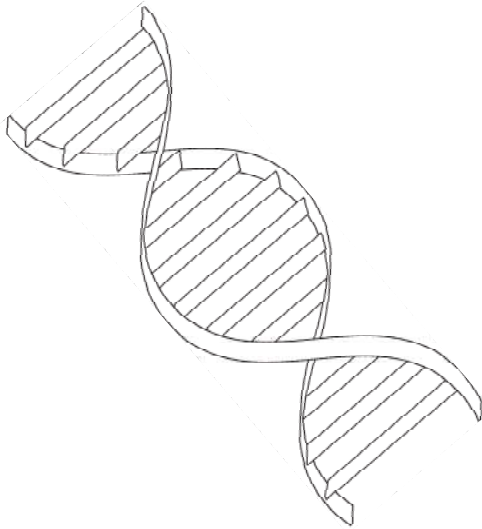


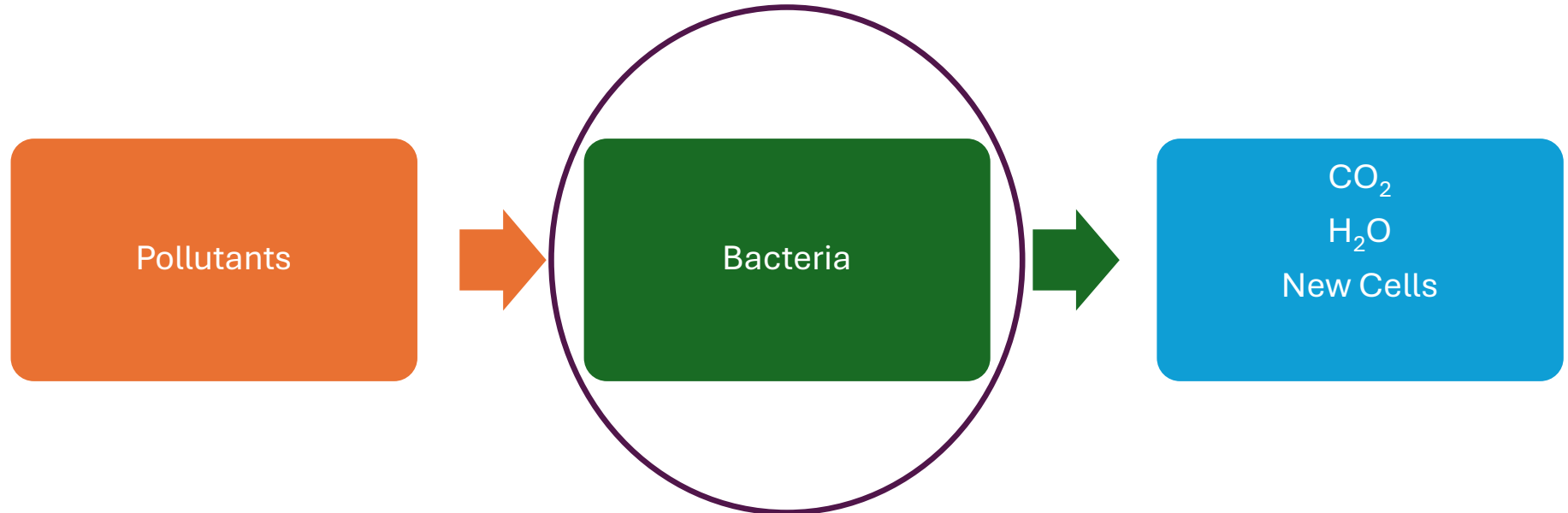
# **Advancing Microbial Monitoring in Biological Wastewater Treatment: Integration of qPCR and Microbial Community Analysis (MCA) via Next-Generation Sequencing**

A & WMA SW LA Spring Conference  
February 2026

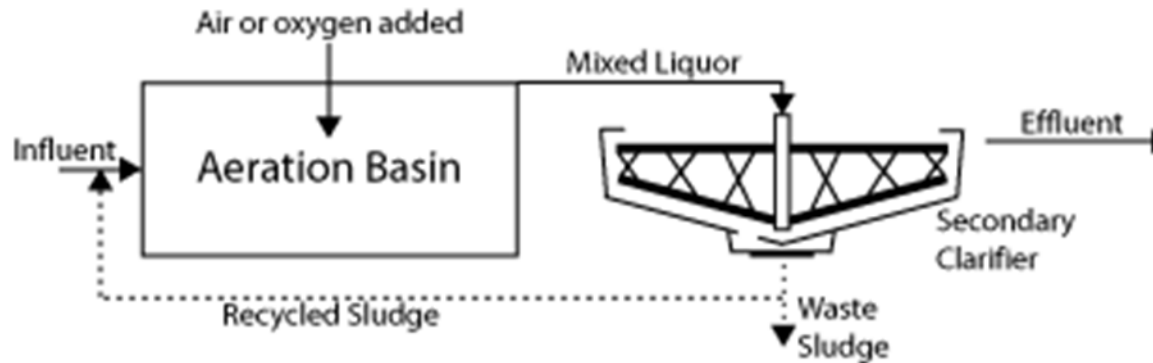
Erik Rumbaugh  
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# The Problem with the Textbook Bioremediation Process



# The Challenge of the “Black Box”

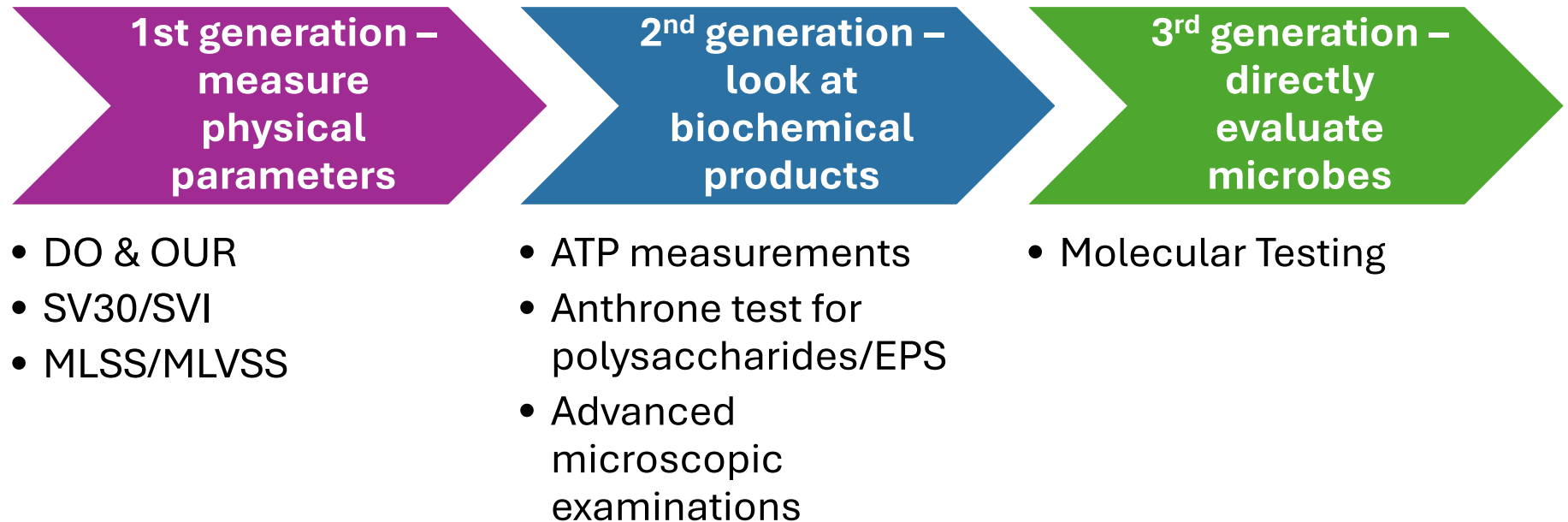


**MLVSS**



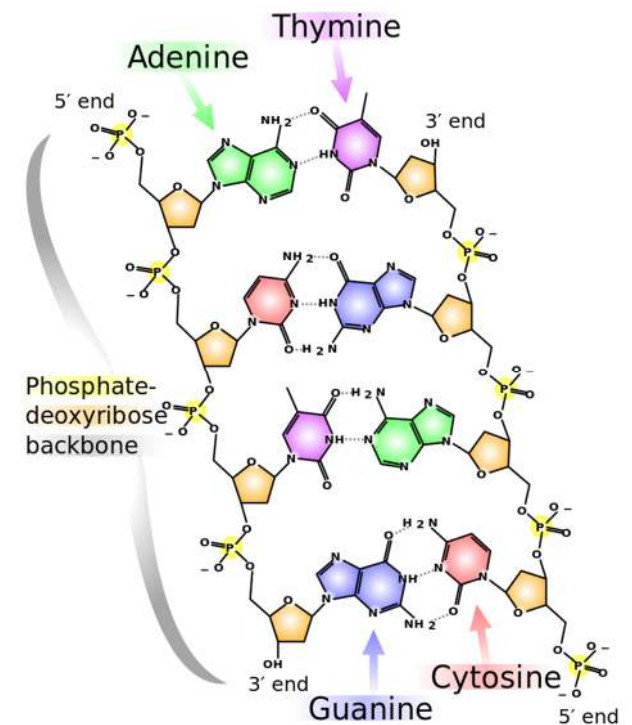
Know what is growing & how to improve operations

# Evolution of Biomass Monitoring



# Environmental Genomics – Molecular Toolkit

- Molecular biology has come a long way in the last few decades
- We can test for
  - DNA & RNA
  - Proteins & Enzymes
- Focused on the processes of replication, transcription, & translation
- Our most common tests use 16s rRNA molecular sequencing to ID bacteria & archaea in environmental samples



# Unpacking the Environmental Microbiome

- Use DNA based technology to get read data on microbial populations
- Moving from Guessing to Knowing
- Most common tests
  - qPCR – fast, targeted and quantitative
  - MCA – total microbial census



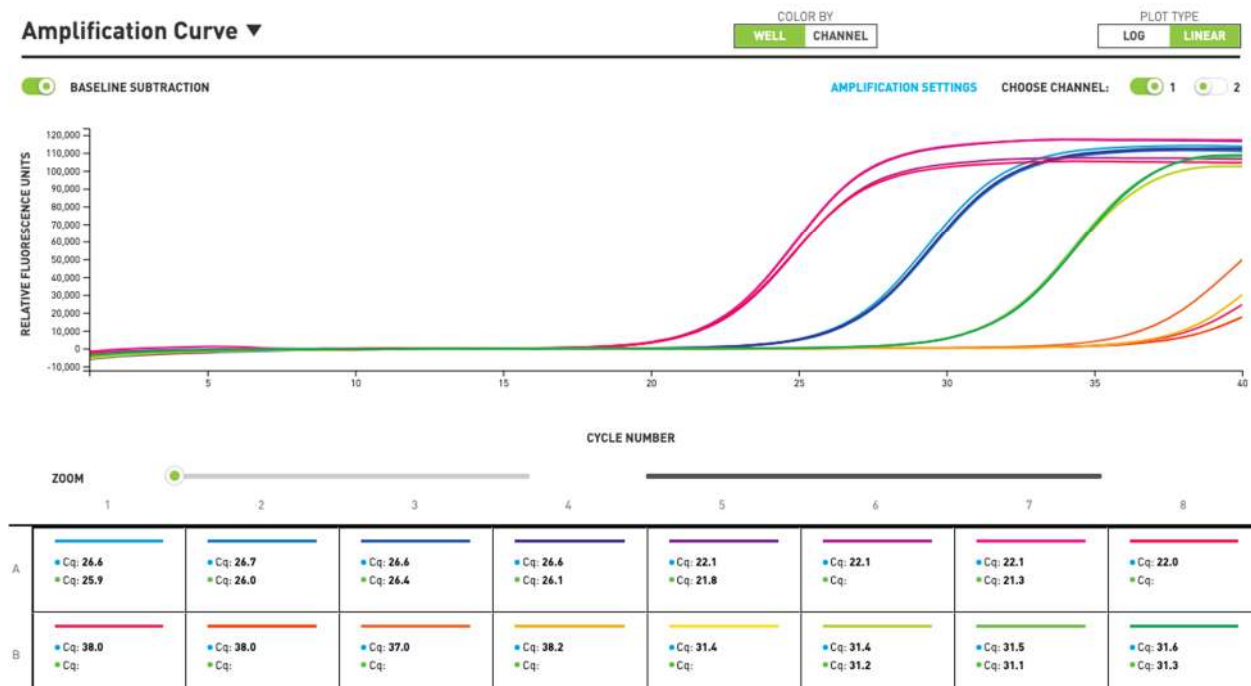


# qPCR

Specific, Rapid, & Quantitative

# Quantitative PCR (qPCR)

- Rapid test
- **Specific** & sensitive
- Monitor for specific organisms or metabolic pathways

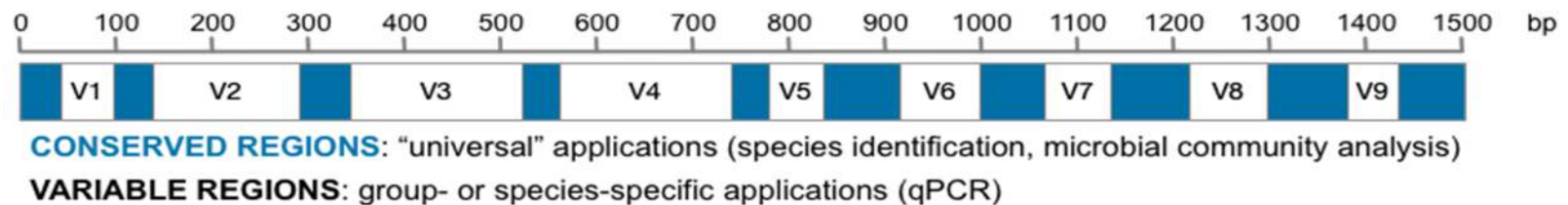


A photograph of several petri dishes containing green microbial cultures, with a pipette tip visible in the foreground. The image is overlaid with a semi-transparent dark grey rectangle containing white text.

# Microbial Community Analysis

Total Microbial Census

# Application of the 16S rRNA Genes



## 16S rRNA Gene is present in all bacteria / archaea

- Conserved – present in all bacteria and archaea
- Variable regions are used to identify bacteria like a barcode
- Environmental Genomics MCA covers V1 to V9



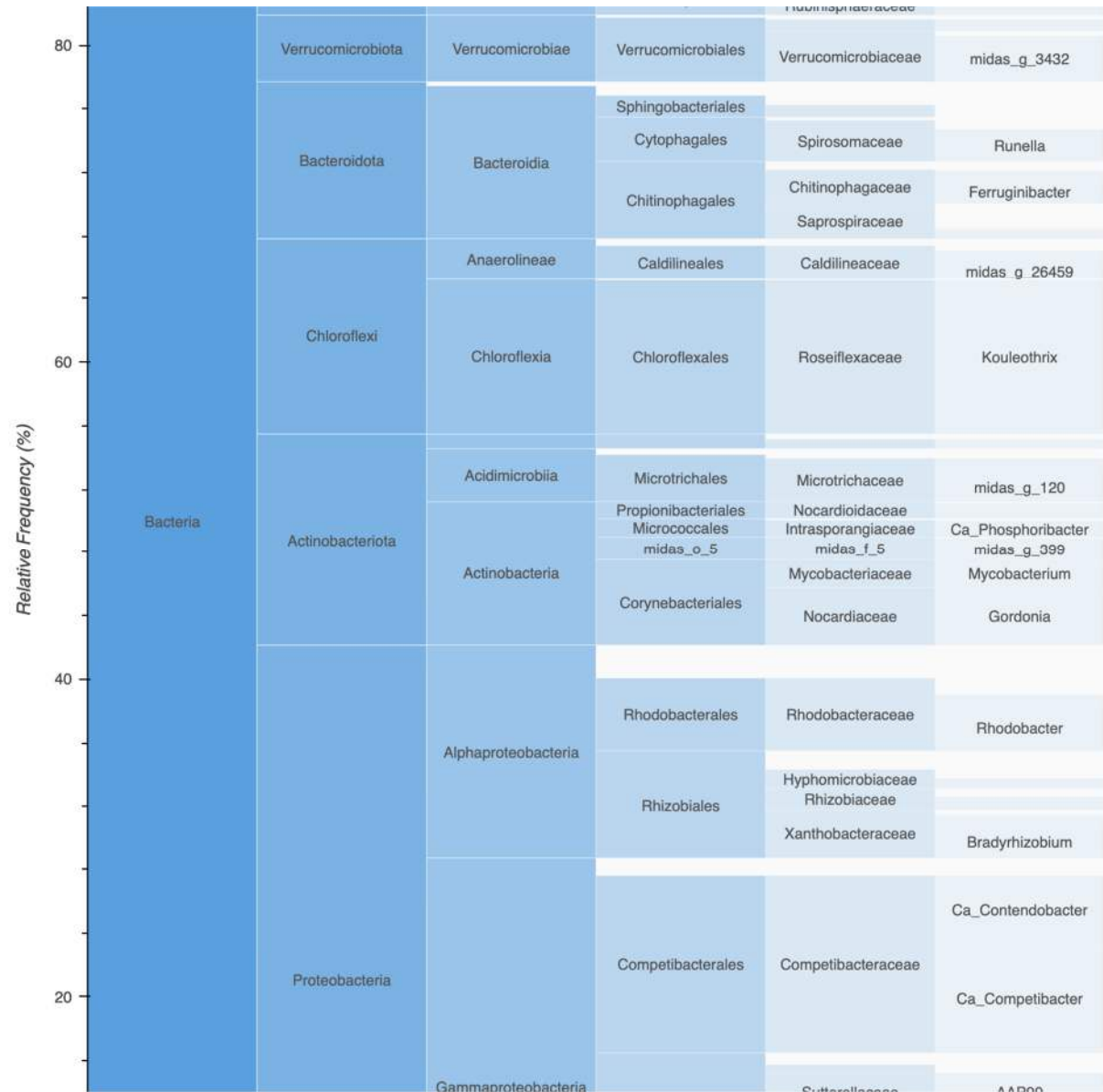
# Microbial Community Analysis (MCA)

- Microbial census
- Break into functional groups for quick interpretation
- Based on NGS sequencing technology
- Deep information on all organisms

	AOB	NOB	NRB	SOX	SRB	PAO	GAO	Bulking	Foaming	Filaments	Methylotrophs
4/15/2021	1.00	3.40	9.80	1.70	0.00	13.80	10.00	0.80	0.70	8.00	0.50
5/13/2021	1.60	5.00	11.70	1.70	0.00	18.00	14.90	0.90	0.30	5.50	0.70
6/17/2021	1.40	4.80	12.80	2.40	0.00	15.70	12.80	1.20	0.40	5.90	1.20

# MCA

- Use Sankey chart to visualize sequencing from Kingdom to Genus level ID
- Allows for monitoring organisms with no official name



## Why Use Molecular Testing

Deep insight into microbial populations

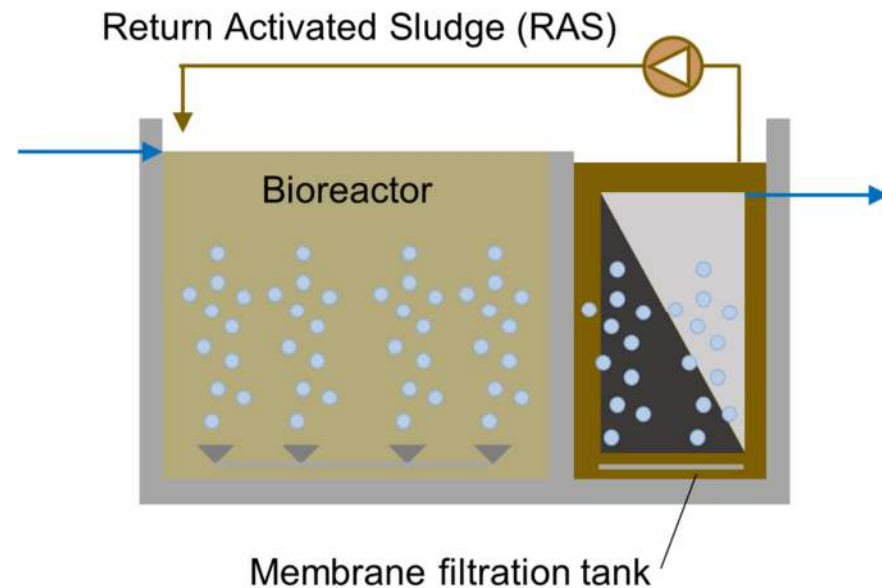
Changes can be noted in days rather than WW sludge ages

Early warning before treatment quality declines

Both time for analysis & price have decreased since introduction

# Example – Landfill Leachate MBR

- Biological fouling of membrane – leading to high transmembrane pressure & limited ability for process water
- The problem was not in design but in the biological makeup of the system
- Questions
  - What is causing the fouling
  - How to control the organisms responsible



# Landfill Leachate MBR

- Used MCA testing to identify sequences correlated to high transmembrane pressure
- Sequences were identified as being in the Genus Thauera (a high EPS producing organism)
- More specific ID found the responsible organism was select species of Thauera rather than the Genus as a whole
- Developed a rapid qPCR test for the problem Thauera – and controlled wasting rates in response to changes in population makeup

	Thauera 1	Thauera 2
11/24/25	0.6%	0.0%
10/8/25	0.0%	0.0%
9/16/25	0.2%	0.0%
5/6/25	0.1%	0.0%
2/27/25	1.3%	0.0%
2/4/25	21.4%	0.0%
1/14/25	15.4%	0.0%

# Example – BNR Municipal System

- An advanced municipal system with anaerobic digester and aerobic BNR system has run monthly MCA tests
- System has always run well without any problems
- Late 2022, switched struvite control chemistry - review of the chemistry predicted no impact on aerobic system

# MCA Data

	<b>AOB</b>	<b>NOB</b>
7/20/23	1.0%	3.9%
6/22/23	0.2%	1.3%
5/25/23	0.3%	2.2%
4/20/23	0.1%	0.3%
3/23/23	0.0%	0.0%
2/23/23	0.1%	0.3%
1/26/23	1.0%	4.7%
12/15/22	1.2%	8.3%
11/17/22	1.4%	6.7%

# What Happened?

Noted decrease in AOB & NOB populations

March 2023 – ammonia breakthrough at effluent

Discovered that the struvite control chemistry also bound trace metals used by AOB & NOB enzymatic pathways.

Stopped new struvite chemistry, wasted heavily, and added WAS from local ASU & bioaugmentation cultures.

# Applications of Molecular Technology

## Industrial & Municipal Impact:

- Tracking the impact of new influents
- Monitoring operational changes in both aerobic and anaerobic digesters

## Value Proposition:

- Cost Reduction: Use chemicals/energy only when the biology needs it
- Compliance: Avoid permit violations

# The Future – Integration with AI & Predictive Modeling



Concept: Moving from Diagnosis to Prediction



Vision: Using longitudinal (time series) MCA data to train AI models



Goal: The system warns of pending problems before treatment efficiency is reduced by monitoring slight changes in biomass makeup

# Summary

- Traditional monitoring is reactive, molecular adds a proactive dimension to monitoring
- qPCR = specific tracking, MCA = total ecosystem view
- This is a paradigm shift toward Precision Microbiology – managing biology with the same precision as we manage pumps & blowers





# Questions

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