# Challenges Assessing and Treating Wastewater from Biotechnology Scale Up Operations

## **Amgen Corporation**

Derek Sykes Jessica Sgrignuoli

### **Tighe & Bond**

Wayne Bates PhD, P.E. William Potochniak, P.E.





## **About Amgen**

Tighe&Bond

- 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

liane&Bond



### **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?





#### 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**

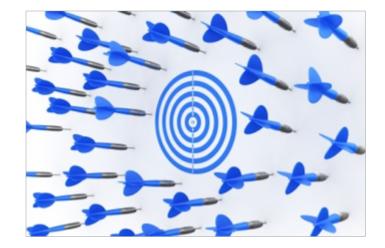
### Use seed from wastewater treatment system

#### 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**

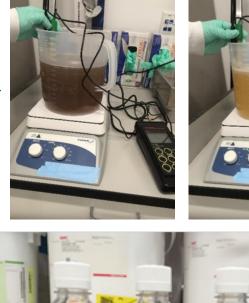






### **Phase 1 – Evaluation - Approach**











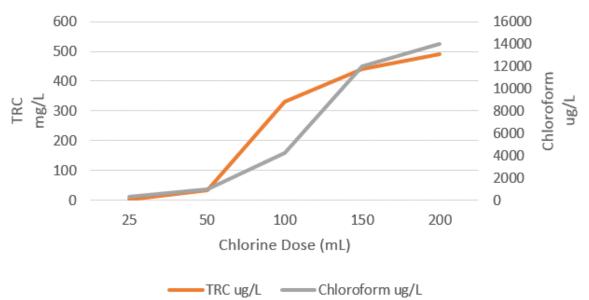


- Higher and lower pH generated less phenol
- SUL wasted did not contain phenol
- Use of chlorine bleach
  - Addressed phenol generation
     » Disinfection or oxidation
  - Generated chloroform



Tighe&Bond

#### Phase 1 - Chlorine Dose vs TRC and Chloroform





### **Phase 2 – Evaluation - Objectives**

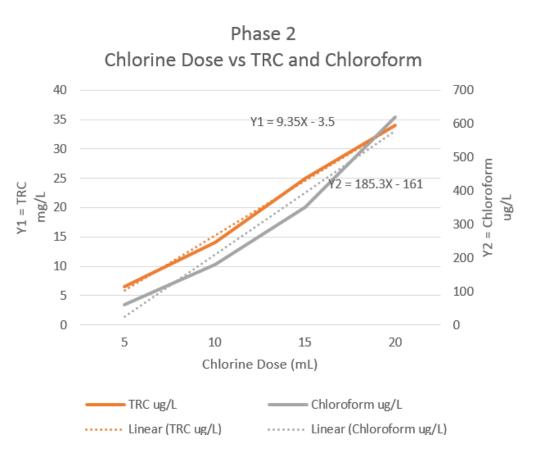
#### Chlorine bleach dosing – additional analysis to fine tune

- Observe odors
- Sample chloroform generation
- Measure TRC vs phenol over time
- Evaluate phenol generation over longer exposure durations
- Identify predominant microbes





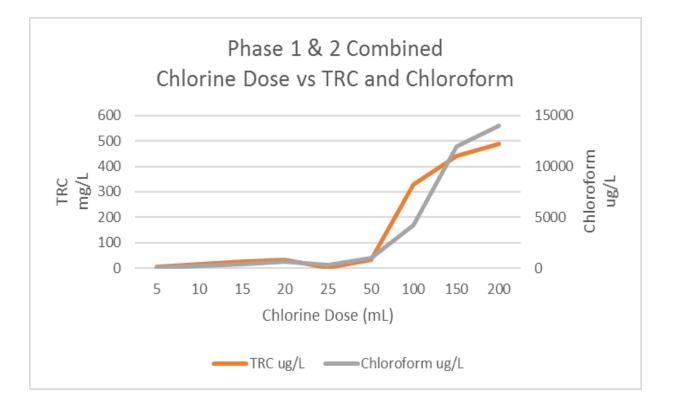
#### Chloroform generation and TRC linear at lower dosages



Tighe&Bond



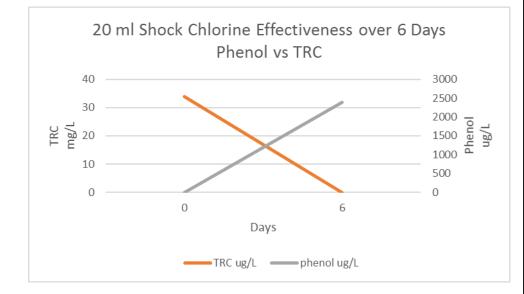
Chlorine dosage closely resembles breakpoint chlorination curve







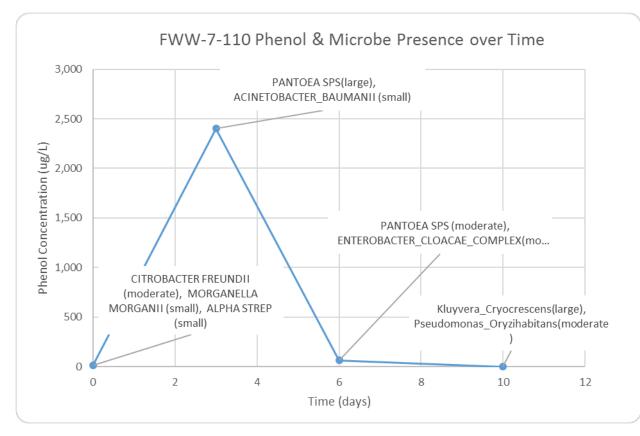
#### Microbes consumed TRC over time







#### Microbes exhibit endogenous decay after 3 days



Tighe&Bond



### **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
- $\blacksquare C_2H_4O_3$

#### Characteristics

- Health 3
- Flammability 2
- Reactivity 2
- Strong odor
- Effective at pH of 7
- Will oxidize cell membranes
- Commonly used in biological laboratories

	OXY	SAN 15 ACID SANITI			
1 :	ermentational fit	Markini sentions of previously disarted net-portors 'ood renter! serfaces in . Ormowies and because Plants	Databases For Use		
	Mile and Peulie Mile and Gairy P Seafood and Pr Free Processin Eco Processing	It is a violation of Federal I product in a manner money Labeling.			
g marels.	* Esting Establish	amands.	Senitiving Herspectrum Field Cards		
	For institutional 1 software such as 1 Exting, Drinker 7 Countertops as	An effective confider openal Staph Suchardus coli and Subwordta e Class equipment increationly after 1. Remove proce particulate me			
sen Acie	* Tableware * Plastic Glass For use as a sa	Autor Such. 2. Wast-equipment with delenger solution			
He Const	FOUND IN A STORE	ania spory for surfaces to control terminari Ministrative in control terminare contrage recording work in	3. Rinse equipment with potable		
en (APOUS D. Golberge De Teatrait	<ol> <li>Prepare product solution by 40 concerns to 5 gathere of potel/in to 200 ppm bachwars as GaCO 125 ppm perceparate and and perceide.</li> <li>Pro alread conterns with divider?</li> </ol>				
N. Of Other Salas, and femalest plan feedback product from plan accts of Other Security, and Other Security, in Barn, if no such nam other Isundy.	* Note and Dat * Seaflood and * Poset Proces * Eag Presses	ing Rooks Farst ACTIVE ACREDIENTS	<ul> <li>and allow a contact serve of Thm e</li> <li>Parc apon or an isotraphicly chose contact sprice and the satisfiest of the satisfiest of a contact time or one chromosome for the satisfiest of the satis of the satis</li></ul>		
the set put on share	1	Parageostic April 18 (24) Hydrogen Macada 13 BCh Barr / Acheciae VIII 78 (34) Tool 18 Ch	relationers of 2 monutes. Carting Entablishment Randitating		
	10	DANGER - PELIGRO	Au effective cashior against Scolorinaee Exclusiona coli and Rationality ontoing		
NTIDE IT HAS BEEN	H See Don F	KEEP OUT OF REACH OF CHILDREN	<ol> <li>Scraps bases of patient, cherech, car att, interven possible</li> <li>Unad at them with a cherepart.</li> <li>Plane transcriptly with prisole water</li> <li>Prepare president unitation de infiliação entres ou la calcura de activos de activos de activos de</li> </ol>		
-			to 201 gors faurtheas as CalCOD. This 123-pps personalic and well 82 gor		
This are repaired		FIRSTAD	presidentille.		
Oftamar out of domar Of recentar pathols.	11	Profil and copy and chief body bid perty with weiter the 12 at resource     Resource contain tensors if annuals, where the first 6 minutes, then     contains thering years     Call any stars installed control at occurs for the start of column	<ul> <li>constant time as execution by the tool of samillaring code.</li> <li>Place all samples interaction of the deale accounting As day down with the research composition. All days for the to- mand compositions. Allow to be for the to- ter days.</li> </ul>		
and and responses of the	1	<ul> <li>Coll a primer control target of 40000 for bitstand advice.</li> </ul>	Sandrag Tablerate		
tenti di concesso di con- tranti di concesso di con- la spontazione constructo di con- tranti di constructo di di con-		Move period to France al:     A previous residence and liter as an amounted. There are an address the previous residence in an address of the previous residence in a second of the	For example on products, the control of the second		
ting d makada Tup y be altaban tup tata ang tup tata ang tup tup tup tup tup tup tup tup tup tup		50 EXPLOSE exceeded controls or desites interactional to Control and Access     4 Hote interactions in parts of desity of control of the Section     50 test interactions are stated of the Section 20 to 20 test test     50 test interactions control or visual to 50 to 20 to 20 test test     50 test interactions control or visual to 50 to 20 to 20 test test     50 test interactions control or visual to 50 to 20 to 20 test test	parente analysis and a book to an environment of the analysis of the a set for any second set of the form of the form parents of a second set for an environment of the form parents of a second set for an environment of a second secon		
a con antes for a constant	A Starting	Thereis are invested and a straight way (path) radiate the use of games length	Biosa		
	1.46	T CONTENTS: 2.5 GALLON	Diosa		

Tighe&Bond



### **Sodium Percarbonate**

#### Commercial products

- Oxyclean
- Laundry detergent
- (Na<sub>2</sub>CO<sub>3</sub>-1.5H<sub>2</sub>O<sub>2</sub>)
- 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%)
- $\blacksquare H_2O_2$

### 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



Tighe&Bond



## Hydrogen Peroxide (35%) + Catalyst

#### Commercial products

- Drug store disinfectant
- Specialty chemical supplier

### ■ H<sub>2</sub>O<sub>2</sub> + ferrous iron catalyst

### Characteristics

- Fenton's Reaction
- Increases oxidation power









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

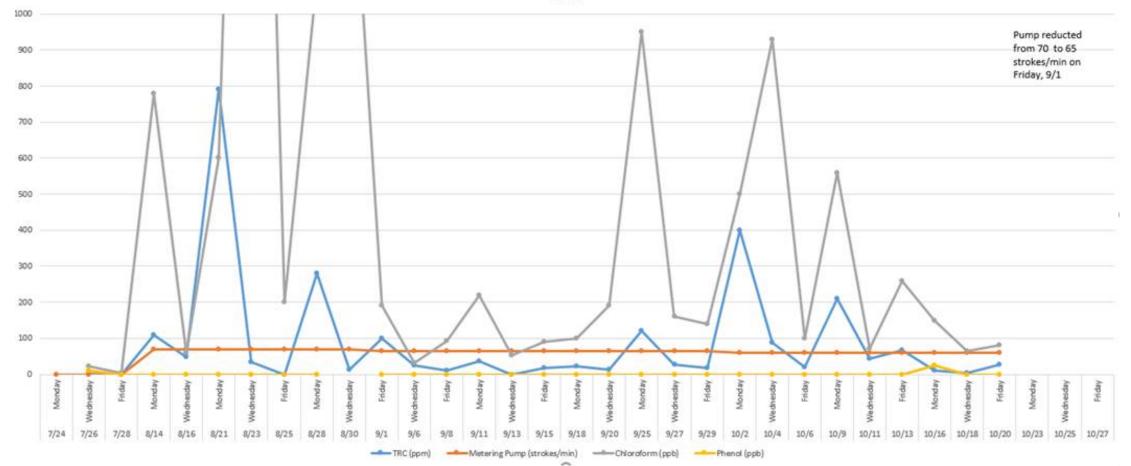
### Long term – WWT Redesign

- Decrease residence time
- Improve chemical addition
- tanks for easy cleaning





### **Short Term – Trend Sampling**

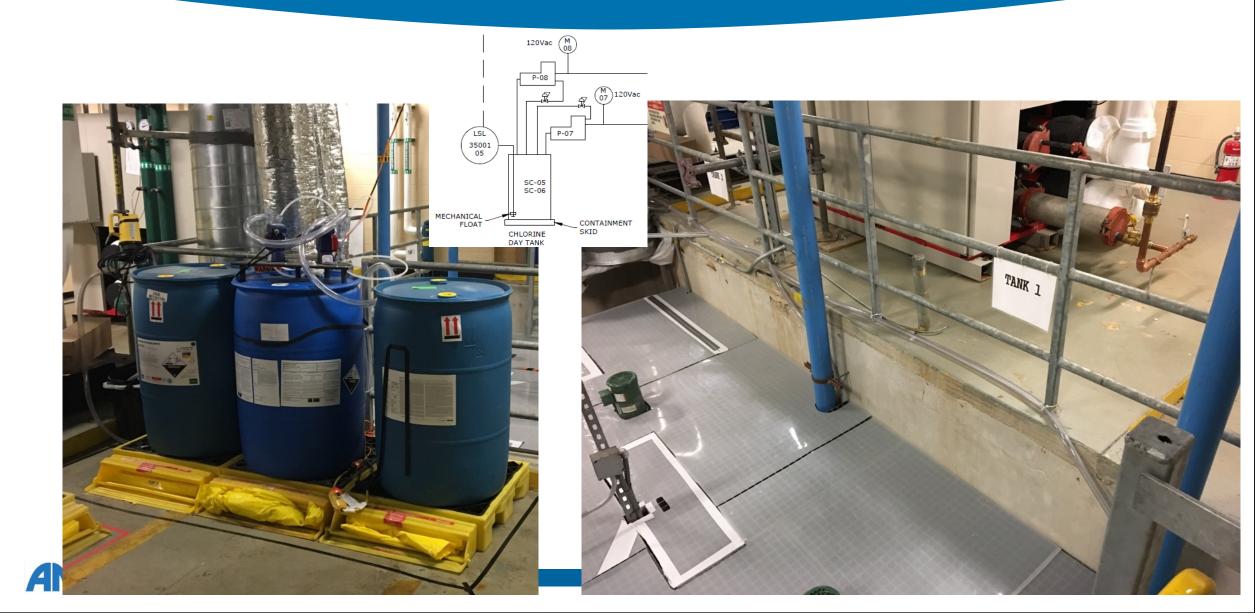


**AMGEN**°

TRC vs. Phenol/Chloroform Tank 1

#### Tighe&Bond

# **Short Term - Temporary Bleach Dosing System**



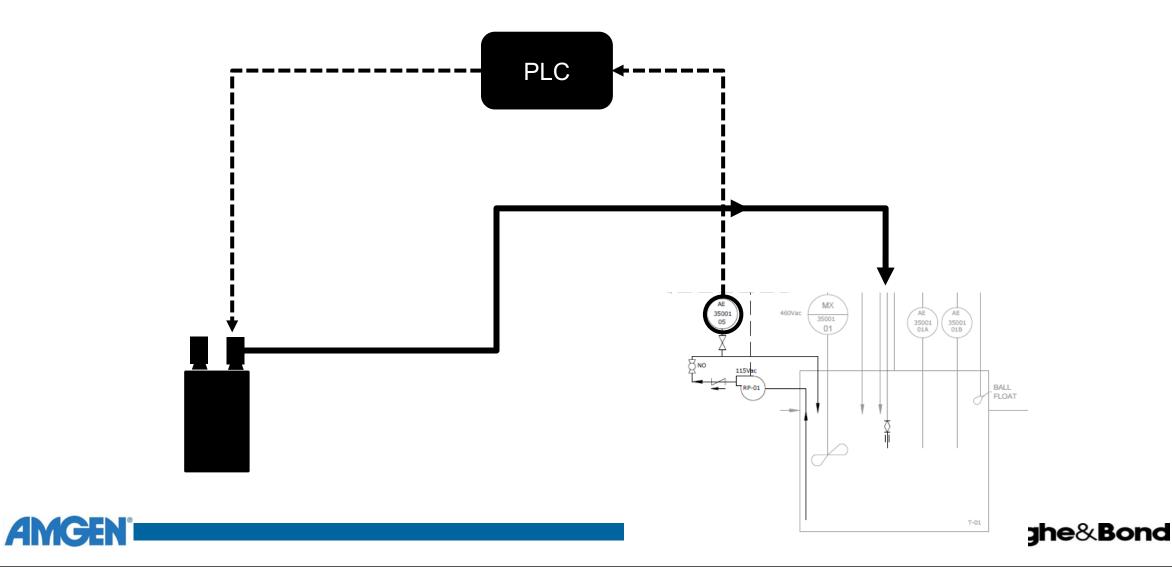
### **Moderate Term – TRC Controller**

- 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"



