NEWEA Annual Conference

Challenges Assessing and Treating Wastewater from Biotechnology Scale Up Operations

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About Amgen

- **Top Biotech/Pharm Companies**
- **10,000+ Employees**
- **Products**
  - Enbrel (RA)
  - Rapatha (Cholesterol)
  - Kyprolis (Chemotherapy)
- **HQ Thousand Oaks CA**
- **Amgen Massachusetts (AMA)**
  - R&D Facility
  - 8 Story Building Kendal Square
  - New Bio, Chem, & Scale Up Labs
  - Doubled Employee Count over last 4 years

Overview

- Existing wastewater pretreatment system
- Wastewater characteristics
- Wastewater challenges
- Root cause evaluation
- Short and long term action
- Questions

Wastewater Pretreatment System

- **Original Design**
  - Installed 2006
  - Typical Dual Stage pH adjustment system
    - 2 X 1,300 Gallon Tank, Mixers, Acid & Caustic Meter Pumps
    - 5000+ GPD flow
    - Run pH 6-8
  - Discharge Streams
    - Vivarium
    - Glass washers
    - Chemistry and Bio labs
  - MWRA Permit
  - Occasional odors

Wastewater Pretreatment System

- **Construction and Changes to Facility Since WWPT Install**
  - Vivarium decommissioned, significant reduction in flow
  - 2014 – 2018 Major Facility Upgrade Program
    - Multiple Floor Renovations
    - Consolidation of Labs from other sites
    - Added two new glasswashers, two Autoclaves, Reactor Washing Room
    - Increased number of chem labs
    - Commissioned one floor dedicated to bioreactor work
    - Commissioned SUL with 1200 L bioreactor
  - Significantly lower flow than design, ~1000 GPD, little to no weekend flow
  - Spike loads of nutrient rich media from bio labs and SUL

Wastewater Pretreatment System

SUL Waste Discharges

- **SUL Operations**
  - 1200 L Reactor, Batch and fed batch operation
  - Daily flow of sterile waste media to drain
  - Batch discharge of entire sterilized reactor at end of run
  - Discharge of dilute buffers from downstream and cleaning water

- **SUL Effect on WWPT System**
  - Daily “trickle” of high BOD, N, and micronutrients even on weekends
  - Spike loads of full bioreactor tankfuls, spikes of dilute cleaning waste
  - Noticeable change in odor and complaint frequency
  - No shift in wastewater system operational or sampling parameters

Wastewater Challenges

- Approximately 3-6 months after commissioning of new labs
- Elevated phenol levels detected at WWTP discharge
- Internal sampling of both WWT tanks confirmed upward shift in phenol levels
- Preliminary data seemed to correlated phenol to SUL reactor discharge
- Internal EHSS site team assembled to investigate root cause

Preliminary Root Cause

- **Did the chemists start discharging phenol from their lab?**
  - No - no new chemical, not enough phenol in existing material
- **Did the cleaners start using phenol based material?**
  - No - no new chemicals, not enough phenol in existing material
- **Did construction or maintence discharge phenol?**
  - No - no chemical discharge, not enough to produce observed levels
- **Did the SUL discharge phenol?**
  - No - chemical and disinfectants did not contain phenol
  - No - analysis of reactor waste and fresh media indicated no phenol
  - No - components in feed media such as tyrosine did not “trick” phenol lab test

Preliminary Root Cause

- **Hints**
  - Phenol level & odors appeared to correlate with SUL reactor discharge
  - Phenol levels & odors dropped off when SUL stopped discharging
  - Phenol levels & odors abated for 1 to 2 weeks after WWPT drain & scrub
  - During a series of no flow days, phenol rose from ND to 2-3ppm, then back to ND
  - During no flow periods, pH in tanks & discharge would “wander” up 1-1.5 units
- **Working theory**
  - Native microbes in WWPT converting SUL wastes into phenol?

Preliminary Root Cause

- **Experiment**
  - Take 1.8 L from WWPT tank 1 add 0.2 L SUL waste, mix 24 hours, test for phenol
- **Results**

Sample	Mix Time	Phenol Level (PPM)	Comment
WWPT Tank 1	0 hrs	5	Control
WWPT Tank 1	24 hrs	5	Control
WWPT Tank 1 + SUL	24 hrs	11	Microbial Action?
SUL	24 hrs	ND	No Phenol in SUL
WWPT Tank 1 + SUL + Bleach	24 hrs	ND	Oxidation?

Next Steps

- **Short Term Containment**
  - Plumb SUL drain to tote
  - Haul Waste Offsite for treatment

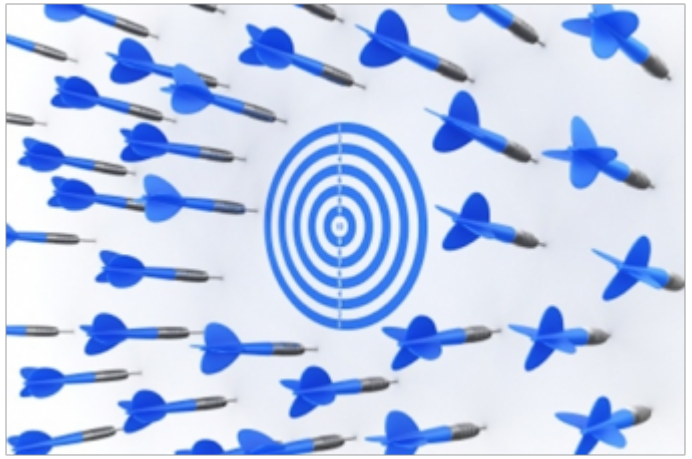
- **Contacted Tighe & Bond**
  - Duplicate Initial Experiments
  - Confirm or Refute Initial Working Theory
  - Fully Characterize Phenol Generation Mechanism
  - Propose and Test Pretreatment Alternatives
  - Provide Short Term and Long Term Treatment Options

Phase 1 – Evaluation - Theory

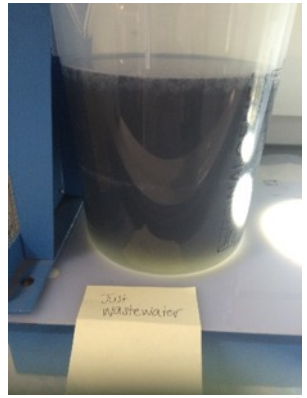
- High strength waste (N, C, P)
- Indigenous microbes
- Long retention times
- Creation or release of phenol under varying conditions

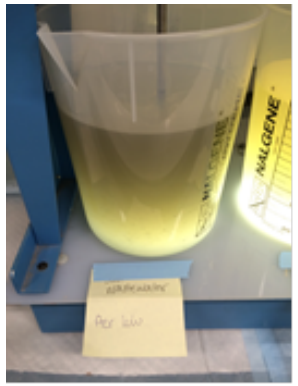
Phase 1 – Evaluation - Approach

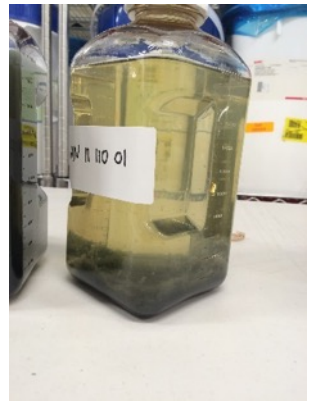
- **Use seed from wastewater treatment system**
- **Samples tested**
  - Raw wastewater
  - SUL permeate
  - 1:10 dilution of ww with SUL
  - 1:10 dilution with oxidizer (chlorine bleach)
- **Conditions controlled**
  - pH (hi, neutral, low)
  - Aerobic conditions
  - Anaerobic conditions



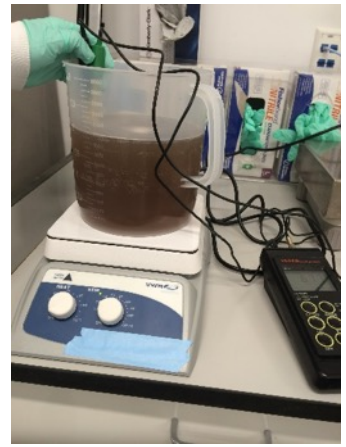
Phase 1 – Evaluation - Approach

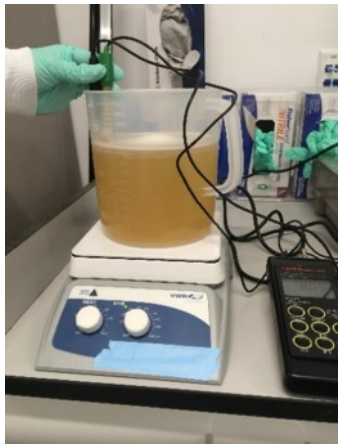


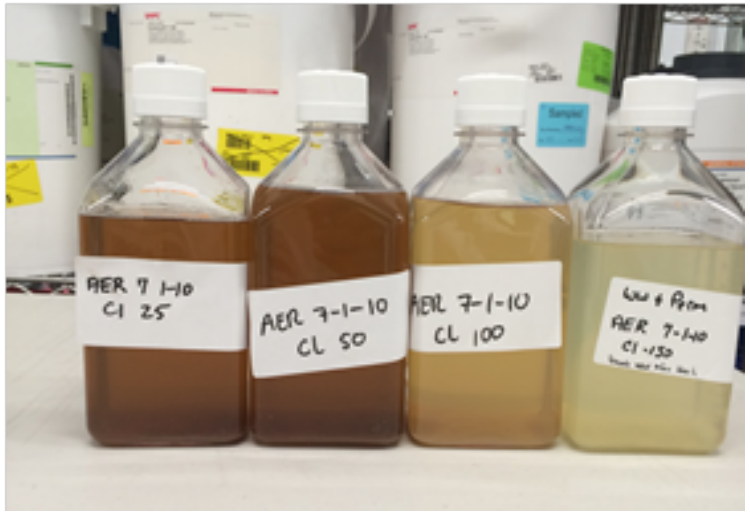




Phase 1 – Evaluation - Approach

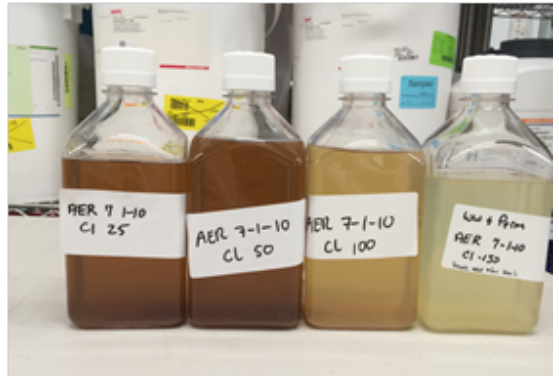


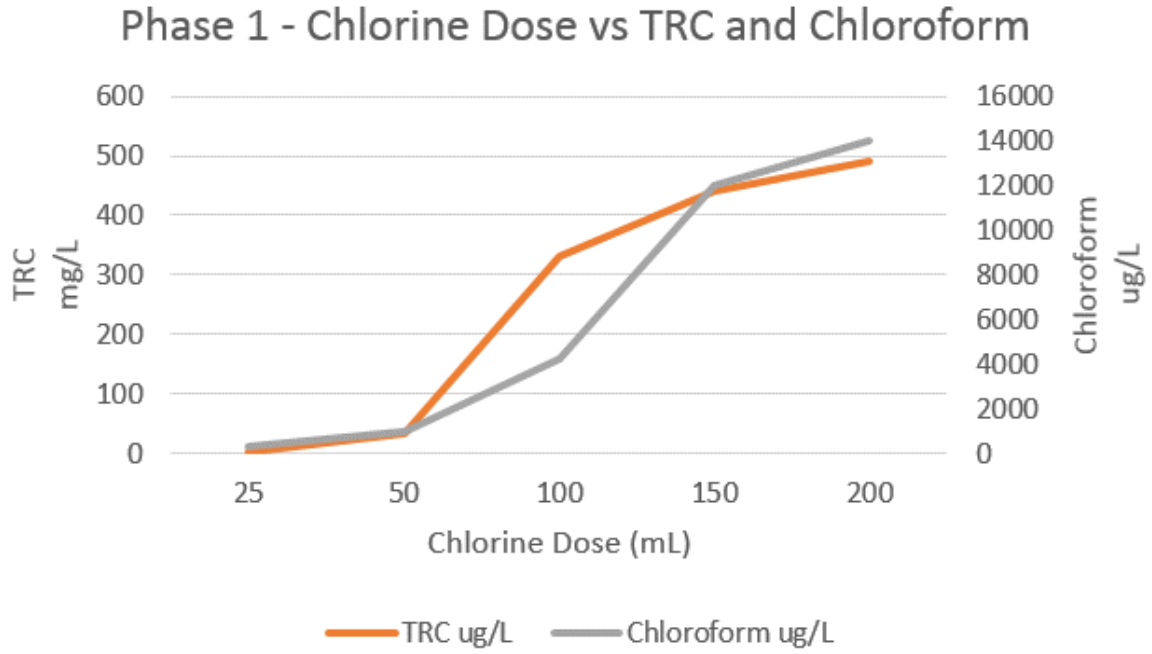




Phase 1 – Evaluation - Findings

- **Higher and lower pH generated less phenol**
- **SUL wasted did not contain phenol**
- **Use of chlorine bleach**
  - Addressed phenol generation
    - » Disinfection or oxidation
  - Generated chloroform



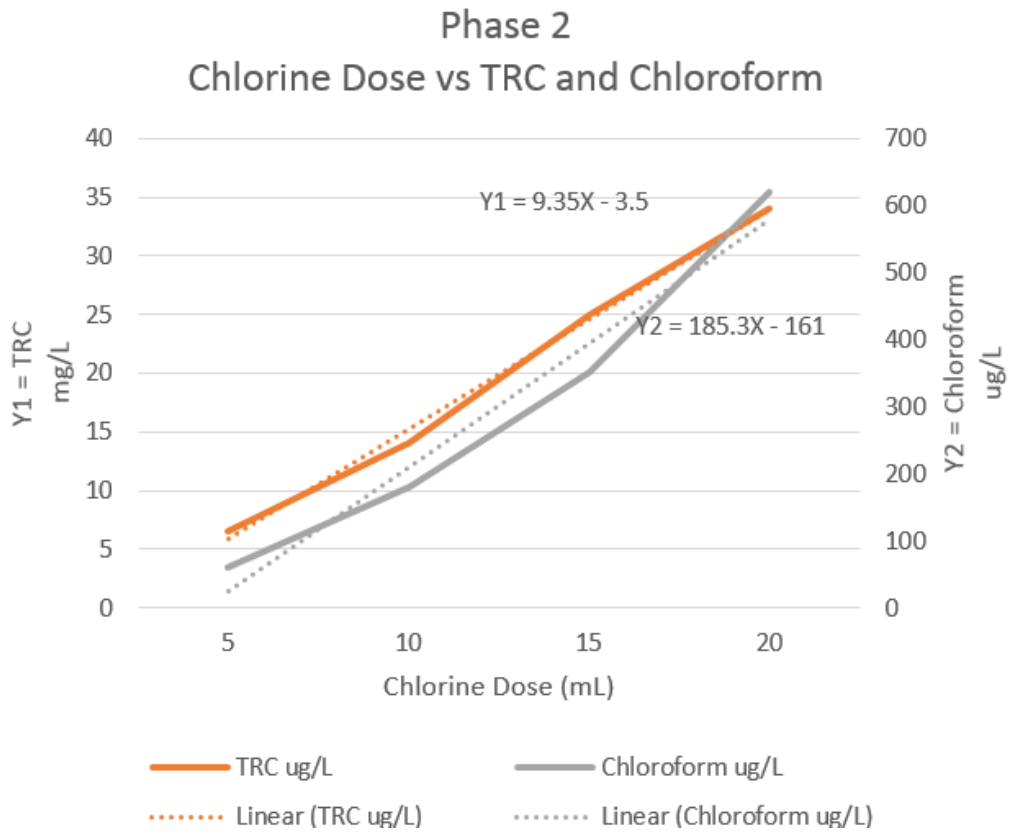


Phase 2 – Evaluation - Objectives

- **Chlorine bleach dosing – additional analysis to fine tune**
  - Observe odors
  - Sample chloroform generation
  - Measure TRC vs phenol over time
- **Evaluate phenol generation over longer exposure durations**
- **Identify predominant microbes**

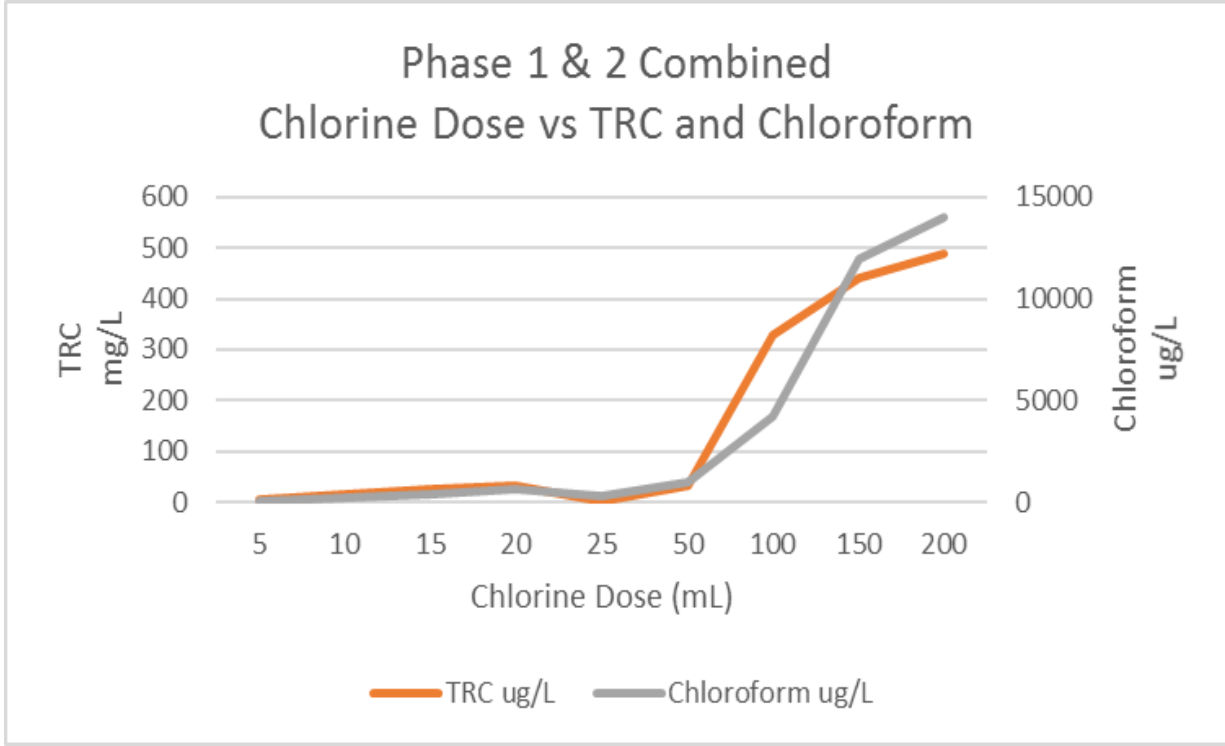
Phase 2 – Evaluation - Findings

- **Chloroform generation and TRC linear at lower dosages**



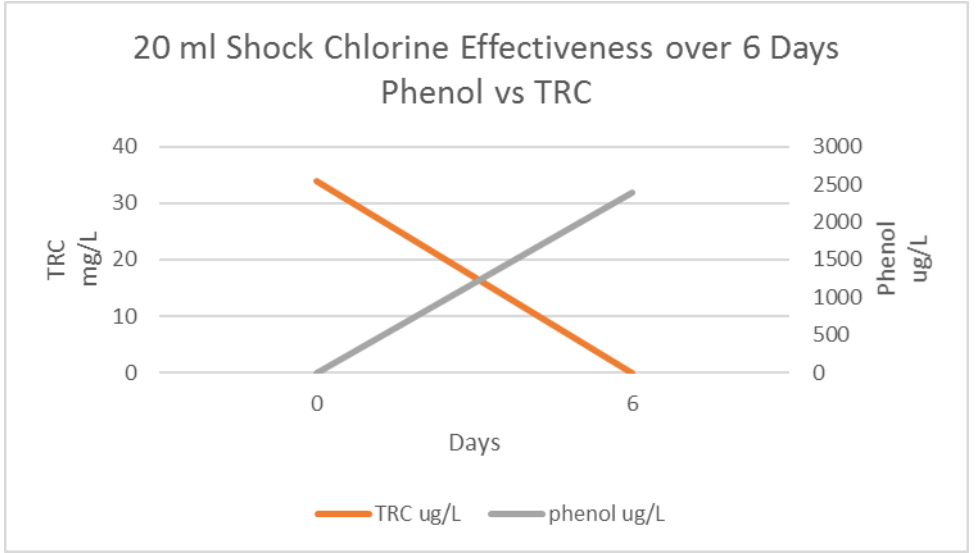
Phase 2 – Evaluation - Findings

- **Chlorine dosage closely resembles breakpoint chlorination curve**



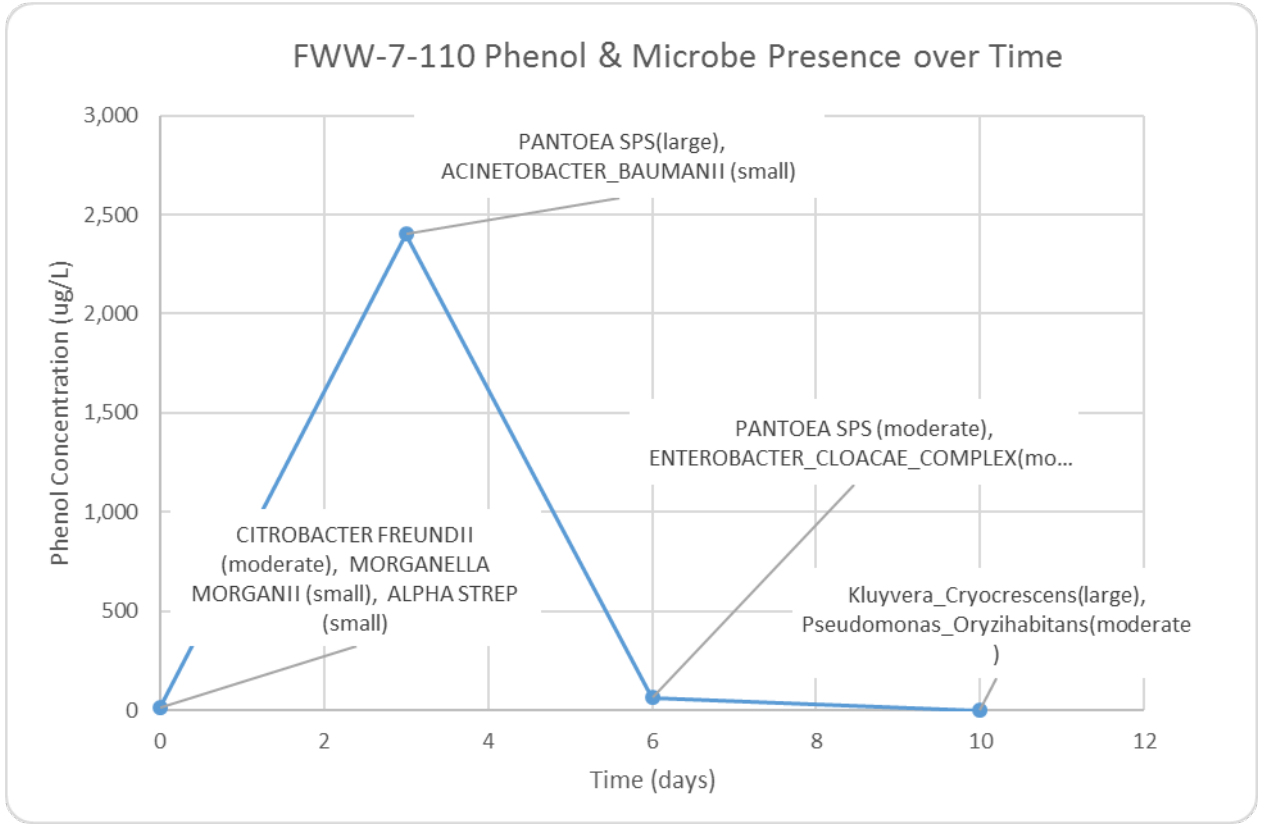
Phase 2 – Evaluation - Findings

- **Microbes consumed TRC over time**



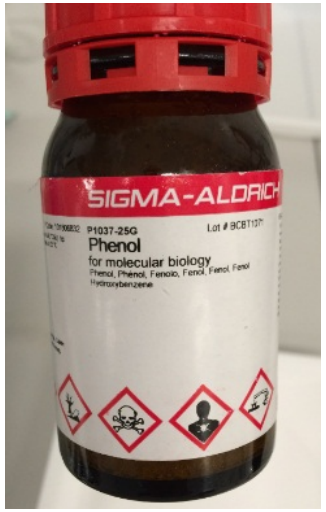
Phase 2 – Evaluation - Findings

- **Microbes exhibit endogenous decay after 3 days**



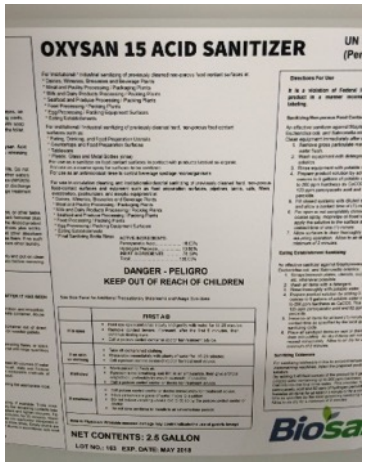
Phase 3 – Evaluation - Objectives

- **Disinfection power of alternatives**
  - Short term exposure
  - Long term exposure
- **Oxidizing power of alternatives on phenol spike**



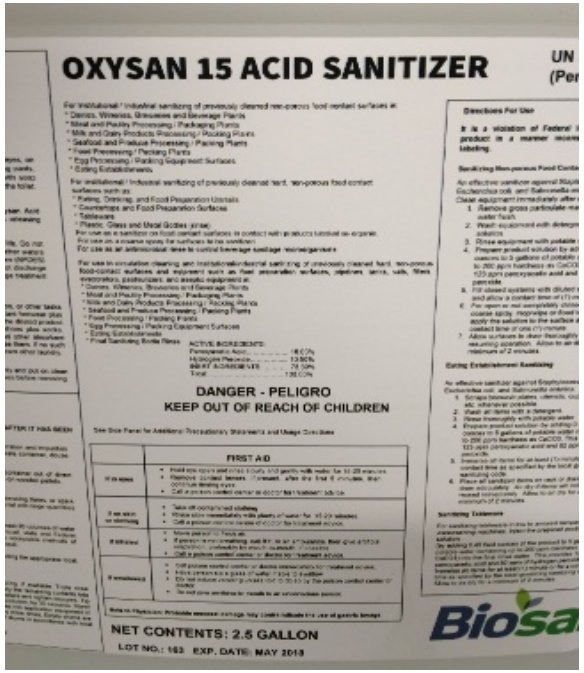
Phase 3 – Chemical Alternatives

- **Selected**
  - Peracetic acid
  - Hydrogen peroxide
  - Hydrogen peroxide + catalyst
  - Sodium Percarbonate
- **Reviewed**
  - Disinfection
  - Oxidation
  - Hazard classifications



Peracetic Acid

- **Commercial products**
  - Oxysan
  - Mincare
- **$C_2H_4O_3$**
- **Characteristics**
  - Health - 3
  - Flammability – 2
  - Reactivity – 2
  - Strong odor
  - Effective at pH of 7
  - Will oxidize cell membranes
  - Commonly used in biological laboratories



Sodium Percarbonate

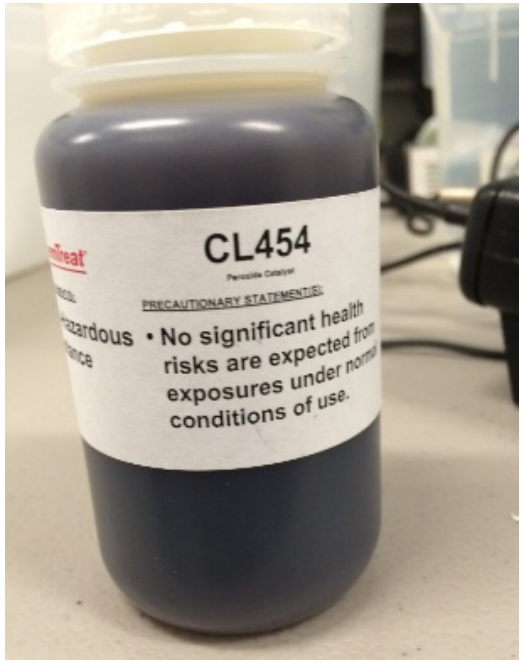
- **Commercial products**
  - Oxyclean
  - Laundry detergent
- **(Na_2CO_3-1.5H_2O_2)**
- **Characteristics**
  - Health - 2
  - Flammability – 0
  - Reactivity – 1
  - Strong oxidizer
  - Disassociates into hydrogen peroxide and sodium carbonate (strong buffer)
  - Takes time to dissolve (residual solids)

Hydrogen Peroxide (35%)

- **Commercial products**
  - Drug store disinfectant (3-5%)
- **H_2O_2**
- **Characteristics**
  - Health – 3
  - Flammability – 0
  - Reactivity – 2
  - Uses free oxygen radicals
  - Very unstable (loses effectiveness with time and temp)
  - Alone not a powerful disinfectant but improves with UV and ozone
  - Oxygen off gassing

Hydrogen Peroxide (35%) + Catalyst

- **Commercial products**
  - Drug store disinfectant
  - Specialty chemical supplier
- **H_2O_2 + ferrous iron catalyst**
- **Characteristics**
  - Fenton's Reaction
  - Increases oxidation power



Phase 3 – Evaluation – Findings

Disinfectant/ Oxidizer	Phenol inhibition over time	Instantaneous oxidation of phenol	Hazard Level	Odors	Other Considerations
Chlorine Bleach	Yes	Yes	Moderate	No	Easy to purchase
Peracetic Acid	Yes	Incomplete	High	Strong	Common lab chemical
Sodium Percarbonate	Yes	Yes	Low	No	Residual solids Off-gassing
Hydrogen Peroxide	Yes	Incomplete	Low	No	Off-gassing
Hydrogen Peroxide + Catalyst	Yes	Yes	Low	No	Heavy off-gasing discoloration

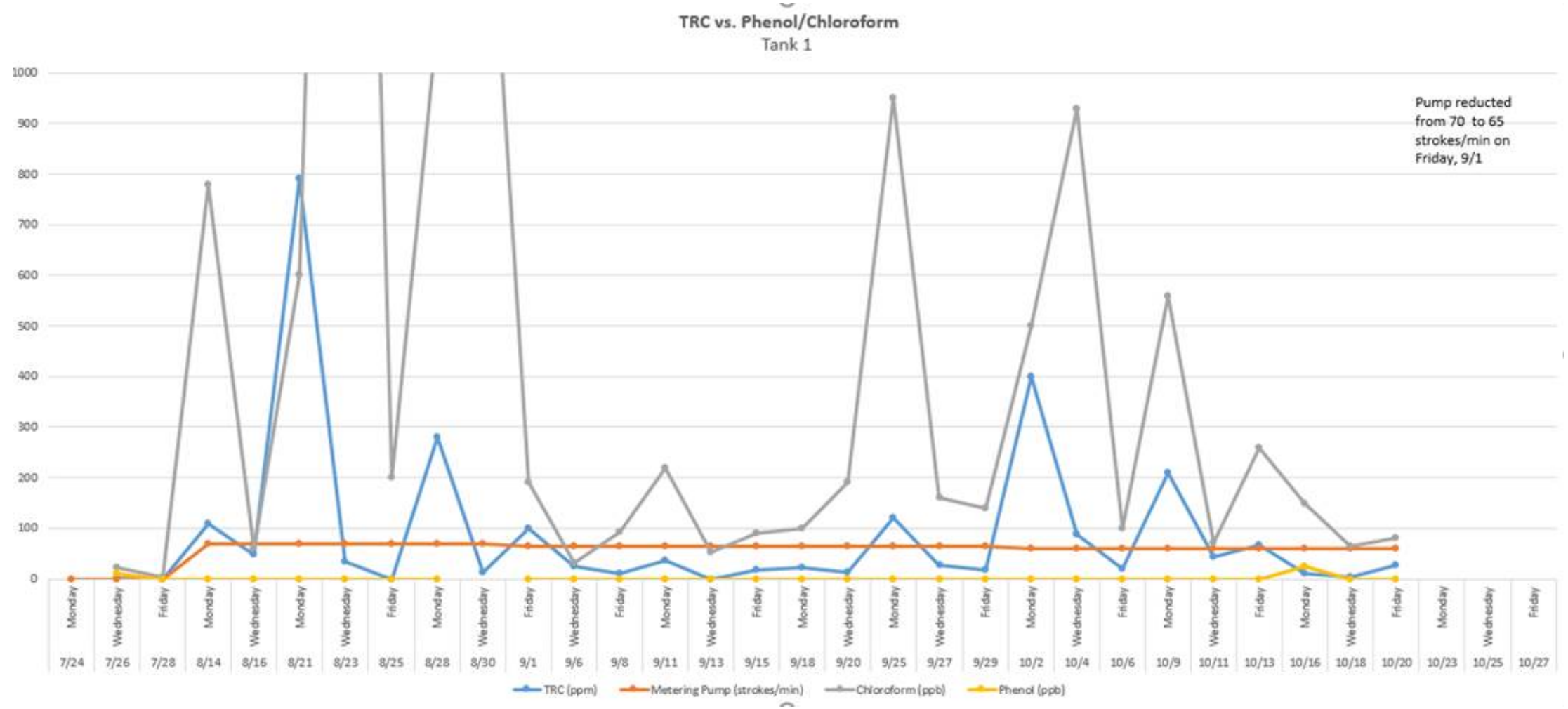
Testing Conclusions

- **Chlorine bleach most effective**
  - Oxidizes phenol
  - Inhibits microbial activity
- **Maintain optimum TRC**
  - Inhibit microbial growth
  - Minimize chloroform generation

Moving Forward

- **Short term**
  - Monitor TRC, phenol and chloroform levels
  - Install temporary chlorine dosing system
- **Moderate term**
  - Design chlorine dosing system with TRC controller
- **Long term – WWT Redesign**
  - Decrease residence time
  - Improve chemical addition
  - tanks for easy cleaning

Short Term – Trend Sampling



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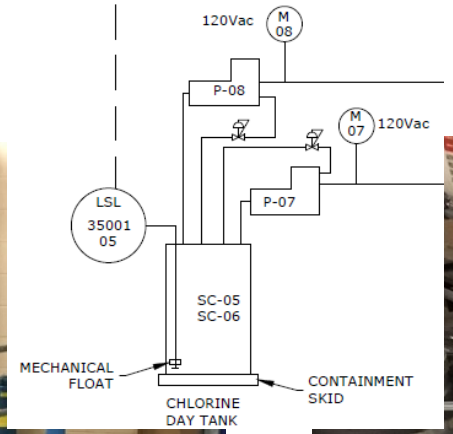
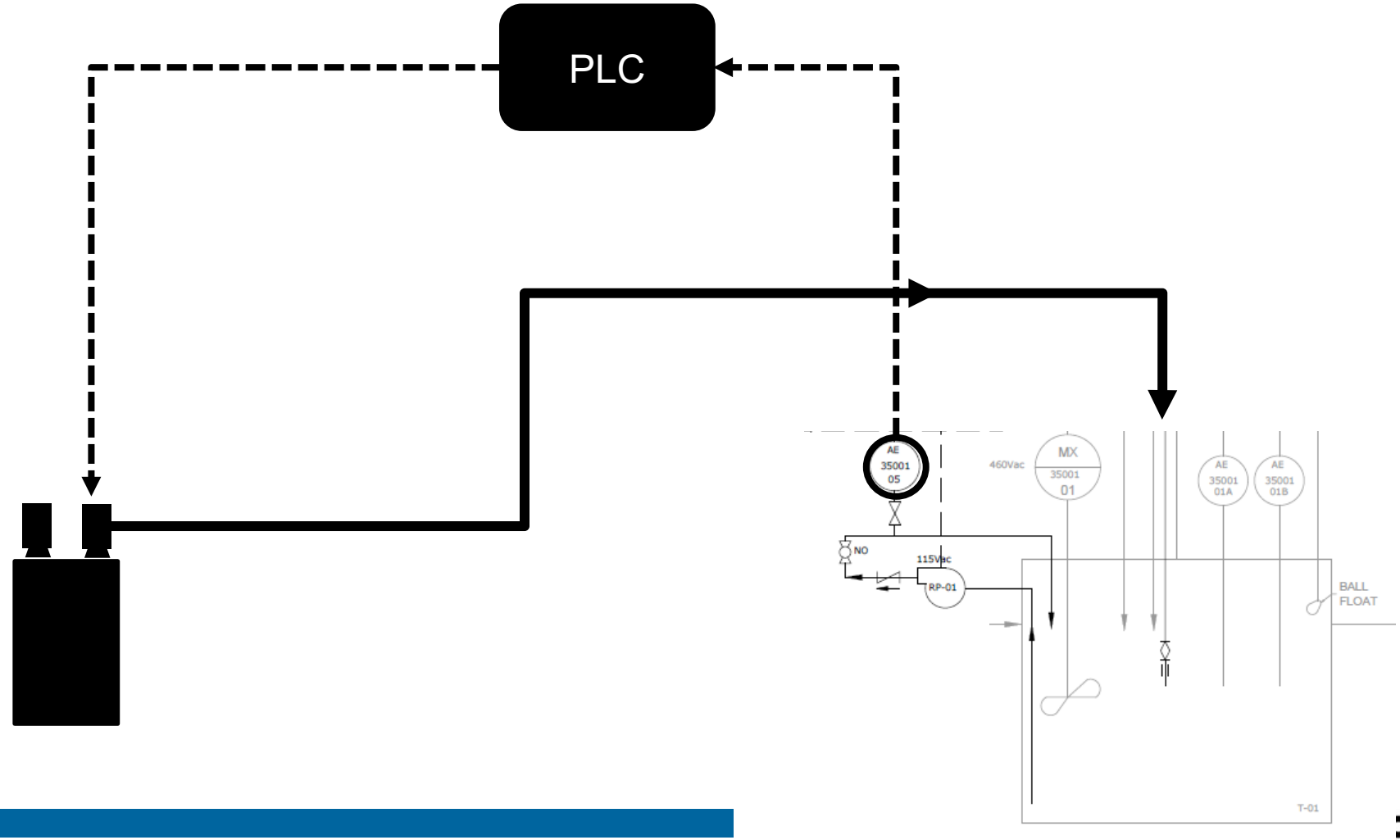
Short Term - Temporary Bleach Dosing System



Moderate Term – TRC Controller

- In-line chlorine analyzer
- Feedback control to PLC
- Maintain total residual chlorine at 20-30 mg/L
- Continue monitoring and trending
  - Phenol
  - TRC
  - Chloroform

Moderate Term – TRC Controller



Long Term – System Redesign

- Reduce reactor sizes to minimize detention time
- Active ventilation
- Process-like equipment
- Automated disinfection controls
- Minimize surfaces for biological growth

Conclusions

- Biological activity can have adverse impacts
- Bigger is not always better
- Approach wastewater treatment as a “process”