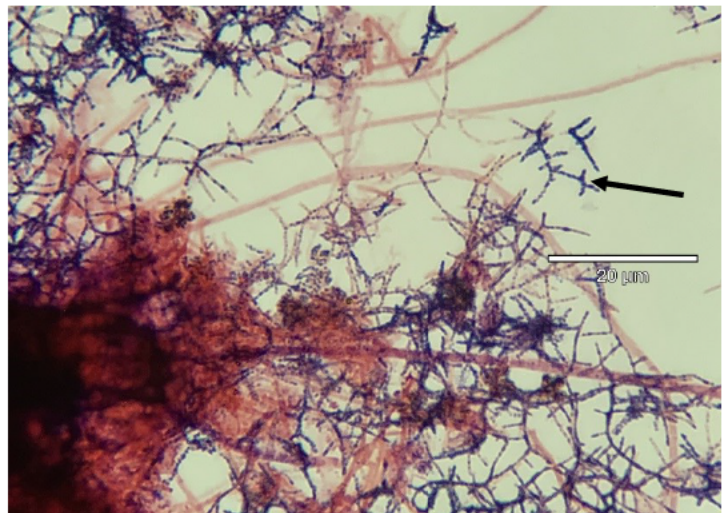


Figure 22-1000x (m) Gram Stain: Moderate to high levels of *Nocardia*-like organisms (arrow) were observed in the "Mixed Liquor: [redacted] sample.

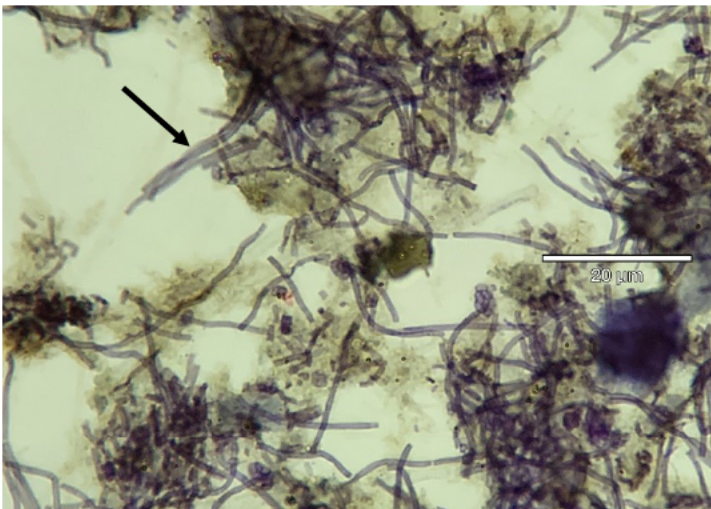


Figure 23-1000x (m) Neisser Stain: Moderate to high levels of Type 0092 (arrow) were observed.

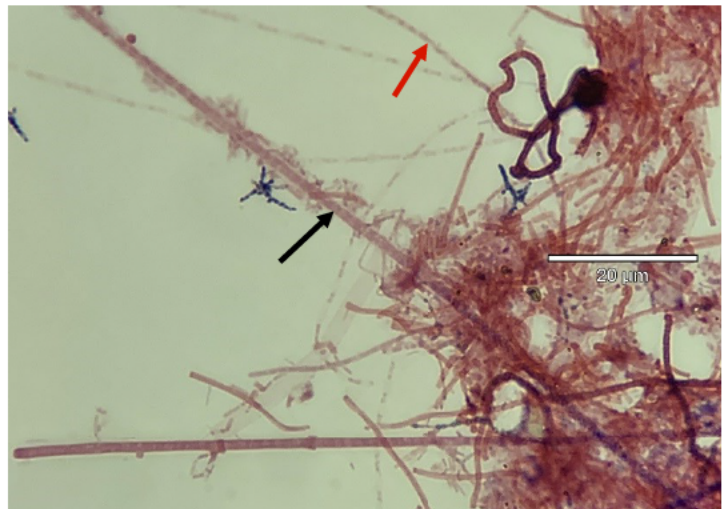


Figure 24-1000x (m) Gram Stain: Moderate levels of Type 0041/0675 (black arrow) were observed in the sample. Low to moderate levels of *Thiothrix*/Type 021N (red arrow) were observed.

Microscopic Observations: Foam: [REDACTED]

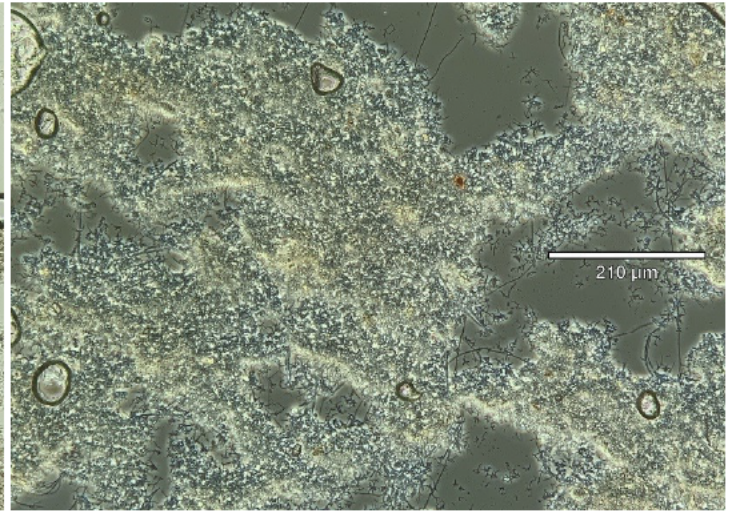
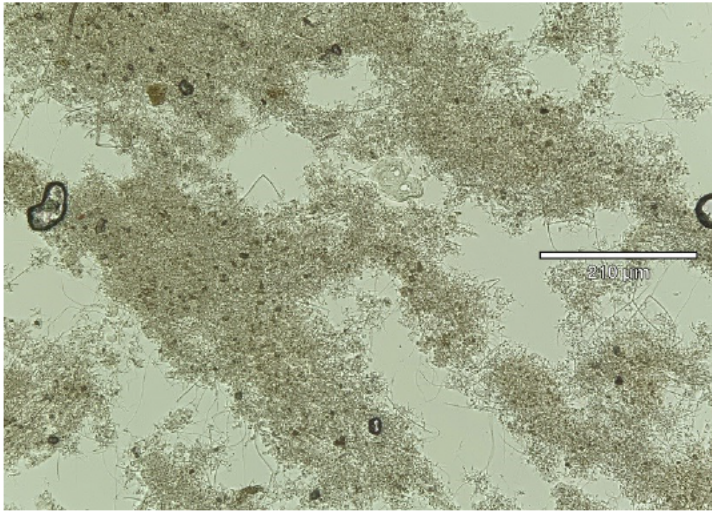


Figure 25-100x magnification (m) & Figure 26-100x (m) Phase Contrast: Very high amounts filamentous bacteria were observed within the "Foam: [REDACTED]" sample. Moderate amounts of suspended solids were observed.

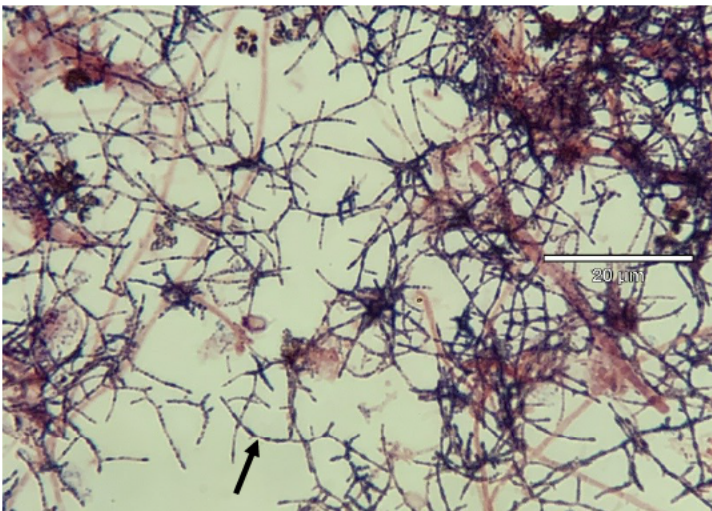


Figure 27-1000x (m) Gram Stain: Very High levels of Nocardia-like organisms (arrow) were observed in the "Foam: [REDACTED]" sample.

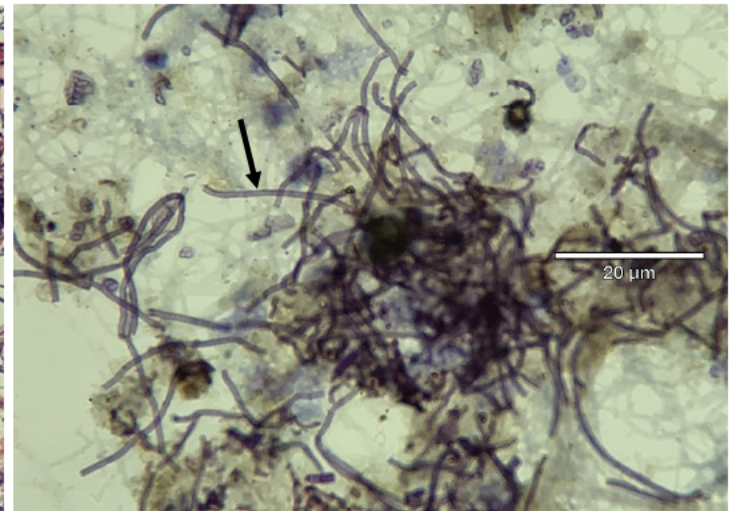


Figure 28-1000x (m) Neisser Stain: Moderate to high levels of Type 0092 (arrow) were observed.

100mL, 30-minute Settling Test

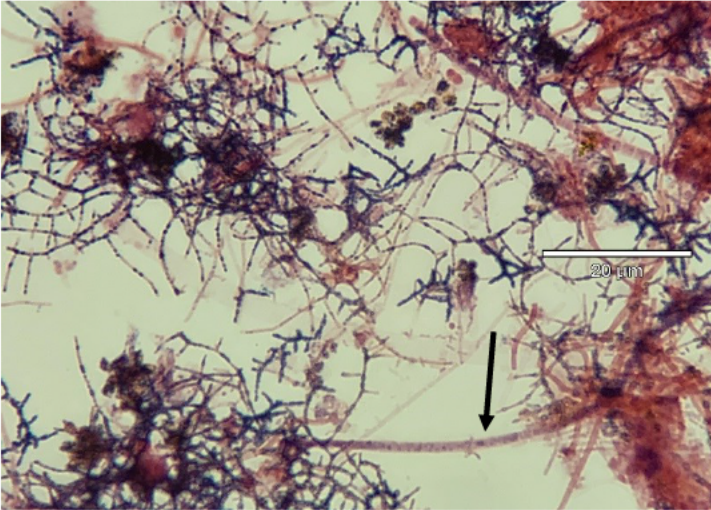


Figure 29-1000x (m) Gram Stain: Low to moderate levels of Type 0041/0675 (black arrow) were observed in the sample.

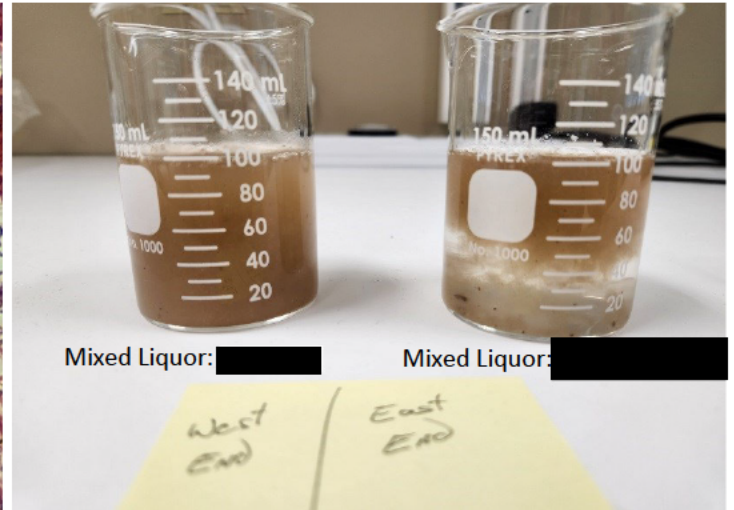


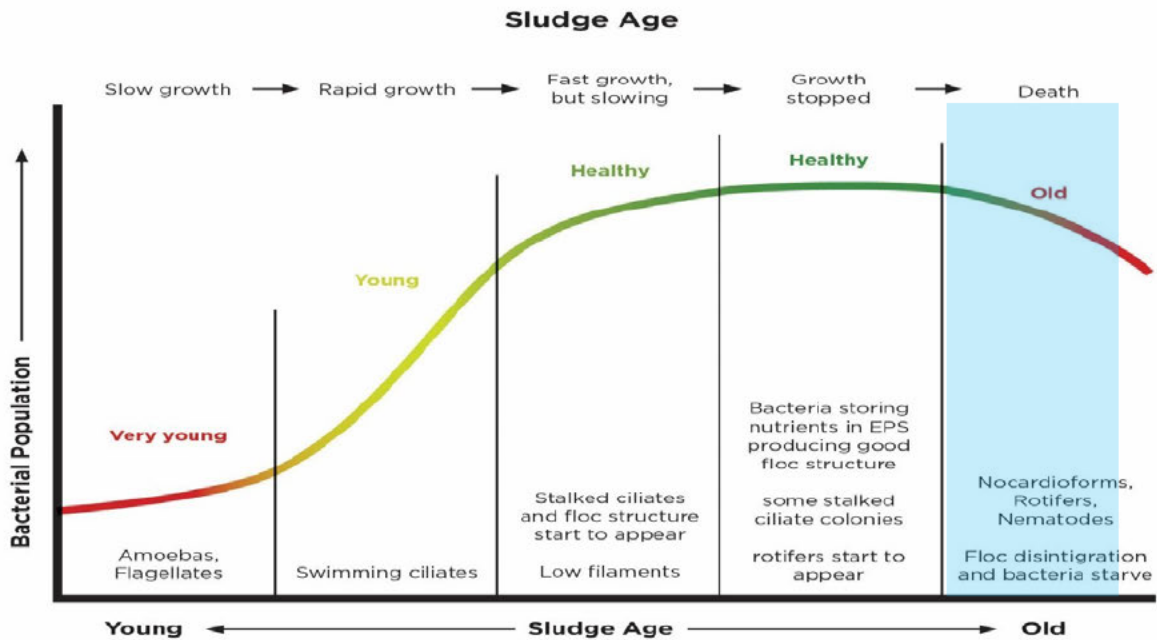
Figure 30: Results of "Mixed Liquor: [redacted]" 100 mL, 30-minute settling test. Zero settling observed in either beaker. Solids appear to be floating in the Mixed Liquor: [redacted] due to high levels of filaments.

Summary:

Floc in both Mixed Liquor samples were primarily open in structure and irregular in shape. These open structures were permitting good oxygen penetration to occur. Due to the high levels of filamentous bacteria creating interfloc bridging, determining the average floc size was difficult. Floc structures were likely further being influenced by the high sludge age. When a sludge age is high, floc begin consuming extracellular polymeric substances (EPS) in place of the limited available nutrients. Since EPS acts as a glue like substance, these decreasing levels result in floc beginning to break apart and disintegrate. A high sludge age can be supported by the presence of moderate amounts of low F:M filaments, rotifers, and Nocardia-like organisms.

Low to moderate amounts of stalked ciliates, with an occasional larger stalked ciliate colony were observed in the mixed liquor samples. Stalked ciliates are typically observed in healthy sludge, however larger colonies are indicative of an older sludge age. Low to moderate levels of rotifers were observed. Rotifers consume dead and decomposing organic matter and are usually characteristic of a older sludge age. Moderate levels of gastrotrichs were observed. Gastrotrichs are a relatively rare organism that can be found in all wastewater plant conditions with older aged sludge. Low to moderate amounts of plant/ paper fibers were also observed throughout the samples. Plant and paper fibers can add structure to sludge interfering with settling properties. However, they do not appear abundant enough to be an issue at this time.

When viewed under phase contrast the floc were mostly white in color with few tan areas. This is indicative of good oxygen penetration into the floc. Moderate levels of EPS were observed within floc. EPS is a glue-like substance that helps floc forming bacteria stick together and form strong floc. EPS was also observed diffusing from floc. Diffused EPS tends to be a sign of stress in a wastewater system.



(The blue shaded area on the diagram above represents the approximant sludge age in the [REDACTED])

Microscopic Observations: Mixed Liquor: [REDACTED]

Rank	Filament	Abundance	Cause
1	Nocardia-like organisms	Moderate to high	Older Sludge Age, High FOG
2	Type 0092	Moderate to high	High FOG & Warm Temperatures
3	Type 0041/0675	Moderate	Low F:M
4	<i>Thiothrix</i> /Type 021N	Low to moderate	Low D.O., Nitrogen deficiency, incoming septicity, and/or sulfur reducing conditions

Microscopic Observations: Foam: [REDACTED]

Rank	Filament	Abundance	Cause
1	Nocardia-like organisms	Very high	Older Sludge Age, High FOG
2	Type 0092	Moderate to high	High FOG & Warm Temperatures
3	Type 0041/0675	Low to moderate	Low F:M

The presence of Nocardia-like organisms in the mixed liquor samples indicate that these filaments are currently growing within the [REDACTED] not just concentrated in the foam. When abundant enough, Nocardia-like organisms form a mat-like structure that floats to the surface due to its low-density fatty cell walls. This structure then helps contribute to the formation of strong foam. Nocardia-like organisms can be controlled by reducing the sludge age and managing levels of fats, oils, and grease. If Nocardia-like organisms are left untreated, excessive foam can quickly result from unchecked Nocardia-like organism growth.

Type 0092 prefers conditions with high levels of incoming fats, oils, and greases (FOG) as well as in temperatures above 65° F. If Type 0092 is observed in a system, it is a strong indication that *Microthrix parvicella* will be present in the system if temperatures drop below 65° F. This is because *M. parvicella* and Type 0092 appear to use similar food substrates. Therefore, if you observe high levels of Type 0092 in the fall you are very likely to see the population shift to high levels of *M. parvicella* in the winter as temperatures begin to cool. An abundance of *M. parvicella* can lead to foaming. Type 0092 was present in all samples.

Within the mixed liquor samples moderate levels of Type 0041/0675 were observed. Type 0041/0675 can cause bulking to occur, however they are primarily an indication of low F:M conditions. It is common to see filamentous bacteria like Type 0041/0675 in systems with an abundance of Nocardia-like organisms. Nocardia-like organisms are able to rapidly uptake BOD. This then creates the ideal low F:M conditions for Type 0041/0675 filaments to grow.

The current low to moderate levels of *Thiothrix* / Type 021N are not of concern at this time.

ATP: Mixed Liquor: [REDACTED]

Date	tATP	dATP	TSS/MLSS	cATP	BSI	ABR
11/16/2022	4066.65 ng/ml	158.82 ng/ml	2200 mg/L	3907.84 ng/ml	3.91%	88.81%

ATP: Mixed Liquor: [REDACTED]

Date	tATP	dATP	TSS/MLSS	cATP	BSI	ABR
11/16/2022	1350.67 ng/ml	110.68 ng/ml	1030 mg/L	1239.98 ng/ml	8.19%	60.19%

Overall, ATP results show relatively high levels of active biomass and low levels of stress on the mixed liquor bacteria. Active Biomass Ratio (ABR) measures the percent mass of live bacteria in a wastewater system. Typically, a healthy active biomass ratio is above 25%. This suggests that levels of inert material are low in the [REDACTED] as the ABR% in the Mixed Liquor: [REDACTED] sample was 88.81% and the ABR% was 60.19% in the Mixed Liquor: [REDACTED] sample.

The Biological Stress Index (BSI) represents the ratio of dissolved ATP versus total ATP. This shows what percentage of bacteria present are currently dying in the mixed liquor samples. Generally, a BSI% above 30% indicates significant stress on a wastewater bacterial population. The BSI% can increase in shipping to some extent, but these numbers suggest that bacterial death is happening relatively slowly in the [REDACTED]. Generally, BSI% numbers are more useful if a baseline has been established in a system to compare to. Relatively rapid bacterial death can reflect an aging sludge population or mildly toxic environmental conditions. We observed a lower BSI% in Mixed Liquor: [REDACTED] versus the Mixed Liquor: [REDACTED] (3.91% versus 8.19%). Both numbers indicate a mildly stressed system rather than a severely stressed system.

Recommendation(s):

- We recommend increasing wasting to lower the sludge age in the [REDACTED] and to help stimulate the growth of new floc forming bacteria. This can also help prevent Nocardia-like organisms from increasing in prevalence.
- We recommend the addition of Qwik-Zyme L. Qwik-Zyme L helps to break down fats into short-chain fatty acids which can be more easily degraded by floc forming bacteria. This allows floc forming bacteria to compete more effectively against Nocardia-like organisms and Type 0092.
- We recommend the addition of Foam Buster. Foam Buster contains a blend of proteins, amino acids, and micronutrients that help simulate the uptake of short-chain fatty acids by floc forming bacteria. This helps them to compete more effectively with filamentous organisms.
- If possible, we recommend mechanical removal of foam to speed the treatment process.
- We recommend the addition of Qwik-Zyme D as a boost to help degrade plant/paper fibers. Qwik-Zyme D is a biocatalyst product that assists in the degradation of plant and paper fibers which contribute to poor settling properties, and fats, oils, and greases which contribute to overloading.