# Biofilm Generation and Remediation

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# Biofilm Generation and Remediation

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## ➢ Microbial Contamination Control

- Biofilm
  - Generation
  - Remediation
  - Case Study

# Introduction

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## Types of Microbial Contamination

- “Yeasts, molds, bacteria, viruses or other similar microscopic organisms”.
- Includes species that:
  - May have public health significance
  - May cause product to decompose
  - May be indication that product contaminated with filth
  - May cause product to be adulterated

# Introduction

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## Impact of Microbial Contamination

- Disruption to production
- Time & resources for investigation
- Patient safety
- Product recall
- Brand image

# Main Sources of Microbial Contamination

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| | |
|---|---|
| Raw Materials | • Sterilize, filter or pasteurize |
| Packaging Components | • Sterilize or disinfect |
| Personnel | • Greatest source of contaminants<br>• Gowning<br>• Aseptic behaviour |

# Main Sources of Microbial Contamination

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| | |
|---|---|
| Room Environment | • Establish cleaning procedures<br>• Suitable personnel, material, waste flow & material segregation<br>• Control temperature, humidity, air change & particulates |
| Utilities | • Water, steam, compressed air & gases<br>• Qualify & maintain the critical utilities |
| Equipment | • Establish robust cleaning procedures<br>• Address equipment design issues<br>• Establish disinfection / sterilization parameters as applicable |

# Regulations

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- FDA:

*21 CFR 211.84(d)(6) "Each lot of a component, drug product container, or closure with potential for microbiological contamination that is objectionable in view of its intended use shall be subjected to microbiological tests before use."*

*21 CFR 211.113(a) "Appropriate written procedures, designed to prevent objectionable microorganisms in drug products not required to be sterile, shall be established and followed."*

*21 CFR 211.165(b) "There shall be appropriate laboratory testing, as necessary, of each batch of drug product required to be free of objectionable microorganisms."*

- USP:

*<1111> Microbiological Examination of Non Sterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use*

*<61> Microbiological examination of non-sterile products: Microbiological enumeration tests*

*<61> Microbiological examination of non-sterile products: Tests dor specified product*

# Regulations

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Control of Microbial Contamination

- 21 CFR211.13 – Drug
- 21 CFR111.365 – Dietary Supplements
- 21 CFR820.70 – Medical Device
- 21 U.S.C. 361 – Cosmetics

Contamination =

# Warning Letter

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*2. The firm failed to establish and follow appropriate written procedures that are designed to prevent microbiological contamination of drug products purporting to be sterile, and that include validation of all aseptic and sterilization processes (21 CFR 211.113(b)).*

FDA, Mar 2017

# What is an objectionable organism

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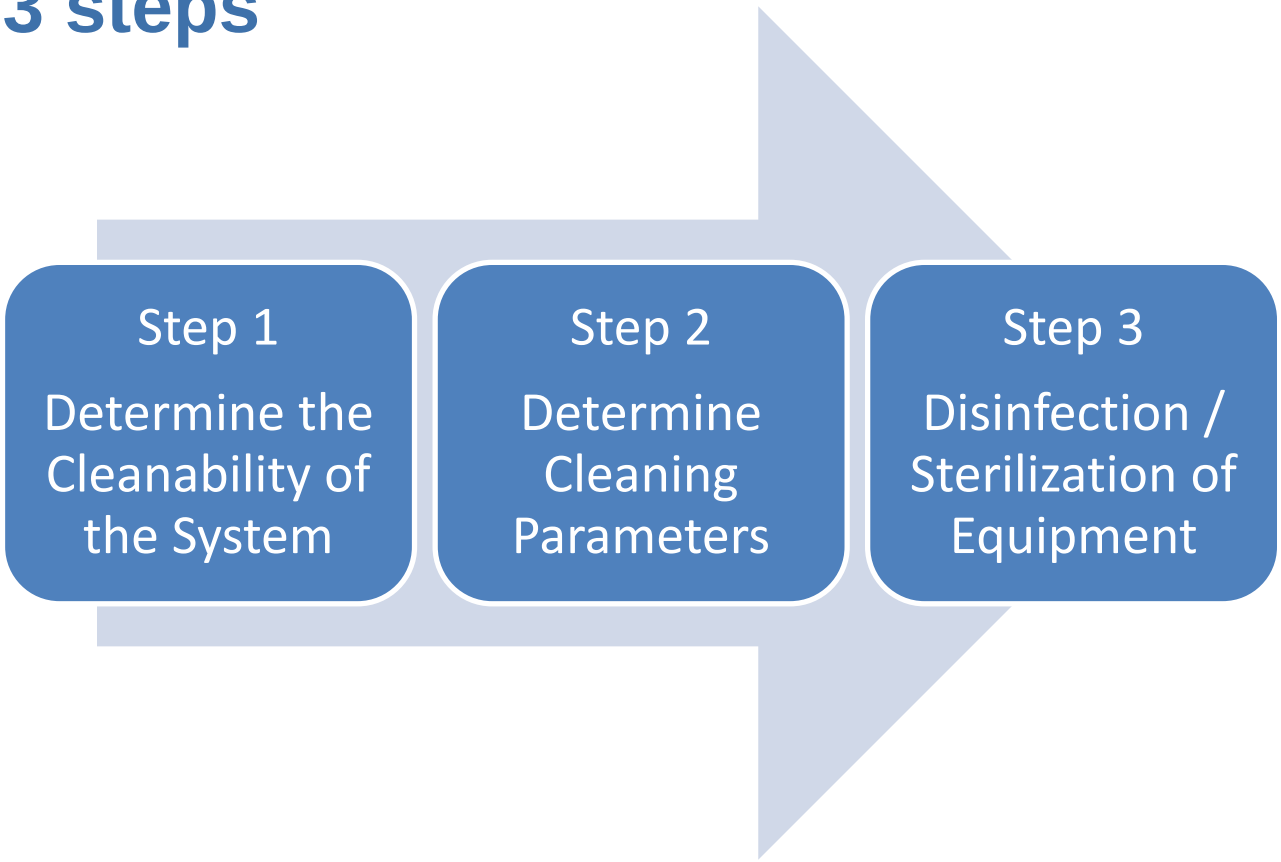
- Count based
- Identification based
- Patient safety

*LIST NON EXHAUSTIVE*

| Gram - | Gram + |
|---|---|
| E. coli | Streptococcus spp. |
| P. spp. | Staphilococcus aureus |
| P. aeruginosa | Enteroccus spp. |
| P. fluorescens | Clostrodium botulism |
| P. Spinosa | B. Cereus |
| Salmonella species | B. species |

Source : What is an “Objectionable Organism”? - Scott VW Sutton, Ph.D. And USP limits

# Removing / Controling microbiological contamination – 3 steps



# Removing / Controling microbiological contamination

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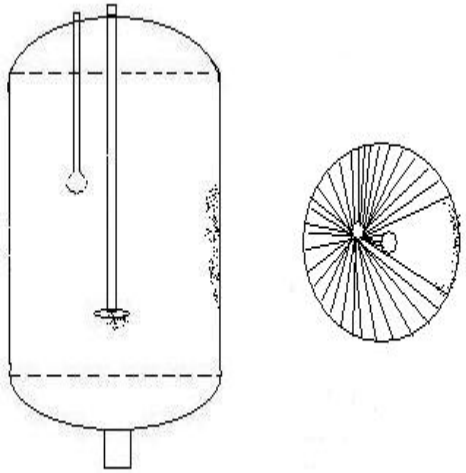
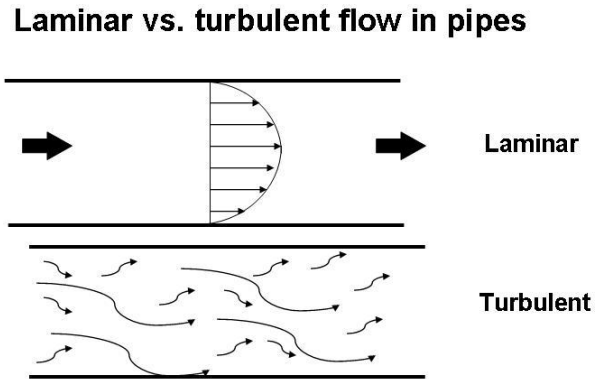
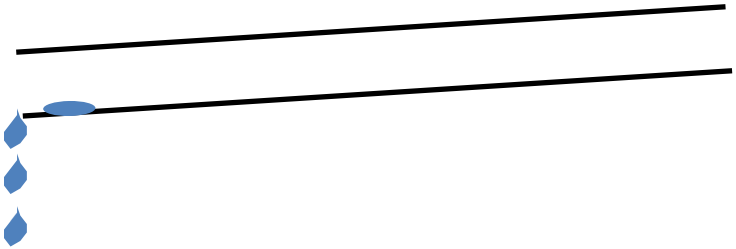
Step 1: Determine Cleanability of System

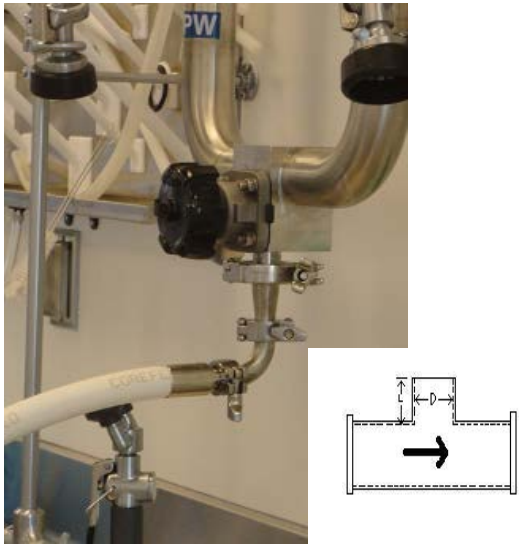
- Spray coverage
- Surface conditions
- Dead legs
- Selection & maintenance of valves, tubings & gaskets
- Cleaning flow velocity
- Slope & drainability (vessel, piping)
- Sampling ports
- Flexible hoses

# Cleanability of the system surface and equipment








Source: photo from Paul L. presentation - STERIS

# Removing / Controling microbiological contamination

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Step 2: Determine Cleaning Parameters

- Time
- Action
- Cleaning chemistry
- Concentration
- Temperature

Source : Lopolito, P. and Rivera, E., An Audit Approach to Address Microbial Contamination in Process Equipment", Chapter 15 in Contamination Control in Healthcare Product Manufacturing, Volume 1, PDA/DHI Publishing (2013)

# Determine Cleaning Parameters

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**Process And Cleaner Evaluation (PACE)**

**T – Temperature**
**C - Chemistry**
**C - Concentration**
**T - Time**


**Visual Check**
**Waterbreak Free Test**
**Gravimetric Test**

# Removing / Controling microbiological contamination

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Step 3: Sterilization / Disinfection

- Steam-in-place (SIP), dry heat, vaporized hydrogen peroxide
- Chemical sanitization utilizing sporicidal / sterilant agents

Source : Lopolito, P. and Rivera, E., An Audit Approach to Address Microbial Contamination in Process Equipment", Chapter 15 in Contamination Control in Healthcare Product Manufacturing, Volume 1, PDA/DHI Publishing (2013)

# Agenda

- Microbial Contamination Control
- **Biofilm**
  - **Generation**
    - Remediation
    - Case Study

# Impact of Biofilm

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- The presence of the Extracellular Polymeric Substances (EPS) increased the micro-organisms' resistance to environmental stresses, antimicrobial agents and cleaning agents
- Frequent microbial excursions
- Production downtime
- Adulterated products

# What is Biofilm ?

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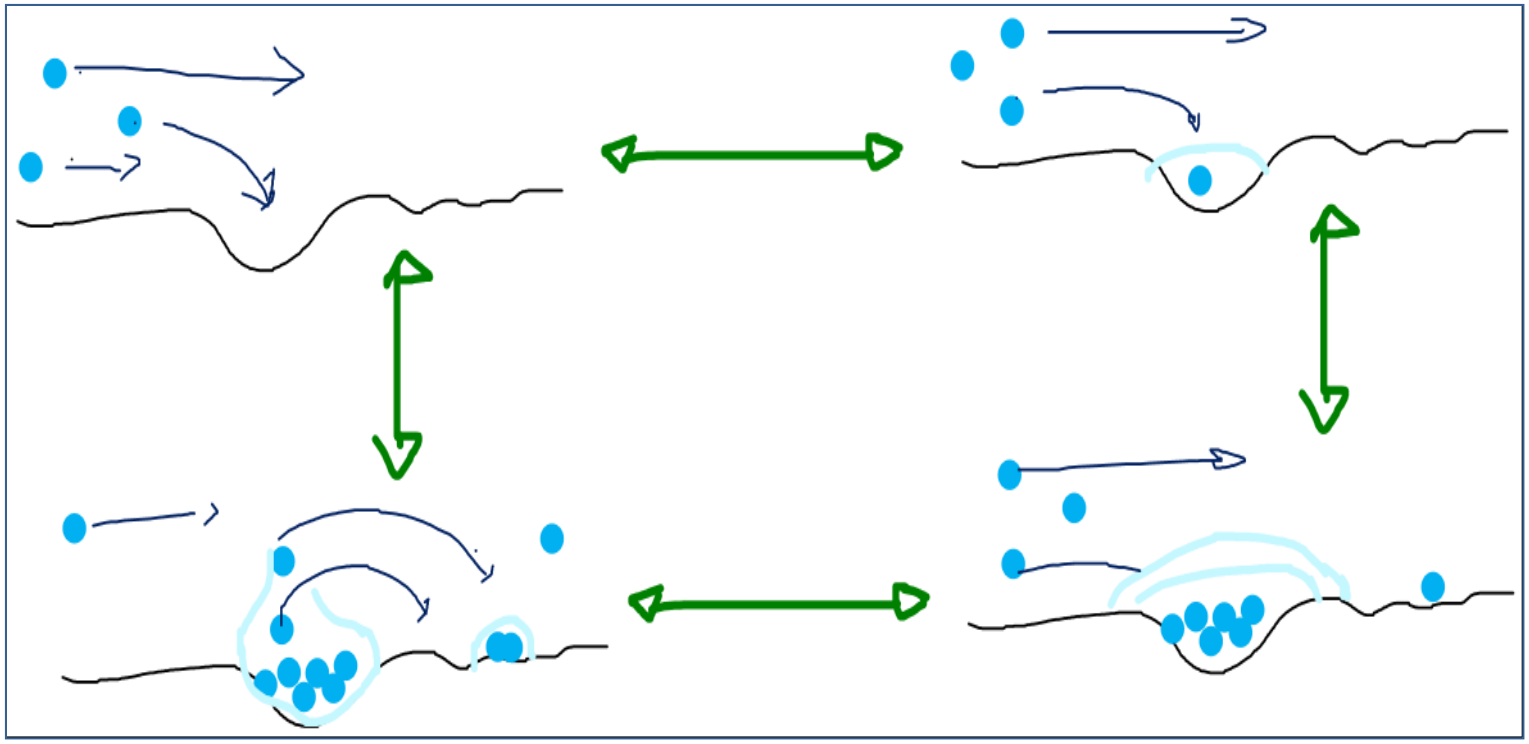
- Biofilm is generally composed of multiple microorganism encased in matrix extracellular polymeric substances (EPS):



# Generation of Biofilm

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- Bacteria adhere to surfaces in moist environment by excreting a slimy, glue-like substance known as “extracellular polymeric substances” or “EPS”
- Can be a single bacterial species, or many species of bacteria, as well as fungi, algae, yeasts, protozoa, other microorganisms, debris and corrosion products

# Generation of Biofilm

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- Can be as thin as a few cell layers or a few inches thick, depending on environmental conditions.
- Composed of multiple microorganisms (such as Pseudonomas or Burkholderia species, Pseudomonas Aeruginosa, Bacillus Cereus)
- Encased in matrix extracellular polymetric susbstance (EPS). Complex mixture of polysaccharides, nucleic acids & proteins

# Biofilm Lifecycle - Attachment

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- Free-floating, or planktonic, bacteria attached to a surface
- Produce slimy extracellular polymeric substances (EPS) and to colonize the surface
- Can anchor more permanently using cell adhesion molecules, proteins on their surfaces that bind other cells in a process called cell adhesion.

# Biofilm Lifecycle – Growth & Detachment

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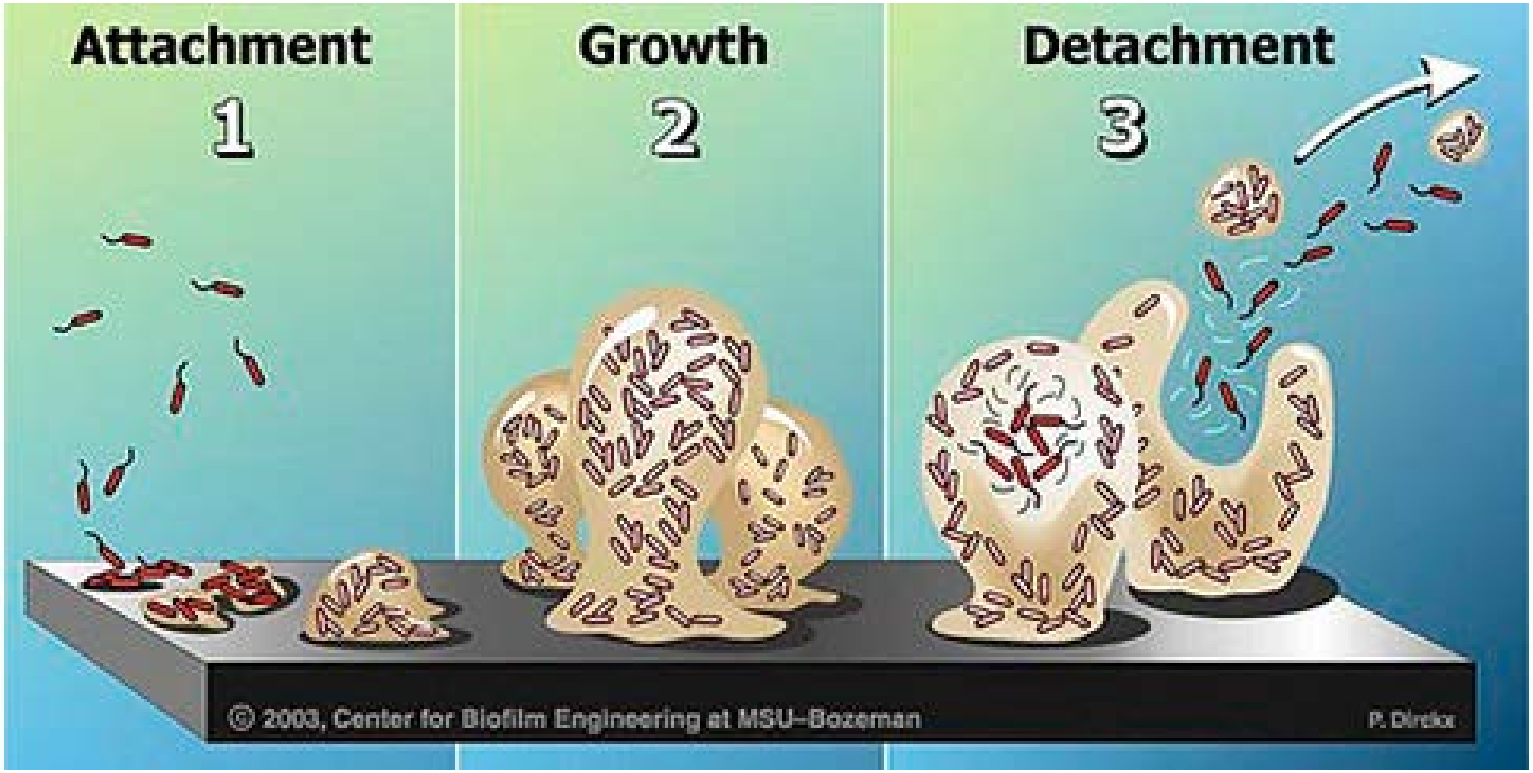
- EPS production allows the emerging biofilm community to develop a complex, three-dimensional structure that is influenced by a variety of environmental factors.
- Biofilms can propagate through detachment of small or large clumps of cells, or by a type of “seeding dispersal” that releases individual cells.
- Detachment allows bacteria to attach to a surface or to a biofilm downstream of the original community

# Biofilm Lifecycle




Source: *Montana State University Center for Biofilm Engineering*

# Factors influencing biofilm generation

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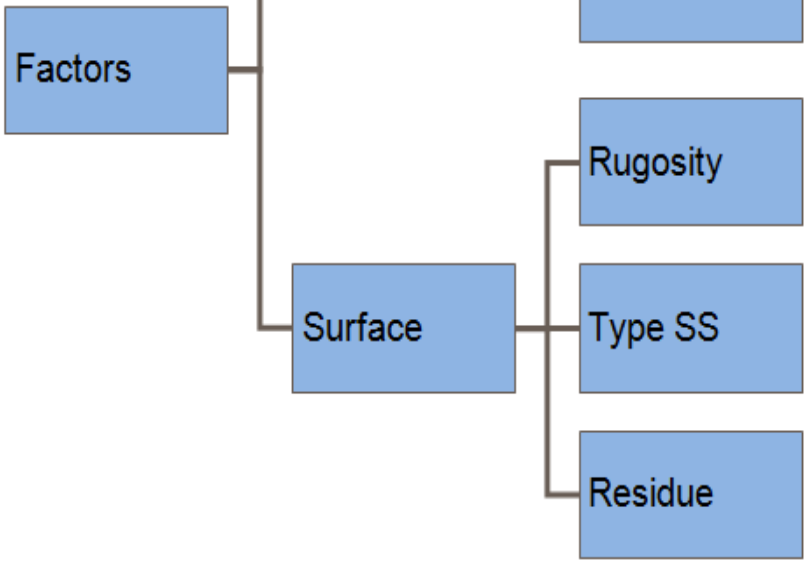


- Laminar velocity (<1.5m/s) increase the probability of microorganism to fix on the surface
- L/D>2 → increase the presence of wet environment and conductive to biofilm growth
- > 0.5 Ra for clean piping and product contact
- Rouging is also a source increasing rugosity
- Biofilm easily generate on 304 SS rather than 316L SS
- poor cleaning procedure will increase residue on the surface, increasing rugosity…

# Effect of Rouge on Cleaning

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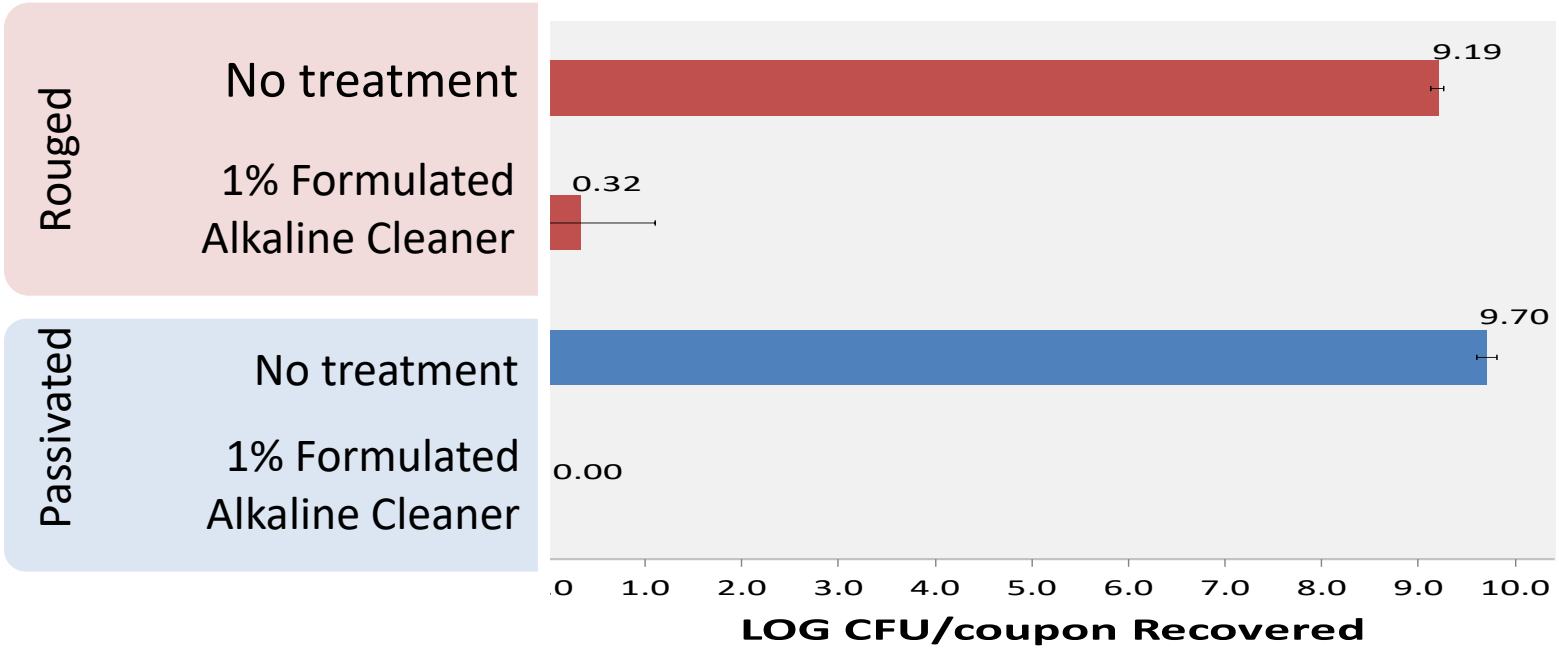
*Treatment 30 minutes at 60°C*




Source: Deal, A., Klein, D., Lopolito, P. and Schwarz, J. (2014) Use of CDC Biofilm Reactor to Test Cleaning and Disinfection on Rouged Stainless Steel , Poster, Center for Biofilm Engineering Conference, Montana State University, July, 2014.

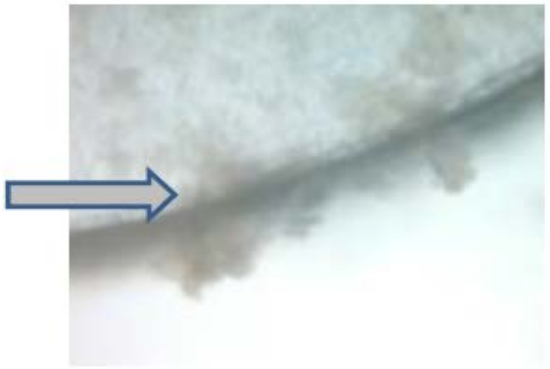
# Agenda

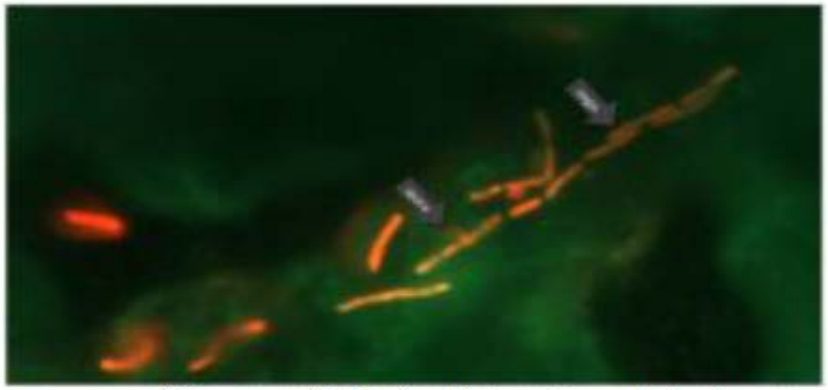
- Microbial Contamination Control
  - **Biofilm**
    - **Generation**
    - **Remediation**
      - Case Study

# Biofilm Remediation

- EPS increases the micro-organisms ability to resist environmental stresses, anti-microbial agents and cleaning agents
- Critical to remove EPS prior to disinfection or sterilization
- The presence of biofilm requires a modification of the cleaning procedure to remove microbial residue.

# Biofilm Residue





Pictures provided by Dan Klein and Amanda Deal

- EPS can be harder to clean than process residue
- Residual organic material can reduce the efficacy of biocides
- Residual EPS can reduce penetration of biocides

# Determine cleaning parameters : Laboratory development for biofilm

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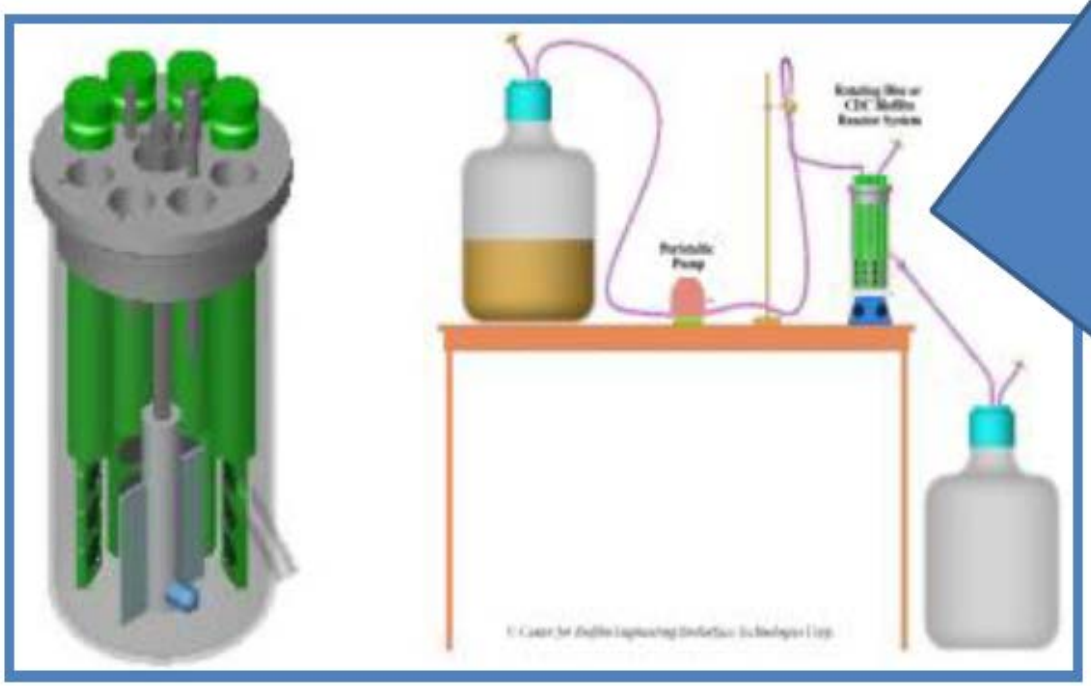
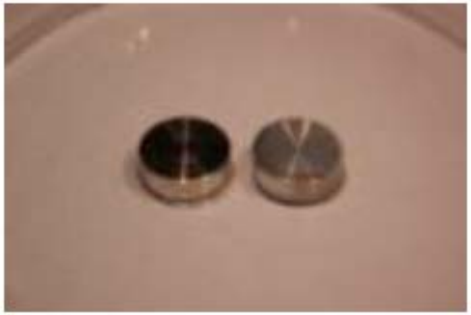
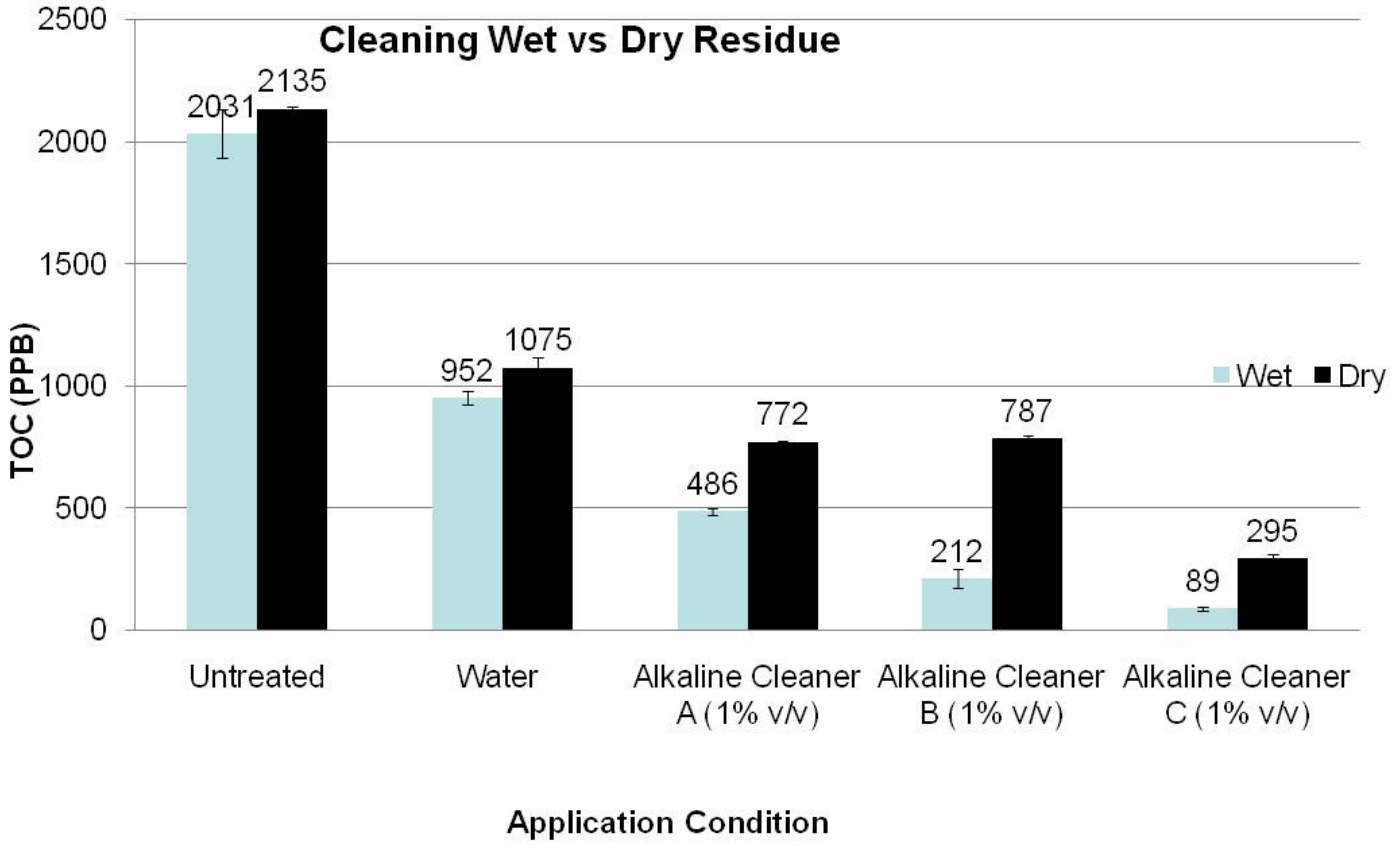
- CBE/ASTM/EPA Standardize methods
- MB-19-02 US EPA SOP (date revised 08-06-13)
- MB-20-01 US EPA SOP (date published 08-06-13)





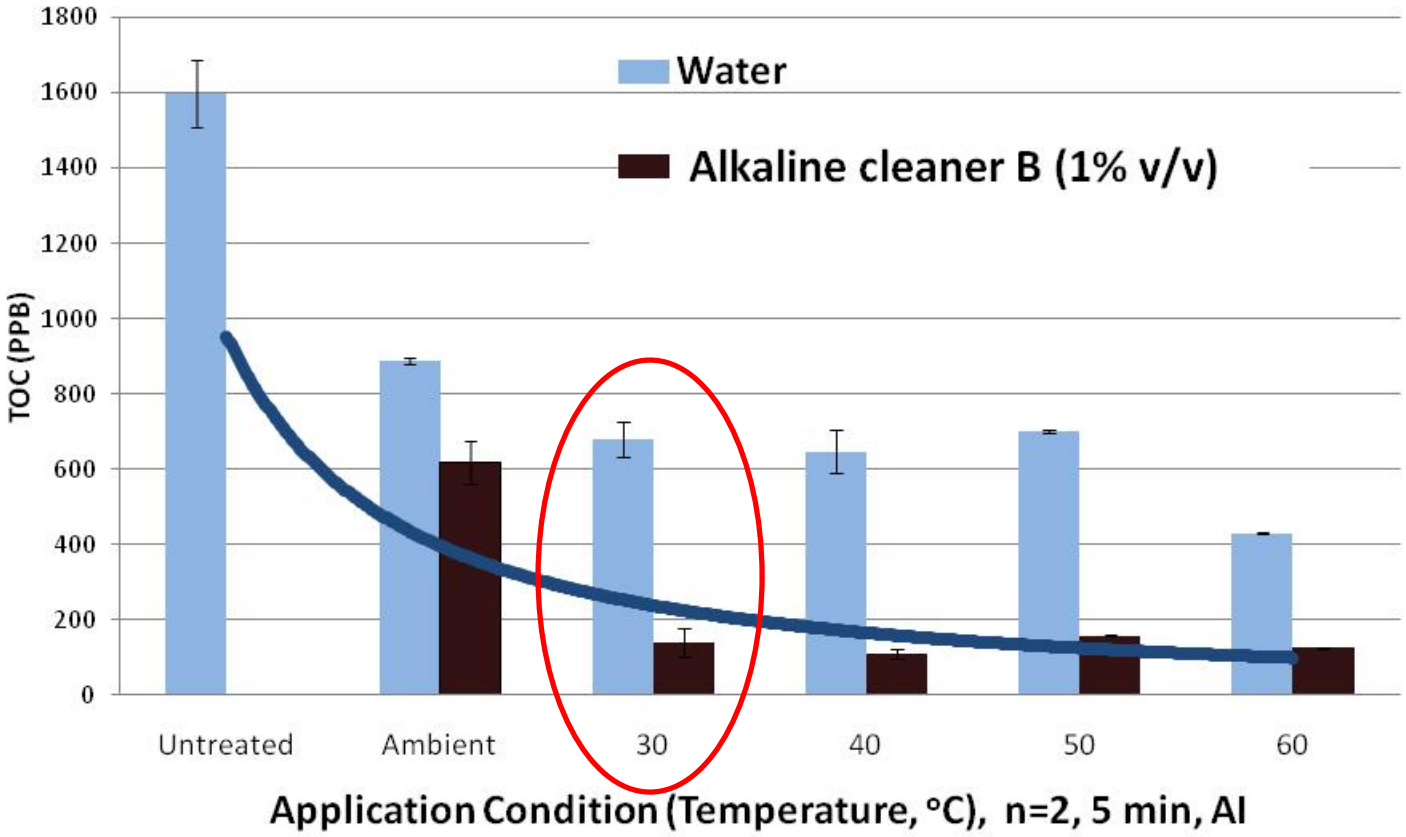
Center for Biofilm Engineering BioSurface Technologies Corp.

# Determine cleaning parameters : Laboratory development for biofilm – residue cleanability



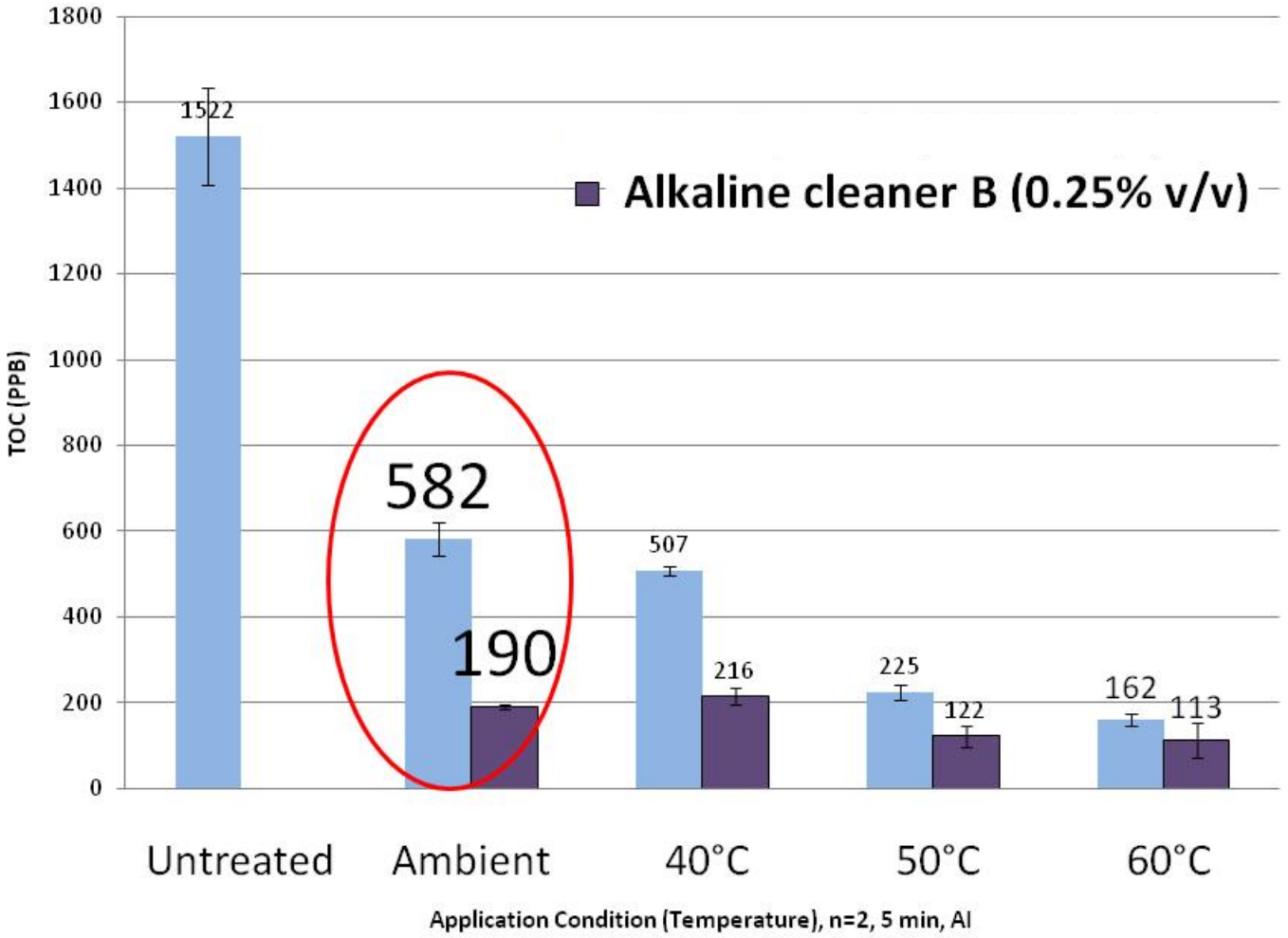

Source: Dell'Aringa, B., Deal, A., Klein, D., and Lopolito, P., (2013) The Use of CDC Biofilm Reactor to Test Cleaning Agents, Poster, Center for Biofilm Engineering Conference, Montana State University, Feb 5-6th, 2013.

# Determine cleaning parameters : Laboratory development for biofilm – temperature effect



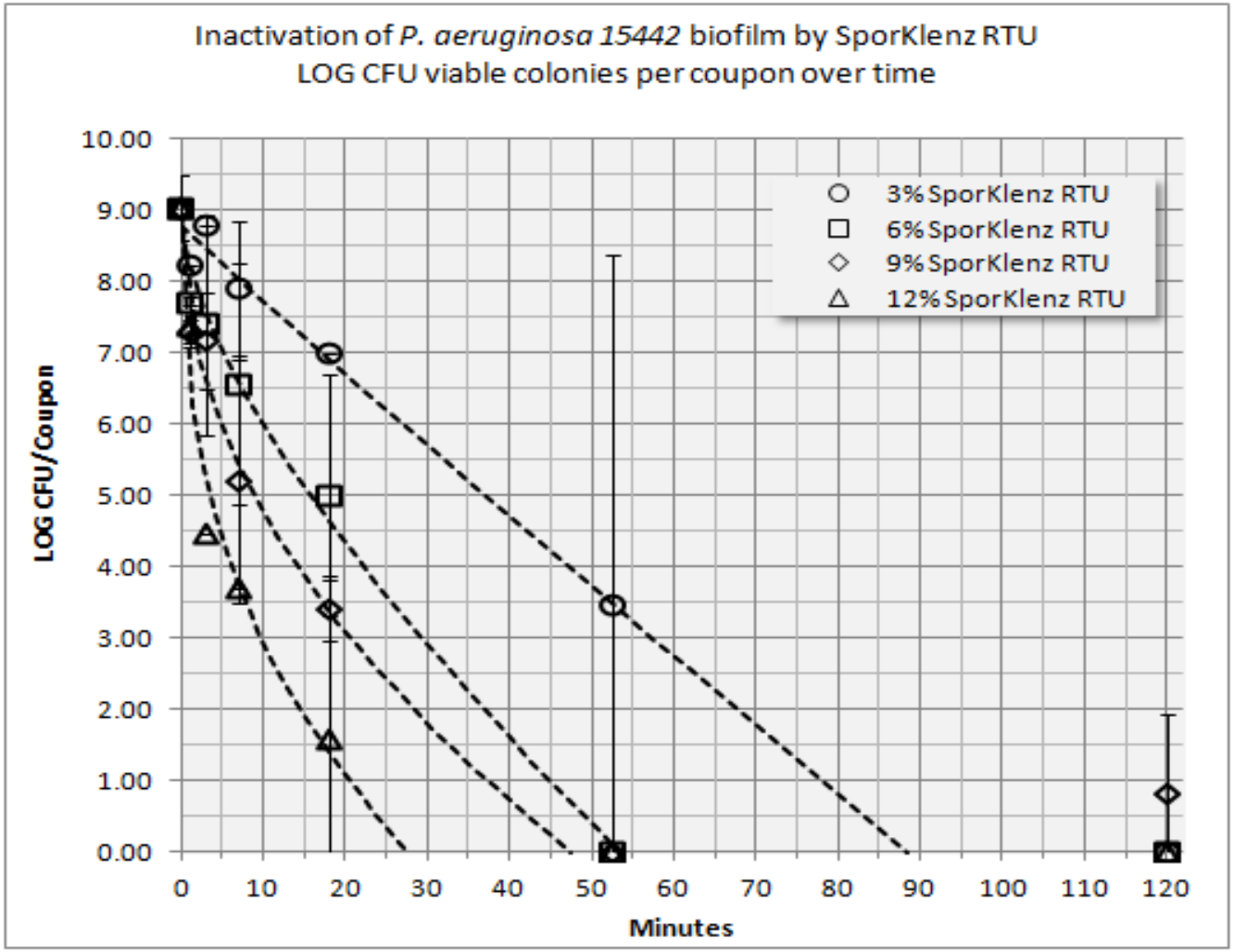

Source: Dell'Aringa, B., Deal, A., Klein, D., and Lopolito, P., (2013) The Use of CDC Biofilm Reactor to Test Cleaning Agents, Poster, Center for Biofilm Engineering Conference, Montana State University, Feb 5-6th, 2013.

# Determine cleaning parameters : Laboratory development for biofilm – chemistry effect




Source: Dell'Aringa, B., Deal, A., Klein, D., and Lopolito, P., (2013) The Use of CDC Biofilm Reactor to Test Cleaning Agents, Poster, Center for Biofilm Engineering Conference, Montana State University, Feb 5-6th, 2013.

# Determine cleaning parameters : Laboratory development for biofilm resistance to disinfectant




Source: Biofilm Remediation Strategies.ppt – Paul L. and Elizabeth R. - STERIS

# Biofilm Remediation

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- Biofilm remediation (2 steps) will always use a combine strategy:
  - Use of alkaline cleaning chemistry to penetrate / denature the EPS
    - Thorough pre-cleaning using 5% formulated alkaline cleaner at 60°C for 3 hours
  - Use of the sporicidal chemistry / SIP to sanitize / sterilize the system
    - Sanitize using 5% hydrogen peroxide & peracetic acid blend at 25°C for 30 minutes, or
    - Steam in place

# Biofilm & Rouge Remediation

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- Biofilm & Rouge remediation (3 steps):
  - Use of alkaline cleaning chemistry to penetrate / denature the EPS
    - Thorough pre-cleaning using 5% formulated alkaline cleaner at 60°C for 3 hours
  - Acidic derouging and passivation using 15% formulated acidic cleaner at 80°C for 5 hours.
  - Use of the sporicidal chemistry / SIP to sanitize the system
    - Sanitize using 5% hydrogen peroxide & peracetic acid blend at 25°C for 30 minutes, or
    - Steam in place

# Biofilm remediation – Case Study

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- Manufacturing site having continual issues with an objectionable organism *Enterobacter cloaceae* in piping, tank and filler.
- Recommended cleaning
  - Thorough pre-cleaning using 5% CIP100 at 60°C for 3 hours.
  - Acid cleaning and passivation using 15%  CIP200 at 80°C for 5 hours.
  - Sanitize using 5% Spor-klenz at 25°C for 30 minutes.
- After cleaning, derouging and sanitization, no microbial excursions have been reported.

# Cleanability of the system surface and equipment : Case study

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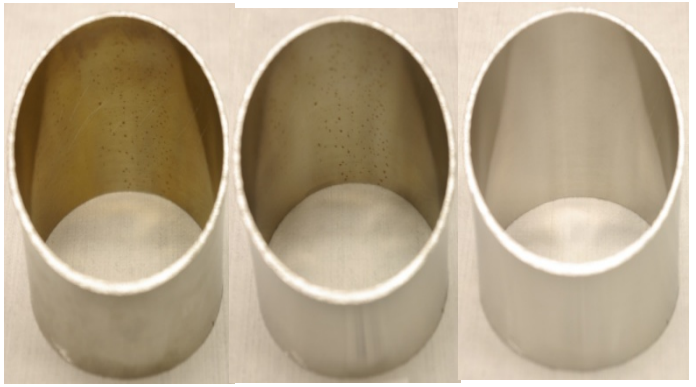
**Microbes:**

*Ralstonia pickettii,*

*Pseudomonas fluorescens,*

*Bacille non revivifiable,*

*Burkholderia cepacia*



Initial

5% v/v Alkaline detergent at 80 C for 1 hr

20% v/v Acid detergent at 80 C for 2 hr

- Cleaning evaluation (laboratory testing)
- Review equipment design and critical parameter limitations
- Alkaline detergent pre-cleaning (8% v/v, 40°C for 3 hours)
- Acid detergent rouge removal (20% v/v, 70°C for 4 hours)
- Sporicide (5% v/v, 20°C )

# In summary:

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## I. Biofilm remediation will always use a combine strategy:

1. **Use of alkaline cleaning chemistry to penetrate/denature EPS**
   - ✓ 5% alkaline formulated cleaner at 60°C for 3 hours
2. **Derouging & passivation, if required**
   - ✓ Acid cleaning and passivation using 15% formulated acidic cleaner at 80 °C for 5 hours
3. **Use of the sporicidal chemistry to sanitize the system**
   - ✓ Sanitization using sporicidal agent at 25 °C for 30 minutes, or
   - ✓ Steam in place

## II. Prevention of biofilm generation is a cycle process

- ✓ Engineering Design
- ✓ Optimal cleaning/sanitization frequency and procedure
- ✓ Optimal disinfection/sterilization frequency and procedure
- ✓ Correct chemistry and disinfectant choice
- ✓ Routine trend analysis is also important

# EPA Biofilm Label Claims

- The United States Environmental Protection Agency (EPA) has two test methods for evaluating the efficacy of antimicrobial germicides against two biofilm bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus*,
  - EPA MLB SOP MB-19: Growing a Biofilm using the CDC Biofilm Reactor
  - EPA MLB SOP MB-20: Single Tube Method for Determining the Efficacy of Disinfectants against Bacterial Biofilm

# EPA Biofilm Label Claims

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- These test methods and guidance provide a framework for germicidal suppliers who wanted to have a biofilm removal claim on hard, non-porous surfaces using a broad-spectrum disinfectant.
- The use of a broad-spectrum disinfectant with a biofilm removal claim is recommended in biofilm remediation strategy

# Biofilm Generation and Remediation

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Questions ?