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

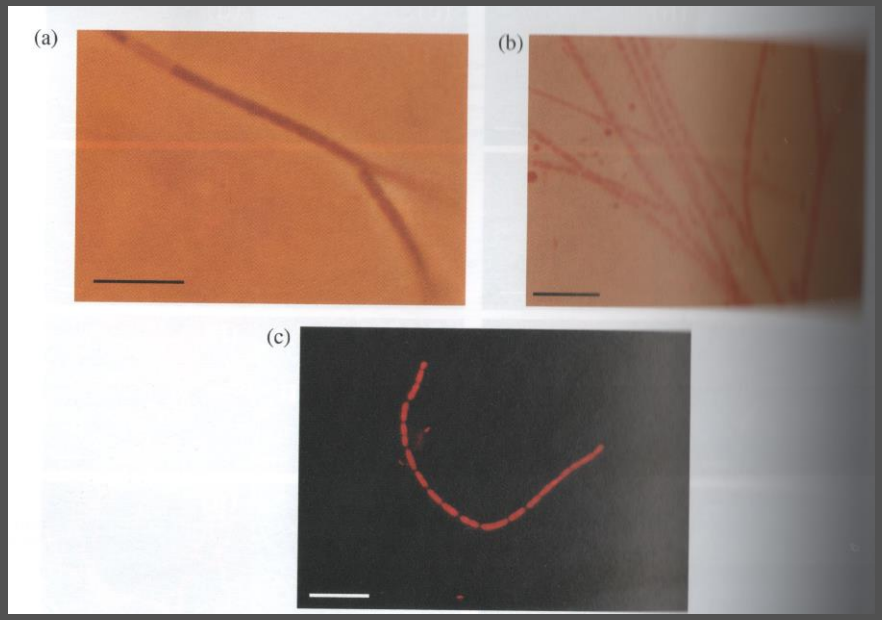
- Less than 1% of bacteria will grow in culture
- Requires days

Moving Beyond the Microscope

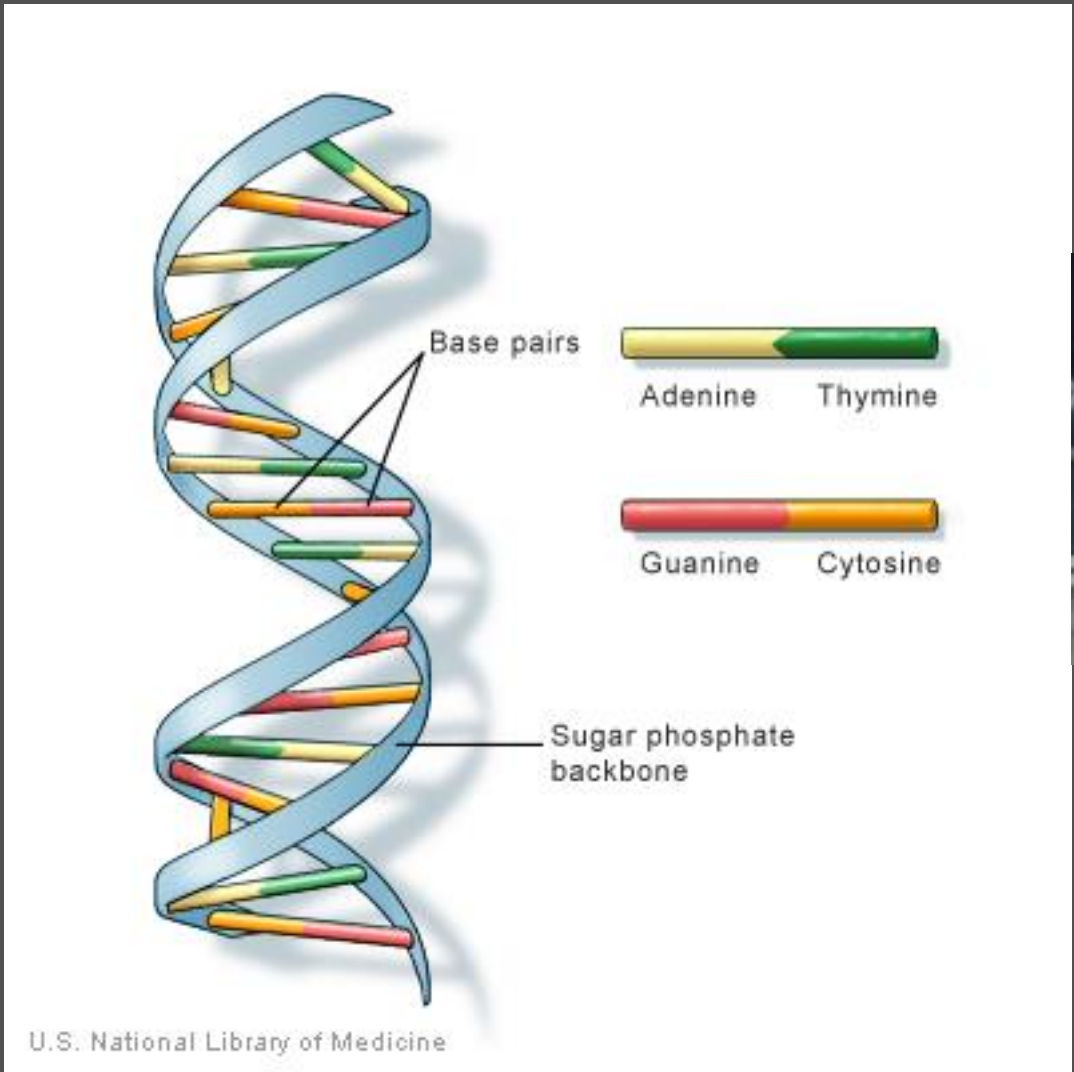
» Many bacteria cannot be identified visually

» Multiple species look identical

» Some species morph between shape based on environmental conditions



DNA Sequencing



Example DNA Data

>594682
TACGGAGGATCCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGTACGTAGGCGGACCCG
TCAGTCAGTGGTGAAAGTTTGCAGCTTAACTGTAAAATTGCCATTGAAACTACGGGTCTT
GAGTGTAAATGAGGTAGGCGGAATGTGTTGTGTAGCGGTGAAATGCTTAGATATAACACA
GAACACCAATTGCGAAGGCAGCTTACTGGGATACAACTGACGCTGAGGCACGAAAGCGTG
GGGATCAAACAGG
>594511
TACGGAAGGTCCGGGCGTTATCCGGATTTATTGGGTTTAAAGGGAACGCAGGCTGGAGAT
TAAGCGTGCTGTGAAATGTACCGGCTCAACCGGTGACGTGCAGCGCGAACTGGTTTCCTT
GAGTGAGTACGACGTCAGCGGAATTCGTGGTGTAGCGGTGAAATGCTTAGATATCACGAA
GAACTCCGATTGCGAAGGCAGCTGACGAGTCCTTTACTGACGCTAAAGCTCGAAAGTGCG
GGTATCGAACAGG
>594448
TACGTAGGGGGCGAGCGTTGTCCGGATTTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGG
TAAGTCGGATGTGAAATCTCCACGCTCAACGTGGAGGGGCCATTCGGTACTGCCATGACT
AGAGTCCGGTAGAGGAATGTGGAATTCCCGGTGTAGCGGTGAAATGCGCAGAGATCGGGA
GGAACACCCGTAGCGAAGGCGGCATTCTGGGCCGGTACTGACGCTGAGGAGCGAAAGCGT
GGGGAGCGAACAGG

Applications

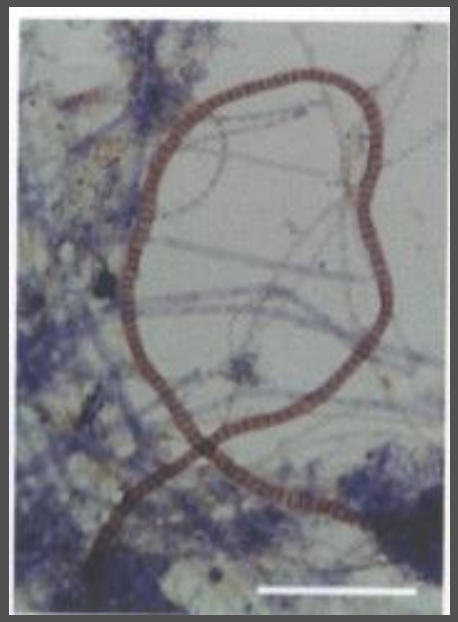
- Drinking water
- Surface Waters
  - Source tracking of fecal pollution
- Remediation
  - Track populations of microbial degraders
- Air quality monitoring
- Food and beverage
  - Safety, quality, longevity

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!

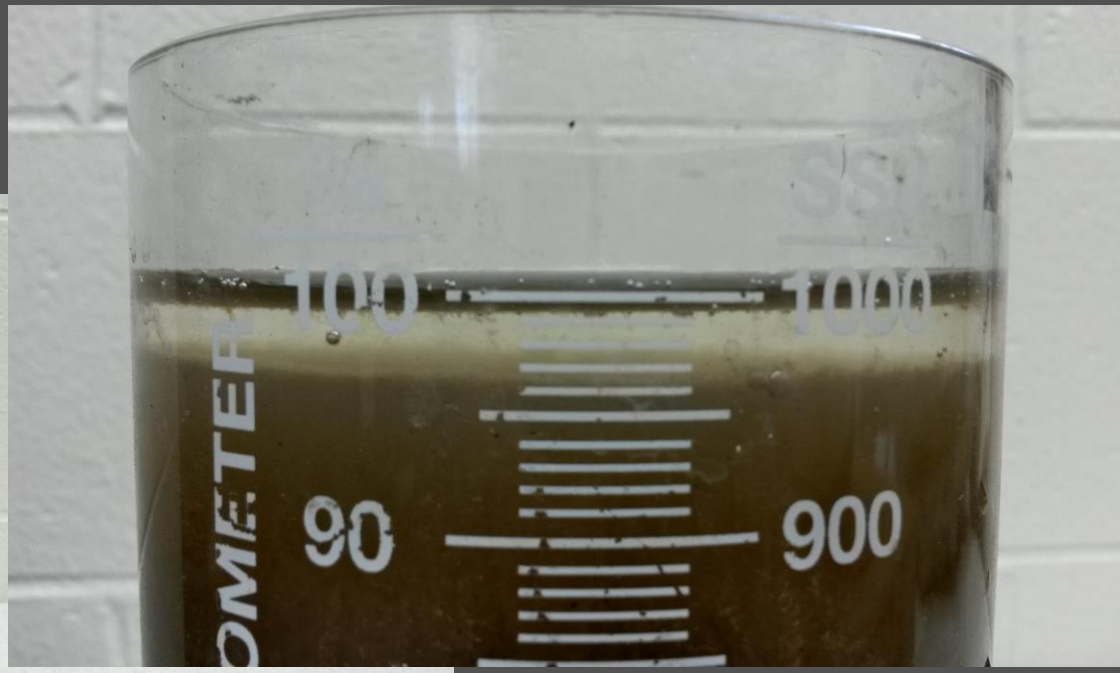
Ho-Chunk WWTF DNA Data

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

Nitrogenous Bacteria Results

Date	4/9/2014	4/15/2014	4/22/2014	4/29/2014	5/6/2014	5/17/2014	5/20/2014	5/27/2014	6/3/2014	6/10/2014	6/17/2014
Rhodococcus	0.01%	0.01%	0.01%	0.00%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.00%
Pseudomonas	0.10%	0.10%	0.06%	0.09%	0.06%	0.18%	0.07%	0.10%	0.07%	0.08%	0.12%
Paracoccus	0.34%	0.45%	0.39%	0.20%	0.36%	0.39%	0.21%	0.32%	0.35%	0.29%	0.06%
Thauera	0.03%	0.08%	0.06%	0.04%	0.08%	0.14%	0.06%	0.17%	0.17%	0.27%	0.15%
Dechloromonas	3.15%	0.85%	1.49%	4.09%	3.40%	4.34%	1.42%	2.05%	1.18%	1.44%	1.79%
Nitrosomonas	0.21%	0.15%	0.21%	0.39%	0.23%	0.27%	0.41%	0.69%	0.38%	0.48%	0.99%
Nitrosococcus	0.28%	0.30%	0.31%	0.41%	0.38%	0.41%	0.45%	0.64%	0.80%	0.73%	0.62%
Nitrobacter	0.06%	0.08%	0.06%	0.05%	0.05%	0.04%	0.08%	0.04%	0.06%	0.05%	0.04%
Nitrospira	0.87%	1.31%	1.72%	1.04%	2.64%	1.57%	1.81%	2.71%	3.42%	3.22%	2.06%
Nitrospina	0.00%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.02%	0.01%	0.00%
Thauera	0.03%	0.08%	0.06%	0.04%	0.08%	0.14%	0.06%	0.17%	0.17%	0.27%	0.15%
Nitratireductor	0.07%	0.09%	0.08%	0.05%	0.05%	0.06%	0.07%	0.05%	0.04%	0.04%	0.02%
Nitriliruptor	0.01%	0.02%	0.01%	0.01%	0.01%	0.01%	0.00%	0.01%	0.01%	0.00%	0.00%

Date	6/24/2014	7/1/2014	7/8/2014	7/15/2014	7/22/2014	7/29/2014	8/7/2014	8/12/2014	8/19/2014	8/26/2014
Rhodococcus	0.09%	0.05%	0.04%	0.03%	0.02%	0.03%	0.03%	0.02%	0.02%	0.01%
Pseudomonas	0.03%	0.02%	0.01%	0.09%	0.02%	0.02%	0.02%	0.02%	0.04%	0.04%
Paracoccus	0.21%	0.26%	0.24%	0.31%	0.21%	0.24%	0.22%	0.20%	0.23%	0.19%
Thauera	0.00%	0.00%	0.00%	0.00%	0.01%	0.00%	0.00%	0.00%	0.00%	0.01%
Dechloromonas	2.19%	2.30%	2.41%	1.97%	1.42%	1.75%	1.53%	2.19%	1.86%	5.26%
Nitrosomonas	0.85%	0.66%	0.24%	0.28%	0.42%	0.32%	0.38%	0.24%	0.25%	0.15%
Nitrosococcus	0.33%	0.29%	0.26%	0.33%	0.40%	0.44%	0.66%	0.62%	0.66%	0.84%
Nitrobacter	0.06%	0.07%	0.05%	0.06%	0.05%	0.06%	0.08%	0.04%	0.04%	0.03%
Nitrospira	1.21%	0.56%	1.68%	1.93%	2.50%	2.10%	0.47%	1.80%	0.97%	3.17%
Nitrospina	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Thauera	0.00%	0.00%	0.00%	0.00%	0.57%	0.00%	0.00%	0.00%	0.00%	0.57%
Nitratireductor	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Nitriliruptor	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Filamentous Bacteria Results

Date	4/9/2014	4/15/2014	4/22/2014	4/29/2014	5/6/2014	5/17/2014	5/20/2014	5/27/2014	6/3/2014	6/10/2014	6/17/2014
Thiothrix	0.01%	0.01%	0.01%	0.02%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
Acinetobacter	0.09%	0.10%	0.03%	0.04%	0.03%	0.02%	0.02%	0.04%	0.05%	0.07%	0.05%
Microthrix	0.14%	0.48%	0.41%	0.31%	0.06%	0.48%	1.74%	1.04%	0.44%	0.69%	1.86%
Runella	0.07%	0.11%	0.11%	0.05%	0.10%	0.08%	0.08%	0.08%	0.11%	0.08%	0.03%
Gordonia	0.01%	0.02%	0.01%	0.01%	0.01%	0.01%	0.02%	0.02%	0.01%	0.01%	0.01%
Haliscomenobacter	0.04%	0.01%	0.03%	0.03%	0.05%	0.03%	0.04%	0.02%	0.03%	0.05%	0.04%
Leptolinea	0.02%	0.02%	0.01%	0.02%	0.02%	0.02%	0.03%	0.02%	0.03%	0.02%	0.03%
Caldilinea	0.25%	0.29%	0.26%	0.28%	0.13%	0.15%	0.27%	0.16%	0.10%	0.11%	0.07%
Kouleothrix	0.25%	0.37%	0.36%	0.34%	0.60%	0.38%	0.63%	0.74%	0.82%	0.73%	0.80%

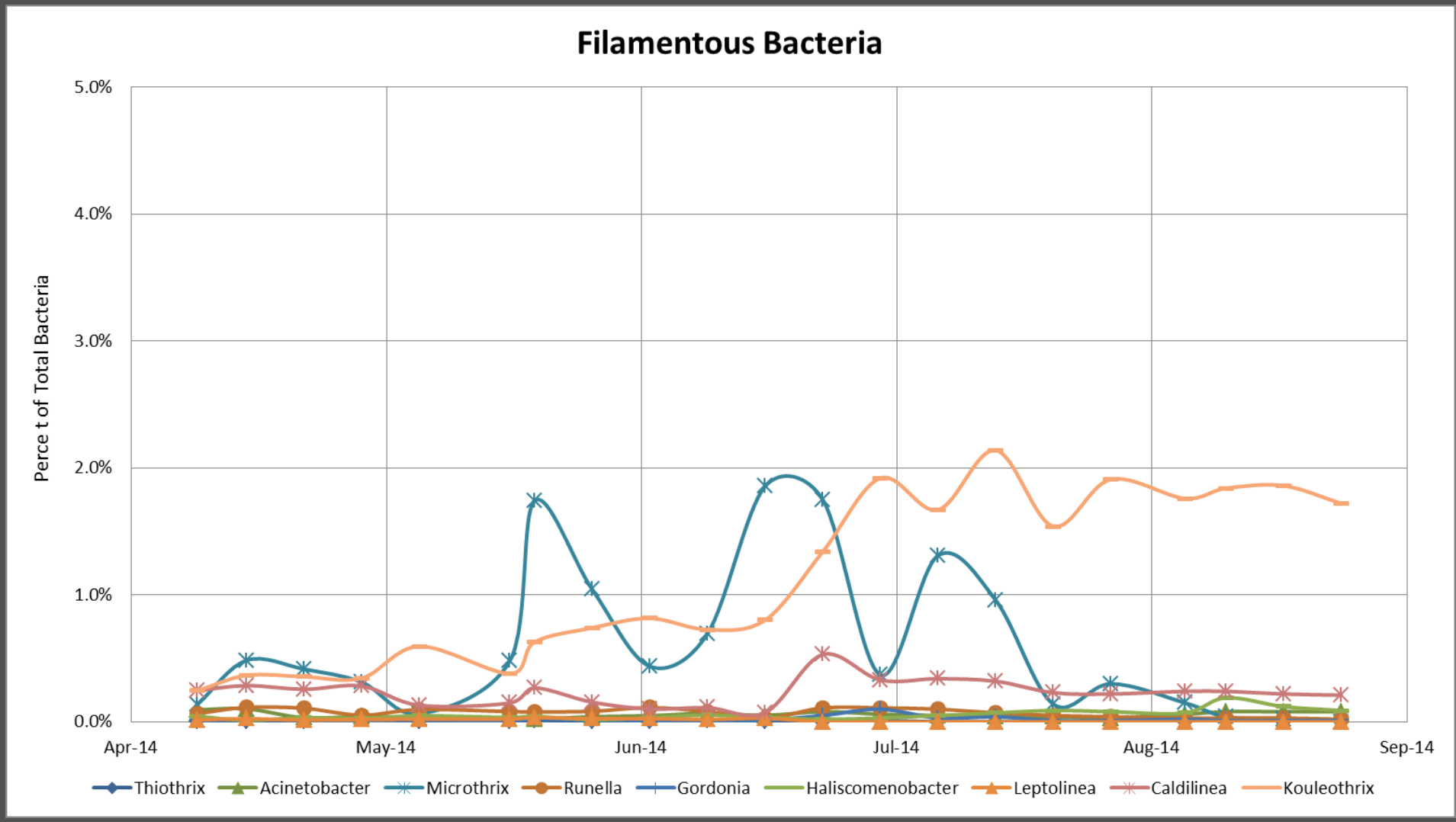
Date	6/24/2014	7/1/2014	7/8/2014	7/15/2014	7/22/2014	7/29/2014	8/7/2014	8/12/2014	8/19/2014	8/26/2014
Thiothrix	0.00%	0.01%	0.00%	0.00%	0.00%	0.00%	0.00%	0.01%	0.00%	0.00%
Acinetobacter	0.08%	0.06%	0.05%	0.04%	0.04%	0.03%	0.06%	0.08%	0.08%	0.08%
Microthrix	1.75%	0.37%	1.31%	0.96%	0.14%	0.30%	0.15%	0.04%	0.02%	0.00%
Runella	0.11%	0.11%	0.10%	0.07%	0.05%	0.04%	0.03%	0.03%	0.03%	0.02%
Gordonia	0.05%	0.10%	0.03%	0.04%	0.01%	0.01%	0.02%	0.01%	0.01%	0.01%
Haliscomenobacter	0.02%	0.03%	0.05%	0.07%	0.09%	0.08%	0.07%	0.19%	0.12%	0.09%
Leptolinea	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Caldilinea	0.53%	0.33%	0.34%	0.32%	0.23%	0.22%	0.24%	0.24%	0.22%	0.21%
Kouleothrix	1.34%	1.92%	1.67%	2.14%	1.54%	1.91%	1.76%	1.84%	1.86%	1.72%

Foaming Bacteria Results

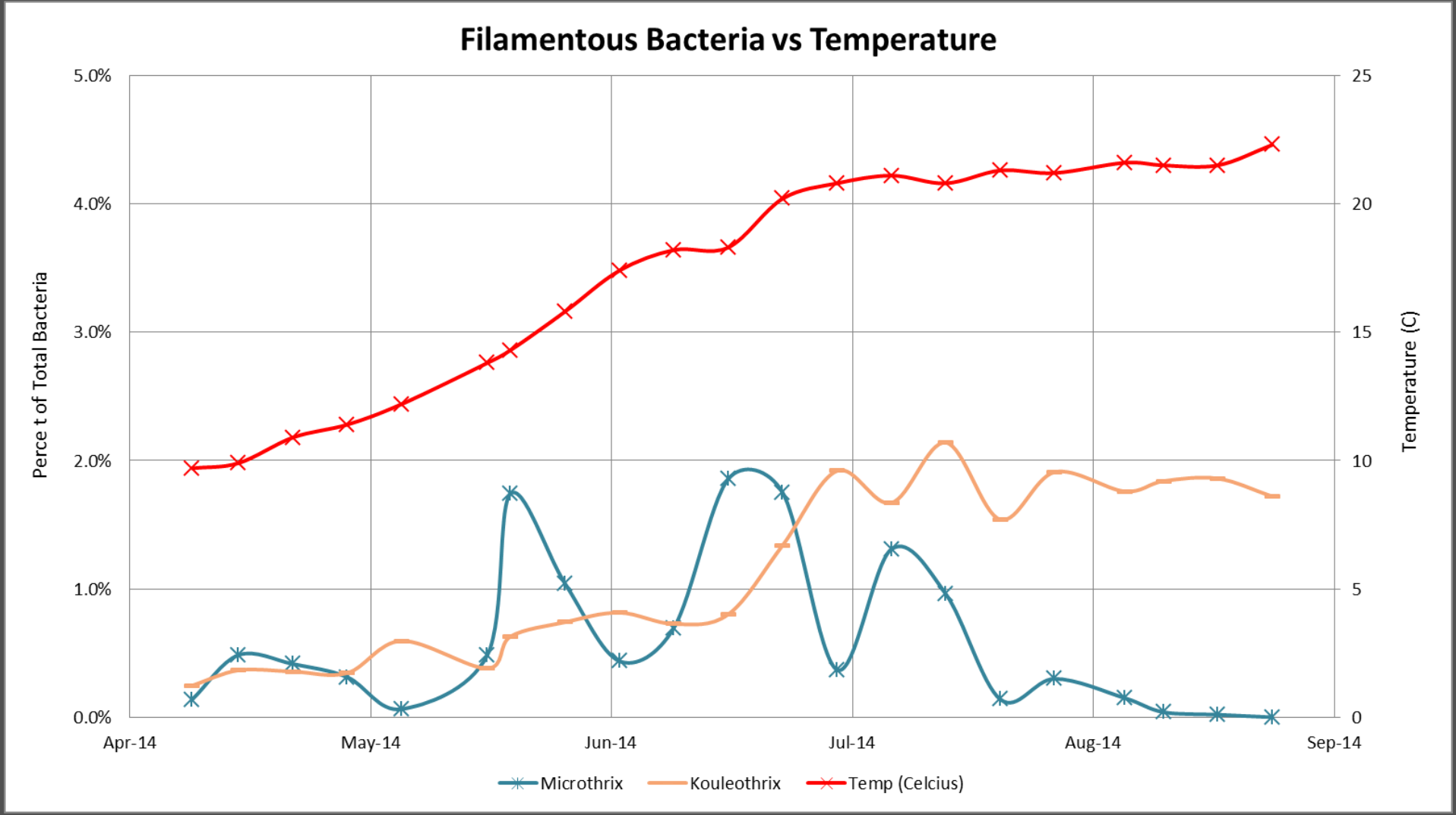
Date	4/9/2014	4/15/2014	4/22/2014	4/29/2014	5/6/2014	5/17/2014	5/20/2014	5/27/2014	6/3/2014	6/10/2014	6/17/2014
Acidimicrobiales	1.69%	2.69%	1.90%	1.62%	0.88%	1.28%	3.17%	1.78%	1.32%	1.36%	2.74%
Microthrix	0.14%	0.48%	0.41%	0.31%	0.06%	0.48%	1.74%	1.04%	0.44%	0.69%	1.86%
Rhodococcus	0.01%	0.01%	0.01%	0.00%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.00%
Actinomycetales	1.25%	1.35%	1.01%	1.07%	1.10%	0.79%	1.25%	0.45%	0.51%	0.73%	0.55%
Gordonia	0.01%	0.02%	0.01%	0.01%	0.01%	0.01%	0.02%	0.02%	0.01%	0.01%	0.01%
Mycobacterium	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.04%	0.02%	0.02%	0.04%	0.02%

Date	6/24/2014	7/1/2014	7/8/2014	7/15/2014	7/22/2014	7/29/2014	8/7/2014	8/12/2014	8/19/2014	8/26/2014
Acidimicrobiales	2.02%	1.39%	1.71%	1.71%	1.52%	1.61%	2.42%	1.66%	1.09%	0.70%
Microthrix	1.75%	0.37%	1.31%	0.96%	0.14%	0.30%	0.15%	0.04%	0.02%	0.00%
Rhodococcus	0.09%	0.05%	0.04%	0.03%	0.02%	0.03%	0.03%	0.02%	0.02%	0.01%
Actinomycetales	1.72%	1.37%	1.02%	1.00%	0.69%	0.63%	0.82%	0.61%	0.34%	0.30%
Gordonia	0.05%	0.10%	0.03%	0.04%	0.01%	0.01%	0.02%	0.01%	0.01%	0.01%
Mycobacterium	0.11%	0.12%	0.08%	0.10%	0.07%	0.07%	0.05%	0.03%	0.03%	0.02%

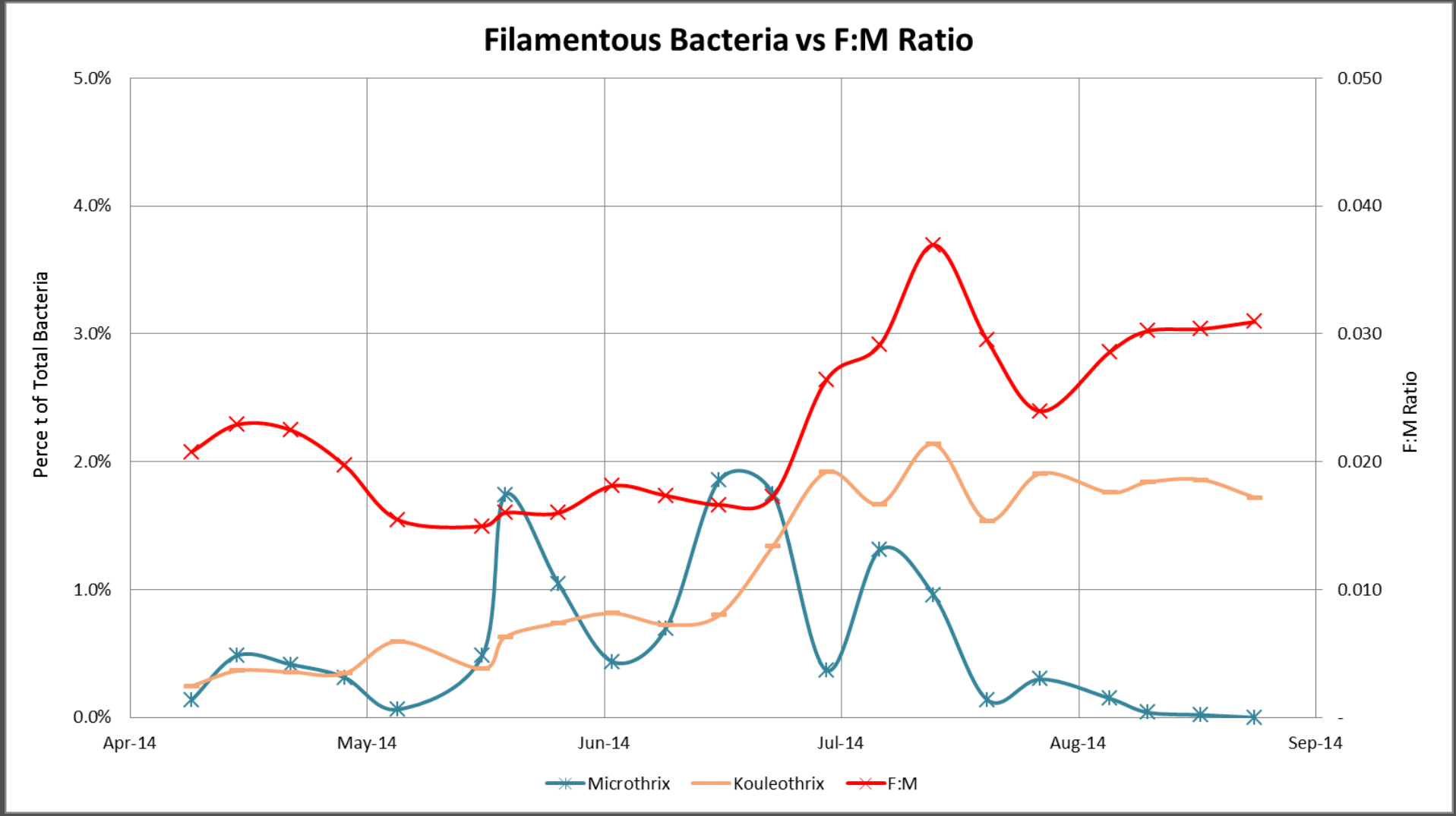
Filamentous Bacteria Results



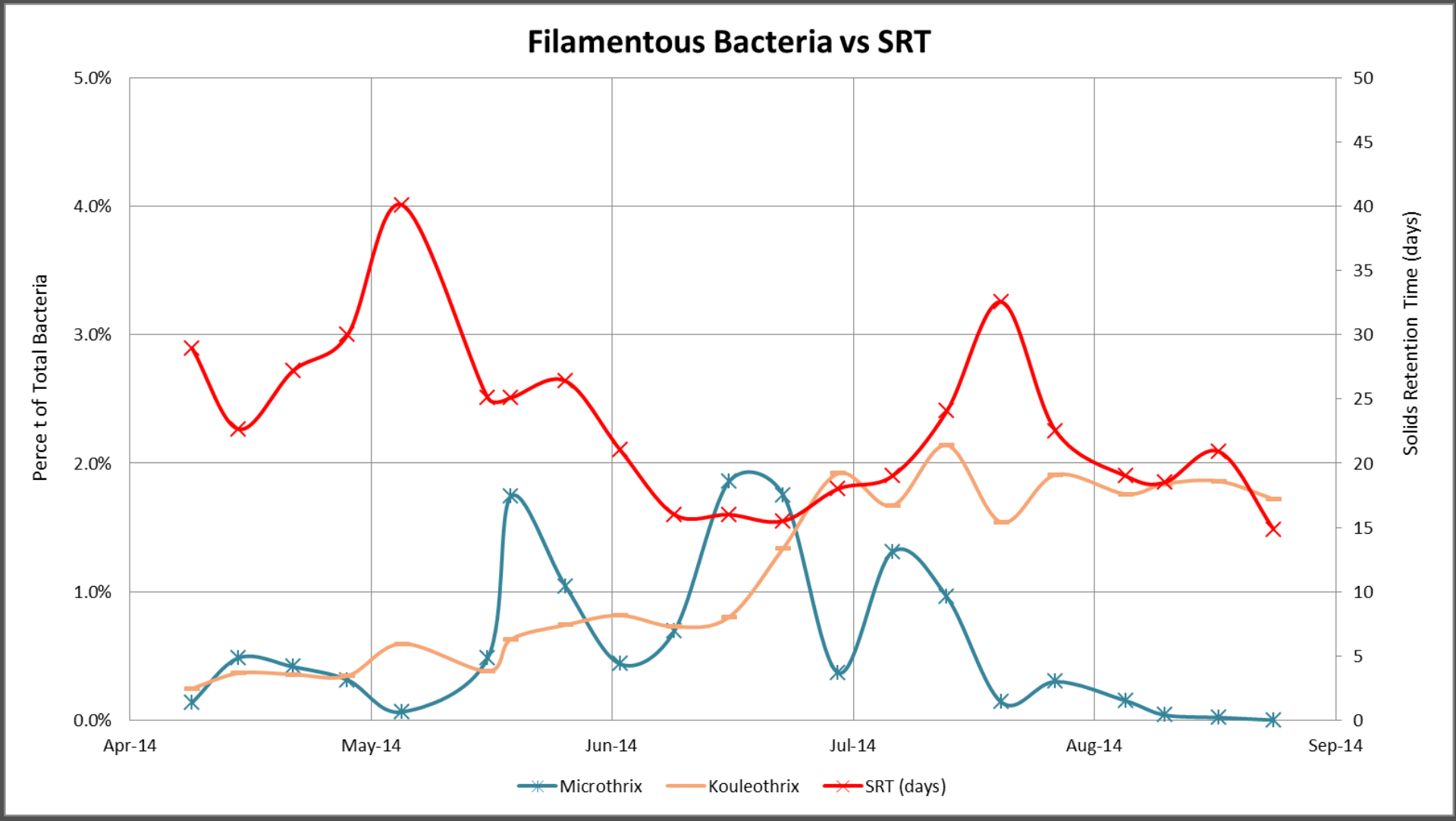
Filamentous Bacteria Results



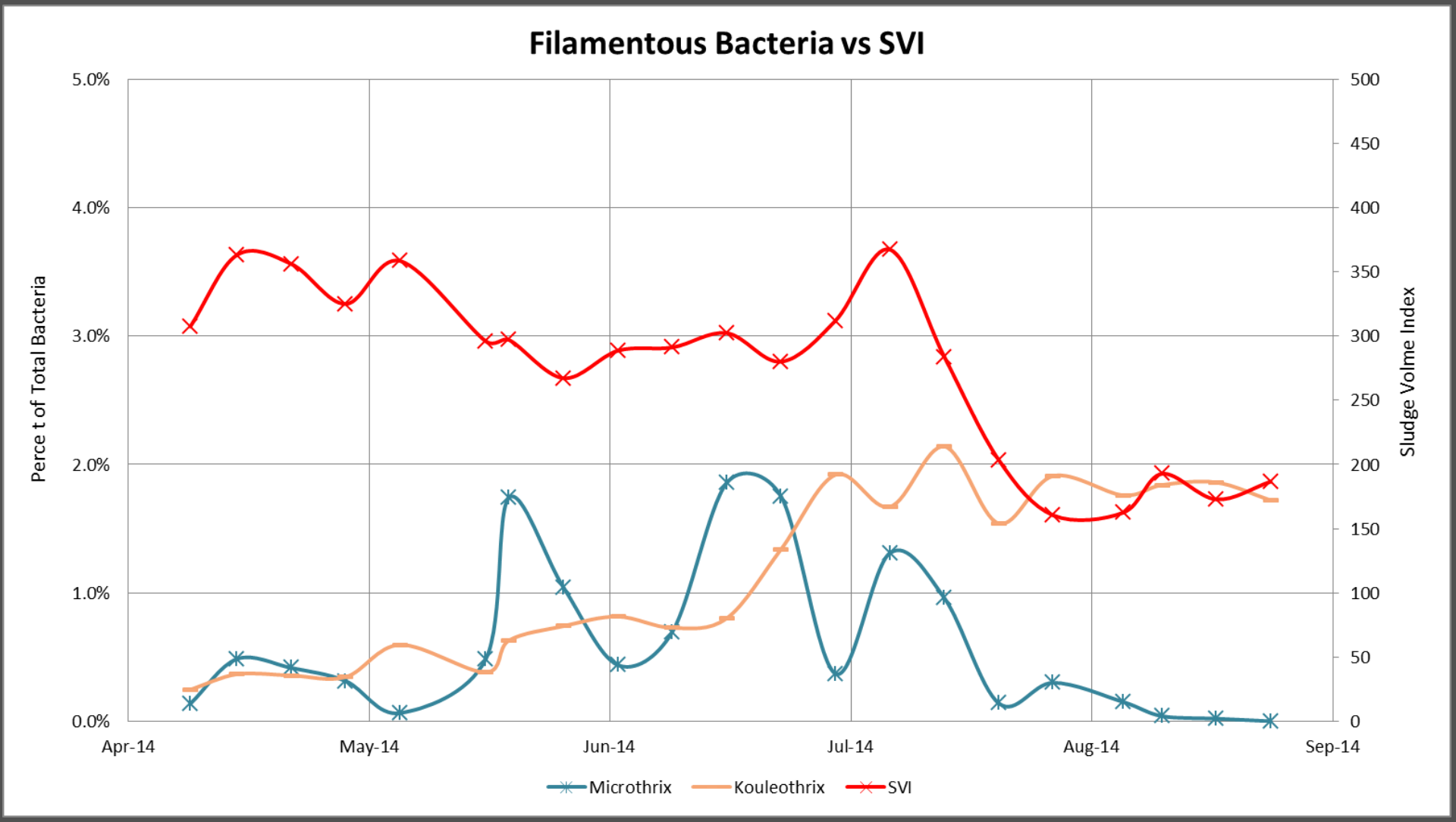
Filamentous Bacteria Results



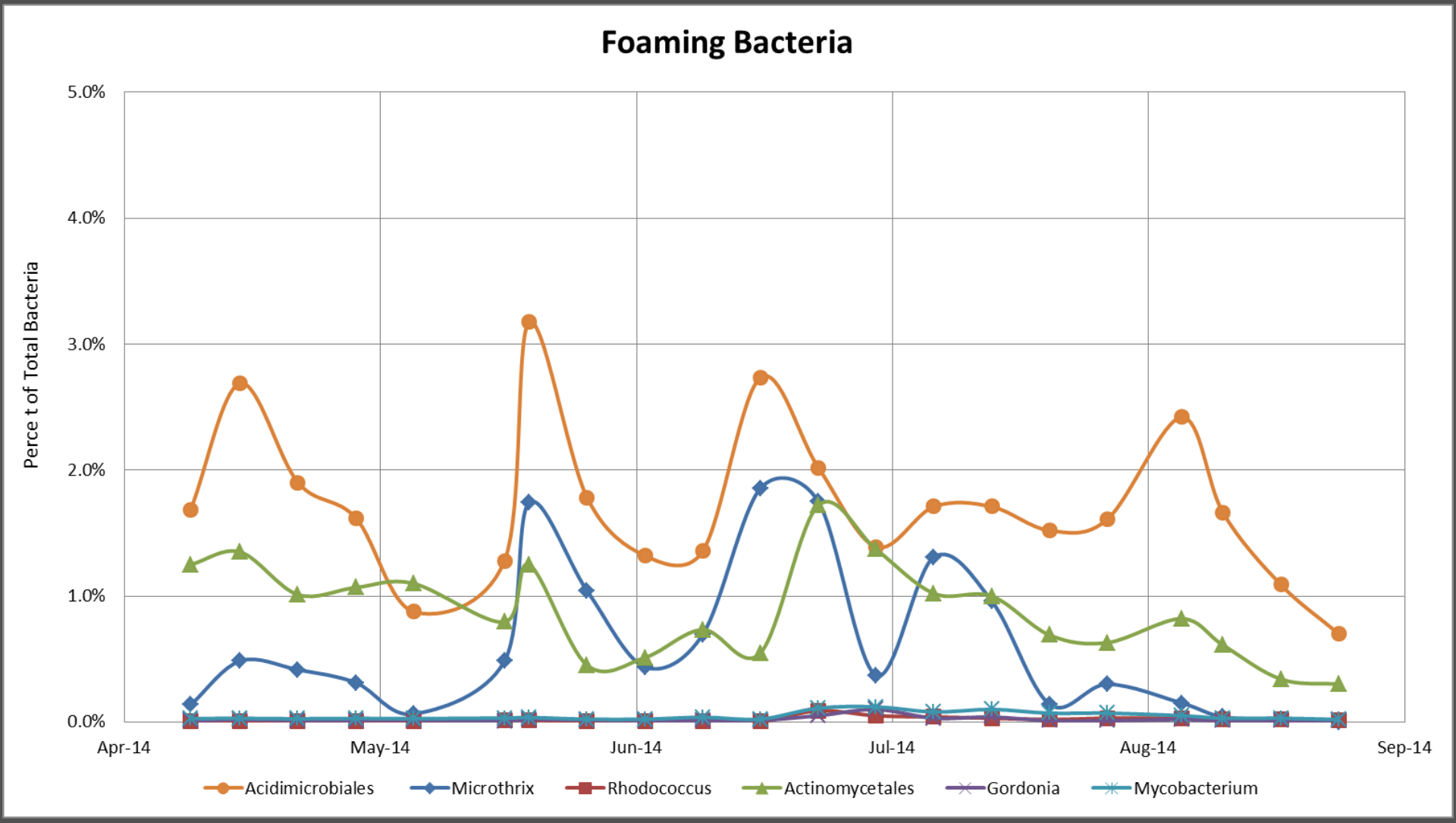
Filamentous Bacteria Results



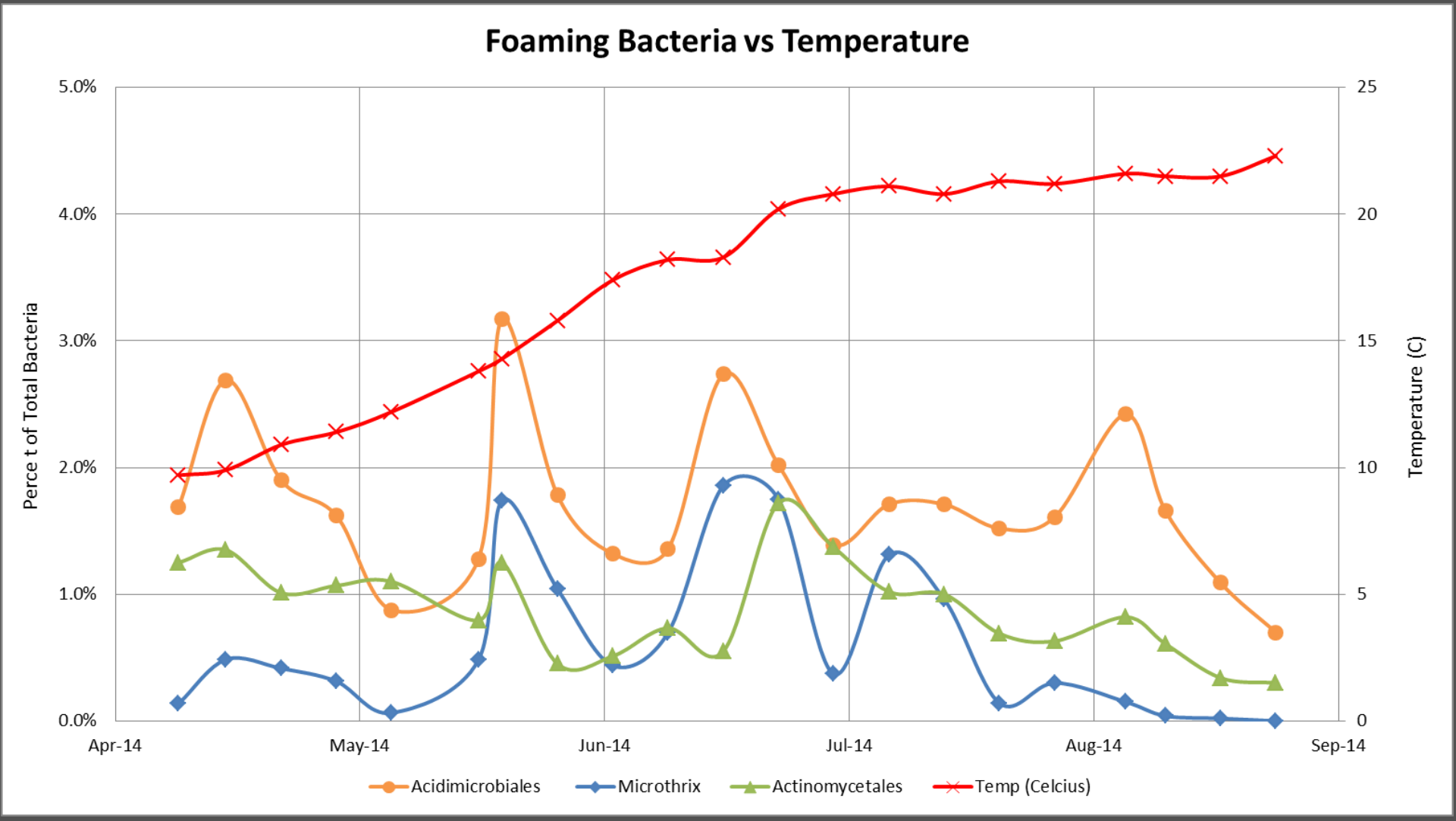
Filamentous Bacteria Results



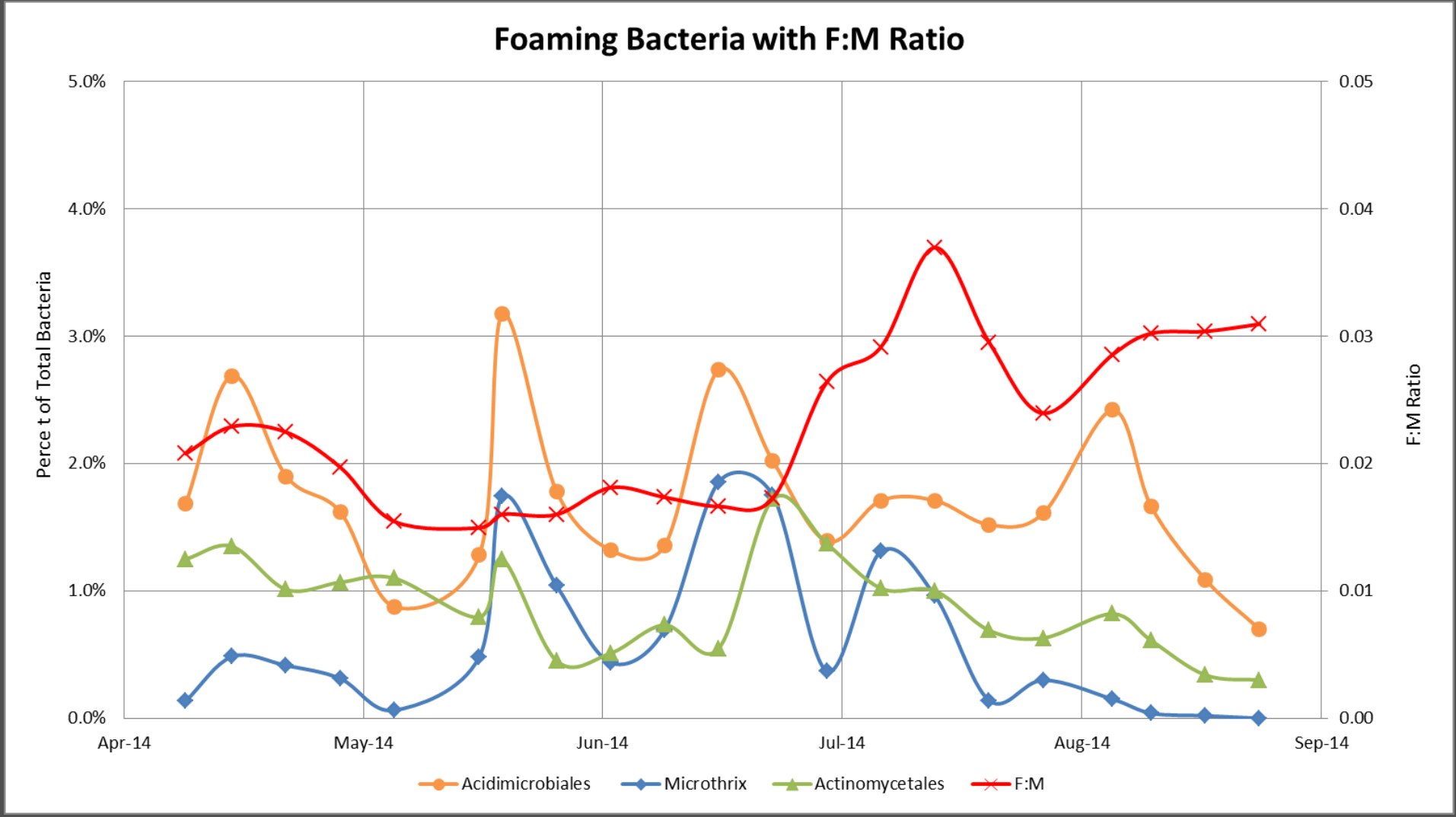
Foaming Bacteria Results



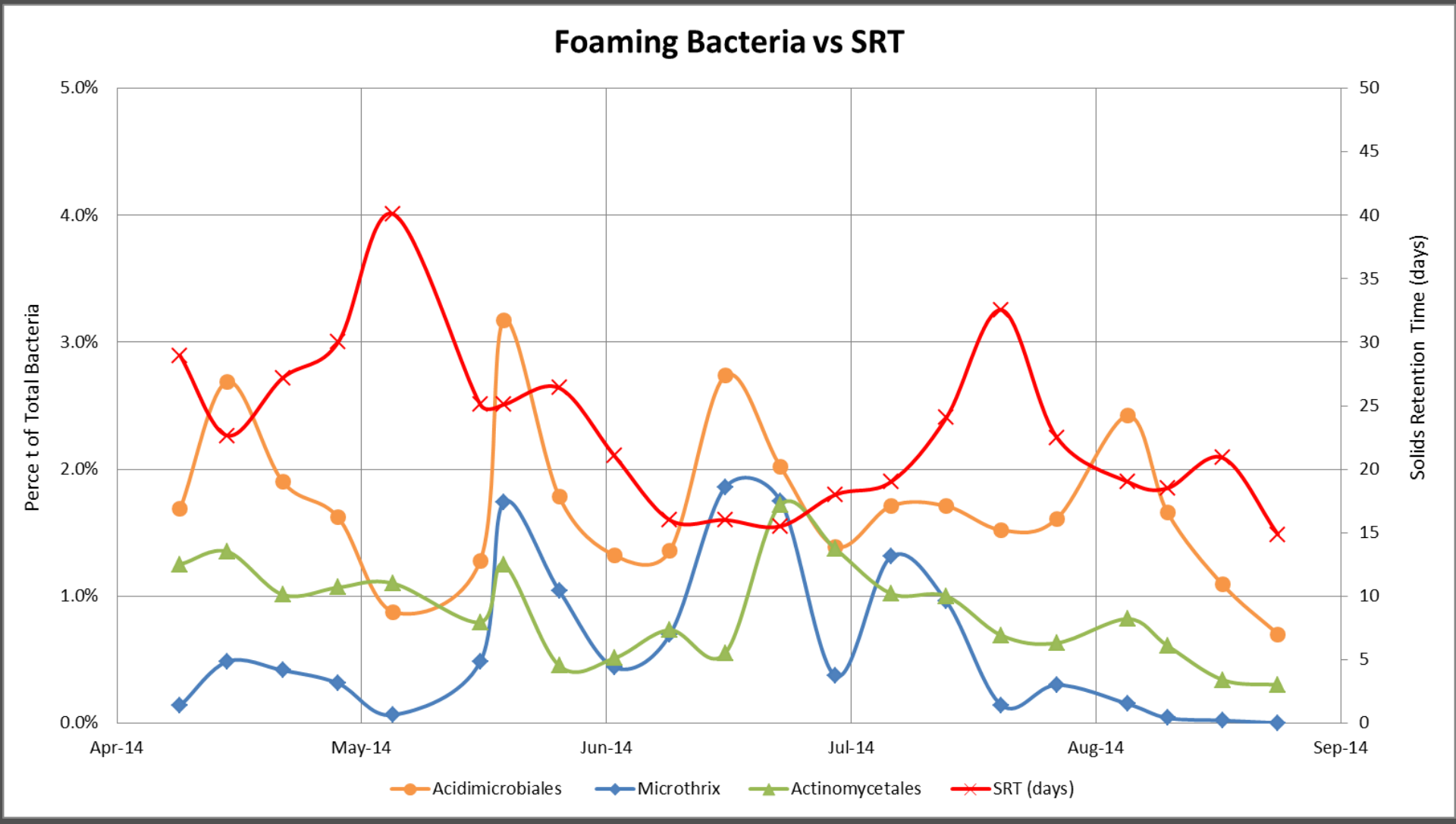
Foaming Bacteria Results



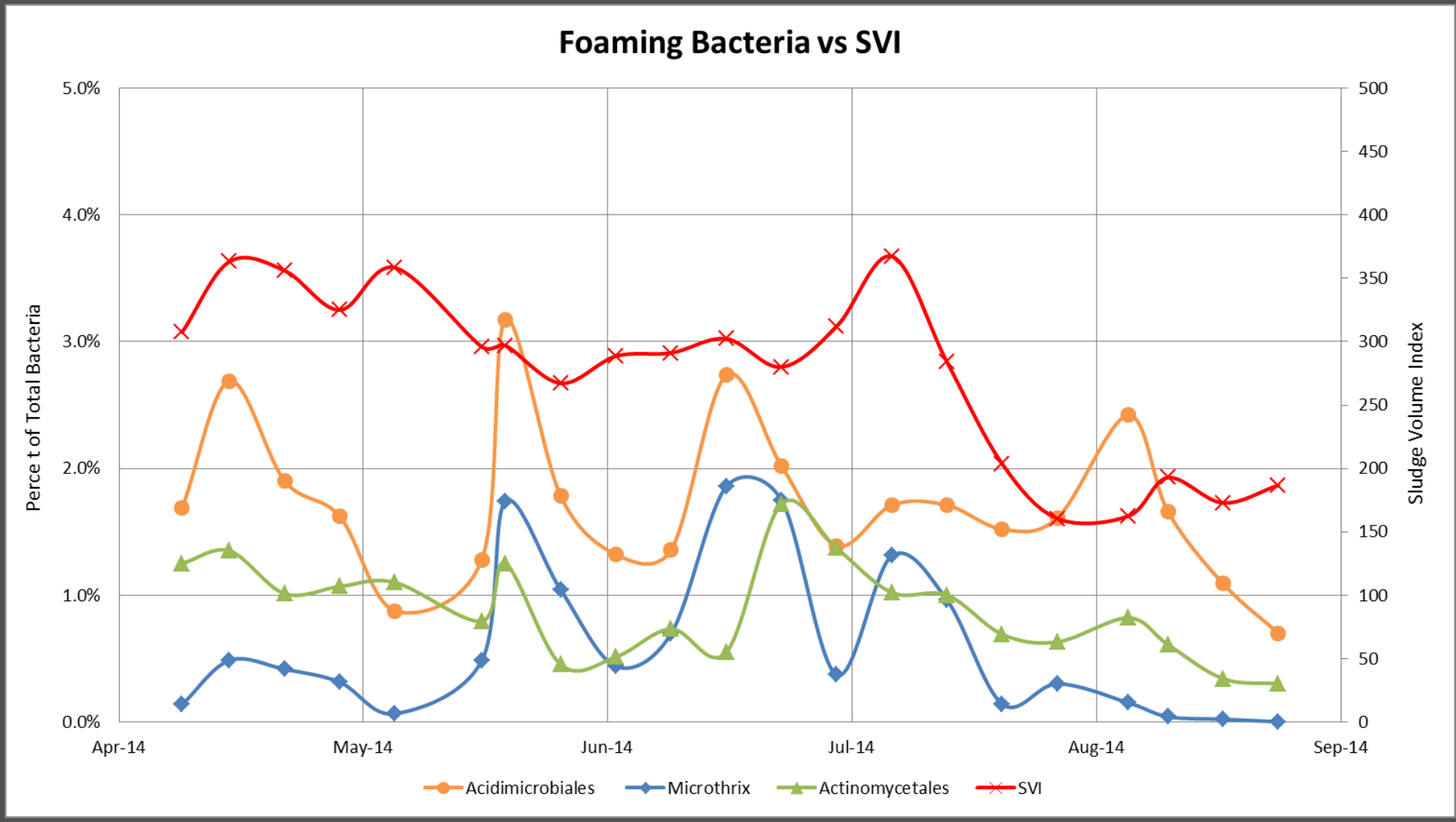
Foaming Bacteria Results



Foaming Bacteria Results



Foaming Bacteria Results



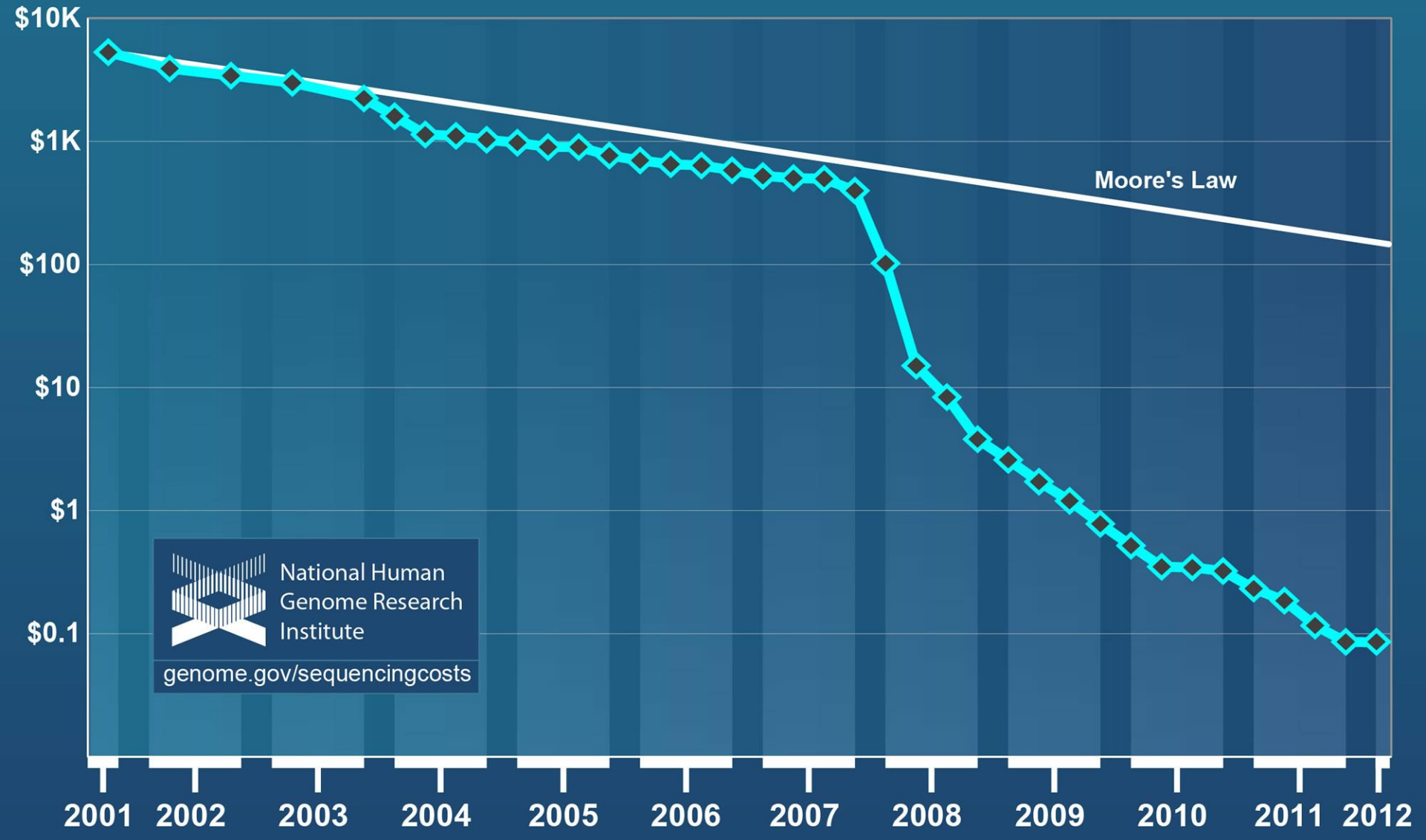
Results - Summary

- 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



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