MICROSCOPY and ACTIVATED SLUDGE PROCESS CONTROL

Mackenzie L. Davis

MWEA Process Seminar
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CREDITS
Identification & Control of Filamentous Bacteria

Toni Glymph, Wastewater Microbiologist
ToniGlymph@msn.com
MANUAL on the CAUSES and CONTROL of ACTIVATED SLUDGE BULKING, FOAMING, and OTHER SOLIDS SEPARATION PROBLEMS

David Jenkins
Michael G. Richard
Glen T. Daigger

LEWIS PUBLISHERS
Activated Sludge Microscopy – Notes & Thoughts

• The ultimate objective of activated sludge microscopy is to identify potential filamentous bacterial causes of solids separation performance problems.
• Microscopy is only one tool.
• The objective of this talk is to make you aware of some of the typical microbial culprits that may be diagnosed with a microscope AND other operational data.
Activated Sludge Microscopy – Notes & Thoughts

• This lecture **will not** explain the mechanics of microscope operation or staining techniques… Refer to WEF text on microscopy.

• This lecture **WILL NOT** make you an expert in activated sludge microscopy!!
FOCUS

BULKING & FOAMING SLUDGE
Aeration tank in service. Faded “orange” pipes are air headers.
Influent to activated sludge aeration tank from primary settling tank and return activated sludge (RAS) from secondary settling tank. This photo was taken in the 1970s before restrictions were placed on using phosphorus builders in detergents.
Oxidation ditch scum baffle and effluent structure. Foam is accumulation of bubbles that have frozen (note white ice layer near scum baffle). When the air temperature rises above 0°C the scum “melts” and dissipates.
Aeration tank in service. Start up problems with *Nocardia* foam.
FACTORS AFFECTING SLUDGE BULKING

• Design Limitations
• Wastewater Characteristics
• Operational Issues
DESIGN LIMITATIONS

- Poor Mixing
- Clarifier Design
- Limited Return Sludge Capacity
- Process Loading
- Internal Plant Overloading
- Limited air supply
Microscopy **CANNOT** Help Identify These Problems
WASTEWATER CHARACTERISTICS

- Flowrate Variations
- Composition/characteristics
- Industrial waste component/composition
- Animal and vegetable FOG
- Sulfur compounds
- pH
- Temperature
- Nutrients
Microscopy CAN Help Identify Composition/Characteristics Problems
OPERATIONAL ISSUES

• MCRT
• Low F/M
• Low Dissolved Oxygen
• Nutrient Deficiency (nitrogen and phosphorus)
Microscopy **CAN** Help Identify These Issues
Causes of Bulking Sludge and Likely Suspects
<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>
<th>20</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/M&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
<td>0.05</td>
</tr>
</tbody>
</table>

- *Type 1701*
- *S. natans*
- *H. hydrossis*
- *Thiothrix spp.*
- *Type 021N*
- *Nocardioforms*
- *Type 0411*
- *N. limicola II*
- *Type 1863*
- *Type 0041*
- *Type 0675*
- *M. parvicella*
- *Type 0092*
- *Type 1851*
- *Type 0914*
- *Type 0803*
- *Type 0581*

<sup>a</sup> F/M as kg BOD<sub>5</sub>/kg MLSS, d.

MCRT VS F/M

MCRT vs F/M

F/M kg BOD/kg MLSS-d

MCRT, d

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9
<table>
<thead>
<tr>
<th>Cause</th>
<th>Filamentous Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low DO concentration</td>
<td><em>S. natans</em></td>
</tr>
<tr>
<td></td>
<td>Type 1701</td>
</tr>
<tr>
<td></td>
<td><em>H. hydrossis</em></td>
</tr>
<tr>
<td>Low F/M</td>
<td>Type 0041</td>
</tr>
<tr>
<td></td>
<td>Type 0675</td>
</tr>
<tr>
<td></td>
<td>Type 1851</td>
</tr>
<tr>
<td></td>
<td>Type 0803</td>
</tr>
<tr>
<td>Elevated low molecular weight organic acid concentration</td>
<td>Type 021N</td>
</tr>
<tr>
<td></td>
<td><em>Thiothrix</em> I and II</td>
</tr>
<tr>
<td></td>
<td><em>N. limicola</em> I, II and III</td>
</tr>
<tr>
<td></td>
<td>Type 0914</td>
</tr>
<tr>
<td></td>
<td>Type 0411</td>
</tr>
<tr>
<td></td>
<td>Type 0961</td>
</tr>
<tr>
<td></td>
<td>Type 0581</td>
</tr>
<tr>
<td></td>
<td>Type 0092</td>
</tr>
<tr>
<td>Hydrogen sulfide</td>
<td><em>Thiothrix</em> I and II</td>
</tr>
<tr>
<td></td>
<td>Type 021N</td>
</tr>
<tr>
<td></td>
<td>Type 0914</td>
</tr>
<tr>
<td></td>
<td><em>Beggiatoa</em> spp.</td>
</tr>
<tr>
<td>Nutrient deficiency</td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>Type 021N</td>
</tr>
<tr>
<td>Phosphorus</td>
<td><em>Thiothrix</em> I and II</td>
</tr>
<tr>
<td></td>
<td><em>N. limicola</em> III</td>
</tr>
<tr>
<td></td>
<td><em>H. hydrossis</em></td>
</tr>
<tr>
<td></td>
<td><em>S. natans</em></td>
</tr>
<tr>
<td>Low pH</td>
<td>Fungi</td>
</tr>
</tbody>
</table>
MOST UNWANTED FOR BULKING AND/OR FOAMING
<table>
<thead>
<tr>
<th>Rank</th>
<th>Filamentous Organism</th>
<th>Percentage of Treatment Plants with Bulking or Foaming in Which Filaments Were:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dominant</td>
</tr>
<tr>
<td>1</td>
<td>Nocardioform organisms</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>Type 1701</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>Type 021N</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>Type 0041</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td><em>Thiothrix</em> spp.</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td><em>Sphaerotilus natans</em></td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td><em>Microthrix parvicella</em></td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>Type 0092</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td><em>Haliscomenobacter hydrossis</em></td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>Type 0675</td>
<td>7</td>
</tr>
<tr>
<td>11</td>
<td>Type 0803</td>
<td>6</td>
</tr>
<tr>
<td>12</td>
<td><em>Nostocoida limicola</em> (types I, II and III)</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>Type 1851</td>
<td>6</td>
</tr>
<tr>
<td>14</td>
<td>Type 0961</td>
<td>4</td>
</tr>
<tr>
<td>15</td>
<td>Type 0581</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td><em>Beggiaota</em> spp.</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>Fungi</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>Type 0914</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: Combined results of Richard et al. (1982) and Strom and Jenkins (1984); 525 samples from 270 treatment plants.
Nocardioform Organisms

Nocardioform organisms include: 
*Gordonia amarae*, 
*Microthix parvicella* and the 
Following pathogens: 
*N. caviae, N brasiliensis, N. asteroides*, and strains of 
*Mycobacterium* and 
*N. farcinica*
MUG SHOTS OF TOP TEN MOST UNWANTED FOR BULKING AND/OR FOAMING
Gram Stain

- Positive (+) => purple
- Negative (-) => pink
No. 1: Nocardia

Photomicrograph of *Nocardia* showing short gram-positive filaments with true branching.
No. 2: Type 1701
No.3: Type 021N
No.4: Type 0041

Photomicrograph of type 0041. Notice attached growth typical of type 0041.
No. 5: Thiothrix

Note: “Thio” = sulfur; sulfur granules are characteristic
No.6: *Sphaerotilus natans*

Photomicrograph of *Sphaerotilus natans*. Notice the false branching.
No.7: Microthrix parvicella

Photomicrograph of Microthrix parvicella. M. parvicella typically stains gram positive.
No. 8: Type 0092

Photomicrograph of type 0092.
No.9: *Haliscomenobacter hydrosis*
No.10 Type 0675
Use the “Dichotomous Key for Identification of Filamentous Organisms”
Gram Stain

- Positive (+) => purple
- Negative (-) => pink
Neisser stain

- Positive (+) = bluish color
- Negative (-) = brownish color
Sulfur granules

Filaments contain sulfur granules in situ or after applying the S test

Sulfur granules "square"

Sulfur granules "spherical"

Gram positive

Strongly Gram positive

True branching

Nocardioforms

No sheath

Motile

Type 021N

Beggiaota spp.

Cell dia. 0.8-1.0 μm

N. limicola I

N. limicola II

N. limicola III

Cell septa present

Cell dia. 1.4 μm

Cell dia. 2.0 μm

M. parvicella

Attached growth; cells square

Type 0675

Cell dia. ≤ 1.0 μm

Type 1851

Cell dia. > 1.0 μm; cell septa present

Cell dia. 1.4 μm

Trichome coiled

Cell dia. 2.0 μm

Trichome straight or smoothly curved

Cell dia. ≤ 1.0 μm

N. limicola II

N. limicola III

Sheath

Cell dia. 1.6 μm; usually false branching

Cell dia. 0.8-1.2 μm; usually attached growth

S. natans

Type 0914

Neisser positive

Neisser positive; inside floc

Type 0092

Cells rectangular; "transparent"

Cells barrel; rectangular or discoid; cell dia. 2.0-3.0 μm; indentations at septa

Type 0961

Neisser negative

Trichome straight, smoothly curved or bent

Cell dia. 0.5 μm; no cell septa

H. hydrossis

Sheath; usually attached growth

Type 1701

Cell septa present

No sheath; no attached growth

Type 0803

Trichome irregularly bent "chain of cells"

No cell septa; trichome coiled

Type 0581

Trichome elongated sausages; cell dia. 0.4-0.6 μm; free in suspension

Cells oval; cell dia. 0.4-0.6 μm

Type 0411

Cells oval;

Type 0211

FIGURE 2.20 Dichotomous key for the identification of filamentous organisms in activated sludge.

Source: Jenkins
Sulfur Granules

Beggiatoa
Gram Stain

Gram (+) Nocardia sp.

Gram (-) Type 1701
Neisser Stain

Neisser (+) 
*Nostocoida limicola*

Neisser (+) granules
Neisser Stain

Neisser (+) Type 0092

Neisser (+) Nostocoida limicola and Neisser (-) Thiothrix
Nocardia
Early Stage Nocardia
Nocardia

Mid - Stage Nocardia
Nocardia

Mid - Stage Nocardia
Nocardia

Late Stage Nocardia
Nocardia
Late Stage Nocardia
Way Past Time to Take Corrective Action!!!
Run for the Hills Foaming is Out of Control!!!
OUT TAKES
MICROSCOPY METHODOLOGY
Sampling point

• Good mixing – end of aeration basin or mixed liquor channel between aeration basin and secondary settling clarifier

Take mixed liquor samples from below surface to exclude foam
Sampling point

• Sample foam from one of following points
• (1) Surface of effluent end of aeration basin
• (2) Surface of mixed liquor channel
• (3) Surface of secondary clarifier
Sampling Frequency

• (1) Routine on site examination
  • About once every MCRT
• (2) Routine off-site laboratory
  • Weekly to monthly
• (3) Daily for critical periods
  • (a) When bulking occurs
  • (b) During RAS chlorination for bulking control
Sample Transport and Storage

1. Examine as soon as possible after collection but not more than several hours.
2. For more lengthy periods store at 4°C.
   - Low F/M; High MCRT – within 7-10 days
   - High F/M; Low MCRT – within 3 to 4 days
3. Transport in small plastic containers (not glass).
4. Do not fill containers more than half full.
Microscope

• Use a research grade, phase contrast microscope with 10x and 100x (oil immersion) phase contrast objectives that yield magnifications of approximately 100x and 1000x respectively. Use phase contrast because biological materials have very low contrast when viewed with direct illumination.

• COST = about $5,000