WHAT’S LIVING IN YOUR ACTIVATED SLUDGE

Presenter:
Tom Fitzwilliams
MSA Professional Services, Inc.
Outline

» Microbial Identification
  • Culturing
  • Microscope
  • DNA Testing
  • DNA Applications

» Case Study: Ho-Chunk WWTF
Moving Beyond Culture ID

» Less than 1% of bacteria will grow in culture
» Requires days
Moving Beyond the Microscope

» Many bacteria cannot be identified visually
» Multiple species look identical
» Some species morph between shape based on environmental conditions
DNA Sequencing

[Diagram of DNA structure with base pairs and sugar phosphate backbone highlighted]

U.S. National Library of Medicine
Example DNA Data

>594682
TACGGAGGATCCAAGCGTTATCCGGGATTATTGCTGTTAAGGATTTGAGCTGTACGATGGCGGAGCCCG
TCAGTCTAGTGTGGAAGGAGTTTGCAGCTTAACCTGTAAAATTTGCGCCATTGAAAACCTAGGCTGGTTCTT
GAGTGTAATCAGGAGGGCGGAATGCTTTGTTCTGTAAGCGGTGGAATGCCTAGATATAACACA
GAACACCAAATTGCAGGAGGCGAGCTTACTGGGATACCAACTGACGCTGAGGCGCAACGAAAGCCGTG
GGGATCAAACAGGG

>594511
TACGGGAAGGTCCCGGGCTGGATTTTATGGGTTTTAAAGGAACGCAGGCTGGGAATG
TAAGCGTGCTGTGAAATGTACCGGCTCAACCCGGGTACGTCAGCGCGAAGCTGGCTTCTT
GAGTGAGTACGACGTCAGCGGAAATTGCTTGTGTAGCGGTGGAATGCTTTAGATATACGAA
GAACCTCCGATTGCGAAGGCAGCTGAGCAGCCCTGTTACTGACGCTAAAGCAGCAAGGCTGCG
GGTATCGAACAGGG

>594448
TACGTAGGGGGCGACGGTTATCCGGGATTATTGCTGTTAAGGCTGCTTACGATGGCGGAGCCGGTTG
TAAGTCCGGATGTGGAATCTCCACGGTCAACCAGGAGGGGCTTCCGGGTACGCTGCAGCGCAGGATGAC
AGAGTCCGGTAGGGAATGCTTCCCGGGTGTAGCGGTGGAATGCCTGAGCAGAGATCGGGAG
GGAACACCGCTAGCAGGAGGCGATTCTGCTGGGCGCTGACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGG

GGGGAGCGAACAGG
Applications

» Drinking water

» Surface Waters
  • Source tracking of fecal pollution

» Remediation
  • Track populations of microbial degraders

» Air quality monitoring

» Food and beverage
  • Safety, quality, longevity
Applications

» Wastewater process trouble-shooting
  • Loss of nitrification
  • Loss of Bio-P
  • Poor digester gas production
  • Pathogens in biosolids or effluent
  • Source tracking of fecal pollution
  • Bulking
  • Foaming
Filaments

» Microscope identification
» >1 species present
» Usually beneficial
» Bulking: Too many filaments
» Selectors
» Diverse, complex causes
Foamers

» Foam
  • Hydrophobic particles (bacteria)
  • Surfactants (excreted by bacteria)
  • Bubbles

» Nocardia

» Microthrix

» Low abundance high impact

» Not necessarily filamentous

» Hard to get rid of it!
Ho-Chunk WWTF DNA Data
Ho-Chunk Village WWTF

» Sequencing Batch Reactor facility

» Design Conditions
  • Flow – 0.30 mgd
  • BOD – 1,270 lbs/day

» Current Loadings
  • Flow – 0.06 mgd
  • BOD – 350 lbs/day

» Effluent Limits
  • BOD < 50 mg/L
  • Total N < 10 mg/L (need to nitrify and denitrify)
Ho-Chunk Village WWTF

» Poor settling during cold weather

» High SVI has lead to elevated effluent TSS
DNA Testing Program

» Samples collected weekly April – August 2014

» All testing was done on one reactor (SBR #4)

» Total of 21 samples were frozen and shipped in batches for bacterial assessment
Sample Results - General

» Nitrogen Removers (13 of 27 detected)
» Filaments (9 of 18 detected)
» Foaming (6 of 9 detected)
» Results are in % of total bacteria in a known volume
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodococcus</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.00%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>0.10%</td>
<td>0.10%</td>
<td>0.06%</td>
<td>0.09%</td>
<td>0.06%</td>
<td>0.18%</td>
<td>0.07%</td>
<td>0.10%</td>
<td>0.07%</td>
<td>0.08%</td>
<td>0.12%</td>
</tr>
<tr>
<td>Paracoccus</td>
<td>0.34%</td>
<td>0.45%</td>
<td>0.39%</td>
<td>0.20%</td>
<td>0.36%</td>
<td>0.39%</td>
<td>0.21%</td>
<td>0.32%</td>
<td>0.35%</td>
<td>0.29%</td>
<td>0.06%</td>
</tr>
<tr>
<td>Thauera</td>
<td>0.03%</td>
<td>0.08%</td>
<td>0.06%</td>
<td>0.04%</td>
<td>0.08%</td>
<td>0.14%</td>
<td>0.06%</td>
<td>0.17%</td>
<td>0.17%</td>
<td>0.27%</td>
<td>0.15%</td>
</tr>
<tr>
<td>Dechloromonas</td>
<td>3.15%</td>
<td>0.85%</td>
<td>1.49%</td>
<td>4.09%</td>
<td>3.40%</td>
<td>4.34%</td>
<td>1.42%</td>
<td>2.05%</td>
<td>1.18%</td>
<td>1.44%</td>
<td>1.79%</td>
</tr>
<tr>
<td>Nitrosomonas</td>
<td>0.21%</td>
<td>0.15%</td>
<td>0.21%</td>
<td>0.39%</td>
<td>0.23%</td>
<td>0.27%</td>
<td>0.41%</td>
<td>0.69%</td>
<td>0.38%</td>
<td>0.48%</td>
<td>0.99%</td>
</tr>
<tr>
<td>Nitrosonococcus</td>
<td>0.28%</td>
<td>0.30%</td>
<td>0.31%</td>
<td>0.41%</td>
<td>0.38%</td>
<td>0.41%</td>
<td>0.45%</td>
<td>0.64%</td>
<td>0.80%</td>
<td>0.73%</td>
<td>0.62%</td>
</tr>
<tr>
<td>Nitrobacter</td>
<td>0.06%</td>
<td>0.08%</td>
<td>0.06%</td>
<td>0.05%</td>
<td>0.05%</td>
<td>0.04%</td>
<td>0.08%</td>
<td>0.04%</td>
<td>0.06%</td>
<td>0.05%</td>
<td>0.04%</td>
</tr>
<tr>
<td>Nitospira</td>
<td>0.87%</td>
<td>1.31%</td>
<td>1.72%</td>
<td>1.04%</td>
<td>2.64%</td>
<td>1.57%</td>
<td>1.81%</td>
<td>2.71%</td>
<td>3.42%</td>
<td>3.22%</td>
<td>2.06%</td>
</tr>
<tr>
<td>Nitrospina</td>
<td>0.00%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.02%</td>
<td>0.01%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Thauera</td>
<td>0.03%</td>
<td>0.08%</td>
<td>0.06%</td>
<td>0.04%</td>
<td>0.08%</td>
<td>0.14%</td>
<td>0.06%</td>
<td>0.17%</td>
<td>0.17%</td>
<td>0.27%</td>
<td>0.15%</td>
</tr>
<tr>
<td>Nitratireductor</td>
<td>0.07%</td>
<td>0.09%</td>
<td>0.08%</td>
<td>0.05%</td>
<td>0.05%</td>
<td>0.06%</td>
<td>0.07%</td>
<td>0.05%</td>
<td>0.04%</td>
<td>0.04%</td>
<td>0.02%</td>
</tr>
<tr>
<td>Nitriliruptor</td>
<td>0.01%</td>
<td>0.02%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.00%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.00%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodococcus</td>
<td>0.09%</td>
<td>0.05%</td>
<td>0.04%</td>
<td>0.03%</td>
<td>0.02%</td>
<td>0.03%</td>
<td>0.03%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.01%</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>0.03%</td>
<td>0.02%</td>
<td>0.01%</td>
<td>0.09%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.04%</td>
<td>0.04%</td>
</tr>
<tr>
<td>Paracoccus</td>
<td>0.21%</td>
<td>0.26%</td>
<td>0.24%</td>
<td>0.31%</td>
<td>0.21%</td>
<td>0.24%</td>
<td>0.22%</td>
<td>0.20%</td>
<td>0.23%</td>
<td>0.19%</td>
</tr>
<tr>
<td>Thauera</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.01%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.01%</td>
</tr>
<tr>
<td>Dechloromonas</td>
<td>2.19%</td>
<td>2.30%</td>
<td>2.41%</td>
<td>1.97%</td>
<td>1.42%</td>
<td>1.75%</td>
<td>1.53%</td>
<td>2.19%</td>
<td>1.86%</td>
<td>5.26%</td>
</tr>
<tr>
<td>Nitrosomonas</td>
<td>0.85%</td>
<td>0.66%</td>
<td>0.24%</td>
<td>0.28%</td>
<td>0.42%</td>
<td>0.32%</td>
<td>0.38%</td>
<td>0.24%</td>
<td>0.25%</td>
<td>0.15%</td>
</tr>
<tr>
<td>Nitrosonococcus</td>
<td>0.33%</td>
<td>0.29%</td>
<td>0.26%</td>
<td>0.33%</td>
<td>0.40%</td>
<td>0.44%</td>
<td>0.66%</td>
<td>0.62%</td>
<td>0.66%</td>
<td>0.84%</td>
</tr>
<tr>
<td>Nitrobacter</td>
<td>0.06%</td>
<td>0.07%</td>
<td>0.05%</td>
<td>0.06%</td>
<td>0.05%</td>
<td>0.06%</td>
<td>0.08%</td>
<td>0.04%</td>
<td>0.04%</td>
<td>0.03%</td>
</tr>
<tr>
<td>Nitospira</td>
<td>1.21%</td>
<td>0.56%</td>
<td>1.68%</td>
<td>1.93%</td>
<td>2.50%</td>
<td>2.10%</td>
<td>0.47%</td>
<td>1.80%</td>
<td>0.97%</td>
<td>3.17%</td>
</tr>
<tr>
<td>Nitrospina</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Thauera</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Nitratireductor</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Nitriliruptor</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
</tbody>
</table>
## Filamentous Bacteria Results

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiothrix</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.02%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>0.09%</td>
<td>0.10%</td>
<td>0.03%</td>
<td>0.04%</td>
<td>0.03%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.04%</td>
<td>0.05%</td>
<td>0.07%</td>
<td>0.05%</td>
</tr>
<tr>
<td>Microthrix</td>
<td>0.14%</td>
<td>0.48%</td>
<td>0.41%</td>
<td>0.31%</td>
<td>0.06%</td>
<td>0.48%</td>
<td>1.74%</td>
<td>1.04%</td>
<td>0.44%</td>
<td>0.69%</td>
<td>1.86%</td>
</tr>
<tr>
<td>Runella</td>
<td>0.07%</td>
<td>0.11%</td>
<td>0.11%</td>
<td>0.05%</td>
<td>0.10%</td>
<td>0.08%</td>
<td>0.08%</td>
<td>0.08%</td>
<td>0.11%</td>
<td>0.08%</td>
<td>0.03%</td>
</tr>
<tr>
<td>Gordonia</td>
<td>0.01%</td>
<td>0.02%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
</tr>
<tr>
<td>Haliscomenobacter</td>
<td>0.04%</td>
<td>0.01%</td>
<td>0.03%</td>
<td>0.03%</td>
<td>0.05%</td>
<td>0.03%</td>
<td>0.04%</td>
<td>0.02%</td>
<td>0.03%</td>
<td>0.05%</td>
<td>0.04%</td>
</tr>
<tr>
<td>Leptolinea</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.01%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.03%</td>
<td>0.02%</td>
<td>0.03%</td>
<td>0.02%</td>
<td>0.03%</td>
</tr>
<tr>
<td>Caldilinea</td>
<td>0.25%</td>
<td>0.29%</td>
<td>0.26%</td>
<td>0.28%</td>
<td>0.13%</td>
<td>0.15%</td>
<td>0.27%</td>
<td>0.16%</td>
<td>0.10%</td>
<td>0.11%</td>
<td>0.07%</td>
</tr>
<tr>
<td>Kouleothrix</td>
<td>0.25%</td>
<td>0.37%</td>
<td>0.36%</td>
<td>0.34%</td>
<td>0.60%</td>
<td>0.38%</td>
<td>0.63%</td>
<td>0.74%</td>
<td>0.82%</td>
<td>0.73%</td>
<td>0.80%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiothrix</td>
<td>0.00%</td>
<td>0.01%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.01%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>0.08%</td>
<td>0.06%</td>
<td>0.05%</td>
<td>0.04%</td>
<td>0.04%</td>
<td>0.03%</td>
<td>0.06%</td>
<td>0.08%</td>
<td>0.08%</td>
<td>0.08%</td>
</tr>
<tr>
<td>Microthrix</td>
<td>1.75%</td>
<td>0.37%</td>
<td>1.31%</td>
<td>0.96%</td>
<td>0.14%</td>
<td>0.30%</td>
<td>0.15%</td>
<td>0.04%</td>
<td>0.02%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Runella</td>
<td>0.11%</td>
<td>0.11%</td>
<td>0.10%</td>
<td>0.07%</td>
<td>0.05%</td>
<td>0.04%</td>
<td>0.03%</td>
<td>0.03%</td>
<td>0.03%</td>
<td>0.02%</td>
</tr>
<tr>
<td>Gordonia</td>
<td>0.05%</td>
<td>0.10%</td>
<td>0.03%</td>
<td>0.04%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.02%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
</tr>
<tr>
<td>Haliscomenobacter</td>
<td>0.02%</td>
<td>0.03%</td>
<td>0.05%</td>
<td>0.07%</td>
<td>0.09%</td>
<td>0.08%</td>
<td>0.07%</td>
<td>0.19%</td>
<td>0.12%</td>
<td>0.09%</td>
</tr>
<tr>
<td>Leptolinea</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Caldilinea</td>
<td>0.53%</td>
<td>0.33%</td>
<td>0.34%</td>
<td>0.32%</td>
<td>0.23%</td>
<td>0.22%</td>
<td>0.24%</td>
<td>0.22%</td>
<td>0.21%</td>
<td>0.21%</td>
</tr>
<tr>
<td>Kouleothrix</td>
<td>1.34%</td>
<td>1.92%</td>
<td>1.67%</td>
<td>2.14%</td>
<td>1.54%</td>
<td>1.91%</td>
<td>1.76%</td>
<td>1.84%</td>
<td>1.86%</td>
<td>1.72%</td>
</tr>
</tbody>
</table>
# Foaming Bacteria Results

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidimicrobiales</td>
<td>1.69%</td>
<td>2.69%</td>
<td>1.90%</td>
<td>1.62%</td>
<td>0.88%</td>
<td>1.28%</td>
<td>3.17%</td>
<td>1.78%</td>
<td>1.32%</td>
<td>1.36%</td>
<td>2.74%</td>
</tr>
<tr>
<td>Microthrix</td>
<td>0.14%</td>
<td>0.48%</td>
<td>0.41%</td>
<td>0.31%</td>
<td>0.06%</td>
<td>0.48%</td>
<td>1.74%</td>
<td>1.04%</td>
<td>0.44%</td>
<td>0.69%</td>
<td>1.86%</td>
</tr>
<tr>
<td>Rhodococcus</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.00%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Actinomycetales</td>
<td>1.25%</td>
<td>1.35%</td>
<td>1.01%</td>
<td>1.07%</td>
<td>1.10%</td>
<td>0.79%</td>
<td>1.25%</td>
<td>0.45%</td>
<td>0.51%</td>
<td>0.73%</td>
<td>0.55%</td>
</tr>
<tr>
<td>Gordonia</td>
<td>0.01%</td>
<td>0.02%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
</tr>
<tr>
<td>Mycobacterium</td>
<td>0.03%</td>
<td>0.03%</td>
<td>0.03%</td>
<td>0.03%</td>
<td>0.03%</td>
<td>0.04%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.04%</td>
<td>0.02%</td>
<td>0.02%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidimicrobiales</td>
<td>2.02%</td>
<td>1.39%</td>
<td>1.71%</td>
<td>1.71%</td>
<td>1.52%</td>
<td>1.61%</td>
<td>2.42%</td>
<td>1.66%</td>
<td>1.09%</td>
<td>0.70%</td>
</tr>
<tr>
<td>Microthrix</td>
<td>1.75%</td>
<td>0.37%</td>
<td>1.31%</td>
<td>0.96%</td>
<td>0.14%</td>
<td>0.30%</td>
<td>0.15%</td>
<td>0.04%</td>
<td>0.02%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Rhodococcus</td>
<td>0.09%</td>
<td>0.05%</td>
<td>0.04%</td>
<td>0.03%</td>
<td>0.02%</td>
<td>0.03%</td>
<td>0.03%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.01%</td>
</tr>
<tr>
<td>Actinomycetales</td>
<td>1.72%</td>
<td>1.37%</td>
<td>1.02%</td>
<td>1.00%</td>
<td>0.69%</td>
<td>0.63%</td>
<td>0.82%</td>
<td>0.61%</td>
<td>0.34%</td>
<td>0.30%</td>
</tr>
<tr>
<td>Gordonia</td>
<td>0.05%</td>
<td>0.10%</td>
<td>0.03%</td>
<td>0.04%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.02%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
</tr>
<tr>
<td>Mycobacterium</td>
<td>0.11%</td>
<td>0.12%</td>
<td>0.08%</td>
<td>0.10%</td>
<td>0.07%</td>
<td>0.07%</td>
<td>0.05%</td>
<td>0.03%</td>
<td>0.03%</td>
<td>0.02%</td>
</tr>
</tbody>
</table>
Filamentous Bacteria Results

Filamentous Bacteria

Percent of Total Bacteria

April-14 | May-14 | June-14 | July-14 | August-14 | September-14

- Thiothrix
- Acinetobacter
- Microthrix
- Runella
- Gordonia
- Haliscomenobacter
- Leptolinea
- Caldilinea
- Kouleothrix
Filamentous Bacteria vs F:M Ratio

- Microthrix
- Kouleothrix
- F:M
Filamentous Bacteria Results

Filamentous Bacteria vs SRT

- Microthrix
- Kouleothrix
- SRT (days)
Filamentous Bacteria Results

Filamentous Bacteria vs SVI

- **X-axis**: Months from Apr-14 to Sep-14
- **Y-axis**: Percentage of Total Bacteria (0.0% to 5.0%)
- **Legend**:
  - Microthrix
  - Kouleothrix
  - SVI

The graph shows the fluctuation of filamentous bacteria types and their impact on the Sludge Volume Index (SVI) over the months from April to September.
Foaming Bacteria Results

Foaming Bacteria

<table>
<thead>
<tr>
<th>Month</th>
<th>Acidimicrobiales</th>
<th>Microthrix</th>
<th>Rhodococcus</th>
<th>Actinomycetales</th>
<th>Gordonia</th>
<th>Mycobacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr-14</td>
<td>1.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>May-14</td>
<td>1.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Jun-14</td>
<td>3.0%</td>
<td>2.0%</td>
<td>0.5%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Jul-14</td>
<td>2.0%</td>
<td>1.5%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Aug-14</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Sep-14</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>
Foaming Bacteria Results

Foaming Bacteria vs Temperature

- Acidimicrobiales
- Microthrix
- Actinomycetales
- Temp (Celsius)

[Graph showing the percentage of total bacteria over time, with a temperature axis on the right.]
Foaming Bacteria Results
Foaming Bacteria Results

Foaming Bacteria vs SRT

- Acidimicrobiales
- Microthrix
- Actinomycetales
- SRT (days)
Foaming Bacteria Results

Foaming Bacteria vs SVI

- Acidimicrobiales
- Microthrix
- Actinomycetales
- SVI

Graph showing the percentage of total bacteria and sludge volume index over time from April to September 2014.
Results - Summary

» Seasonal changes in Temperature, F:M, SRT caused bacteria population shift
  • Is the cause low temp, F:M, SRT?

» Improved SVI (and effluent TSS) as Acidimicrobiales, Microthrix and Actinomyocetales decreased

» What’s up with Kouleothrix?
Cost

» 21 tests = $4,830 ($230 per sample)

» Cost increases with fewer samples
Cost per Raw Megabase of DNA Sequence

Moore’s Law

National Human Genome Research Institute
genome.gov/sequencingcosts
Conclusions

» Bacteria culturing and microscopy have limitations

» DNA speciation can help give operators clues to solving treatment process problems

» DNA technology is an economical and effective method to assess entire microbial communities
Questions

Tom Fitzwilliams, MSA Professional Services

tfitzwilliams@msa-ps.com
608-355-8864

Trevor Ghylin, PhD PE, Microbe Detectives

Trevor.Ghylin@microbedetectives.com

www.microbedetectives.com