The Microbiology of Wastewater Treatment

Life in the Aeration Tank: Bacteria, Protozoa, and Metazoa

USEPA Webinar Series

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Ohio EPA Compliance Assistance Unit
Why Does My Microscopic Work Better than Yours?
Fig. 1. Phase Contrast Microscopy

Specimen

Condenser lens
Objective lens

Direct light
Diffracted light

Image

Ring aperture
Phase plate
Why Perform a Microscopic Analysis?
Essential Resources
Floc Structure
Dispersed Bacteria
Beginning of Flocculation
Dense, Compact Flocs
Internal Filaments
Low Density Flocs
Extending Filaments
Interfloc Bridging
Too Much Mass
Too Much Filament
The Protozoa and Metazoa
Amoeba
Flagellates
Crawling Ciliates
Stalked Ciliates
Stalked Ciliates
Vaginicola
Suctoria
Rotifers
Nematodes
Bristleworm
Paramecium
Water Bear
The Filamentous Bacteria
Filamentous Bacteria

- Filaments grow in under specific conditions
  - Low F/M
  - Low DO
  - Oil and Grease
  - Septicity, sulfides
  - Nutrient Deficiency (usually industrial treatment)
Filamentous Bacteria Commonly Found in WWTPs

Low F/M:
- Type 0041
- Type 0675
- Type 1851
- Type 0803

Oil and Grease:
- Microthrix parvicella
- Nocardia spp.
- Type 1863

Low DO:
- Sphaerotilus natans
- Type 1701
- Haliscomenobacter hydrossis

Septicity:
- Type 021N
- Thiothrix I and II
- Beggiatoa
- Type 0961
- Type 0581
- Type 0411
- Type 0092
- Nostocoida limicola I, II, and III
- Type 0914

Nutrient Deficiency:
- Type 021N
- Thiothrix I and II
- Nostocoida limicola III
- Haliscomenobacter hydrossis
Filamentous Bacteria

• Filaments don’t lie:
  – If you can identify the dominant filaments...
    ...you can identify the growth conditions in the reactors
    ...and you can change the conditions in the reactors
    ...and eliminate the problem
**Type 0041**

- **Growth Conditions:**
  - Low F/M
  - Slowly degradeable (particulate) BOD

- **Response:**
  - Increase Wasting

(Note: Neisser negative difficult to see)
Type 1851

• Growth Conditions:
  – Low F/M
  – Complete mix basin

• Response:
  – Increase Wasting
  – Plug flow / Selector

(Note: Neisser negative difficult to see)
Microthrix Parvicella
Microthrix Parvicella

• Growth Conditions:
  – Oil and Grease (lipids); High Carbon Chain Fatty Acids
  – Low F/M
  – Low DO
  – Cold water temperature

• Response:
  – Oil and Grease control (primary clarifier)
  – Foam trapping eliminated
  – Increase Wasting
  – Maintain adequate DO

  (Note: Neisser positive granules occur)
Nocardia
Nocardia

• Growth Conditions:
  – Fats, Oil and Grease (lipids)
  – Foam trapping
  – Lower organic loading (Low F/M environment)
  – Low aeration tank pH

• Response:
  – Oil and Grease control (primary clarifier)
  – Foam trapping eliminated
  – Increase in-tank pH
  – Waste...a lot

(Note: Neisser positive granules occur)
Foam Trapping

Low pH

### TABLE 3.6

**Relationship of Specific Filamentous Organisms to MCRT and F/M in Activated Sludge**

<table>
<thead>
<tr>
<th>MCRT, d</th>
<th>1.9</th>
<th>2.2</th>
<th>2.5</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>8.0</th>
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<tbody>
<tr>
<td>F/M*</td>
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<td>0.7</td>
<td>0.6</td>
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<td>0.3</td>
<td>0.2</td>
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**Type 1701**
**S. natans**
**H. hydrossis**
**Thiothrix spp.**
**Type 021N**

**Nocardioforms**
**Type 0411**
**N. linicola** II
**Type 1863**
**Type 0041**
**Type 0675**
**M. parvicella**
**Type 0092**
**Type 1851**
**Type 0914**
**Type 0803**
**Type 0581**

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*F/M as kg BOD₅/kg MLSS, d.  

Type 0092

• Growth Conditions:
  – Septicity
  – Breakdown of biomass (clarifier full of solids?)
  – Low F/M environment

• Response:
  – Optimize clarifier operation
  – Optimize digester operation
  – Increase wasting

(Note: Gram negative difficult to see)
Thiothrix
Thiothrix

• Growth Conditions:
  – Septicity, low molecular weight organic acids
  – Sulfides
  – Typically higher F/M environment

• Response:
  – Remove sources of septicity (long forcemains, excessive clarifier sludge blankets, digester decant)
  – Preaeration
Type 021N

• Growth Conditions:
  – Septicity (low molecular weight organic acids)
  – Wide range of F/M environments

• Response:
  – Remove sources of septicity (long forcemains, excessive clarifier sludge blankets, digester decant)
  – Anoxic Selector
Type 0961

• Growth Conditions:
  – Septicity (low molecular weight organic acids)
  – Lower F/M
  – Not very common in domestic wastewater

• Response:
  – Remove sources of septicity
  – Decrease MCRT (waste)
Beggiatoa
• Growth Conditions:
  – Sulfides in wastestream
  – Lower Dissolved Oxygen

• Response:
  – Preaerate to remove hydrogen sulfides in wastestream
Nostocoida Limicola

• Growth Conditions:
  – Septicity (low molecular weight organic acids)
  – Wide range of F/M
  – Nutrient Deficiency

• Response:
  – Investigate source of septicity and organic acids
    • Digesters, force mains, food processing sources

(Note: Gram and Neisser negative occur)
Type 0914

• Growth Conditions:
  – Septicity (low molecular weigh organic acids)
  – Sulfides
  – Low F/M

• Response:
  – Eliminate septicity
  – Eliminate sulfides
Sphaerotilus natans
Sphaerotilus natans

• Growth Conditions:
  – Low Dissolved Oxygen
    • Low DO for the applied load
    • Low DO in the interior of the floc

• Response:
  – Increase bulk Dissolved Oxygen in Aeration
Type 1701

• **Growth Conditions:**
  – Low Dissolved Oxygen in Aeration Tank
  – Wide range of F/M

• **Response:**
  – Increase Dissolved Oxygen in Aeration Tank
Haliscomenobacter hydrosis
Haliscomenobacter hydrosis

• Growth Conditions:
  – Septicity
  – Low dissolved oxygen
  – High influent nitrogen (ammonia)
  – Wide range of F/M

• Response:
  – Remove sources of septicity (long forcemains, excessive clarifier sludge blankets, digester decant)
  – Increase dissolved oxygen in aeration tanks
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- *H. hydrossis*
- *Thiothrix* spp.
- Type 021N
- Nocardioforms
- Type 0411
- *N. limicola II*
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*F/M as kg BOD$_5$/kg MLSS, d.*

22 Filamentous Bacteria Found in WWTPs

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5 Filament Growth Environments

- Filaments grow in under specific conditions
  - Low F/M
  - Low DO
  - Oil and Grease
  - Septicity, sulfides
  - Nutrient Deficiency (usually industrial treatment)
Staining Techniques: Reveal What is Hidden
Why Stain the Bacteria?
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<th>Neisser Positive</th>
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Tools for Staining

• Microscope
  – Bright field (not phase contrast!)
  – Preferably 1000x oil immersion objective
  – Minimum of 200x objective
Tools for Staining

- Microscope Slides
- Clothes pin
- Wash bottle
- Watch with a second hand
- Paper towel
- Sink (preferably a black lab sink) or roasting pan
- Bon Ami Scouring Powder (optional)
Tools for Staining

• Gram Stain Kit
  – Gram Crystal Violet Solution
  – Gram Iodine Solution
  – Gram Decolorizing Solution
  – Gram Safranin Solution
Gram Staining Procedure

1. Gram Crystal Violet Solution
   • Flood slide for 1 minute
   • Rinse with DI water

2. Gram Iodine Solution
   • Flood slide for 1 minute
   • Rinse with DI water

3. Gram Decolorizing Solution
   • Hold slide at 45 degrees and apply dropwise until blue color stops rinsing off (15-20 seconds max)
   • Blast with DI water to stop reaction, blot dry with paper towel

4. Gram Safranin Solution
   • Flood slide for 1 minute
   • Rinse with DI water

5. View Slide at 1000x under bright light (not phase contrast)
Microthris parvicella, Gram Stain 1000x
Tools for Staining

- Neisser Stain Kit
  - Neisser Methyl Blue Solution A
  - Neisser Crystal Violet Solution B
  - Neisser Bismark Brown Solution
  - Transfer pipet
  - Container for mixing Solutions A and B
  - And the Clothes Pin, of course
Neisser Staining Procedure

1. Methyl Blue / Crystal Violet Solution
   - Mix 2 parts Methyl Blue and 1 part Crystal Violet in a small container
   - Flood slide for 30 seconds
   - Rinse with DI water

2. Bismark Brown Solution
   - Flood slide for 1 minute
   - Rinse with DI water and blot dry (do not rub the slide)

3. View Slide at 1000x bright light (not phase contrast)
Mircrothrix parvicella, Neisser Stain, 1000x
Why Stain the Bacteria?

1) Staining is actually very easy
2) Staining bacteria will help determine what is growing
3) Staining will show what is hidden in a wet mount
4) Staining can be effective if a phase contrast microscope is not available
Questions?

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