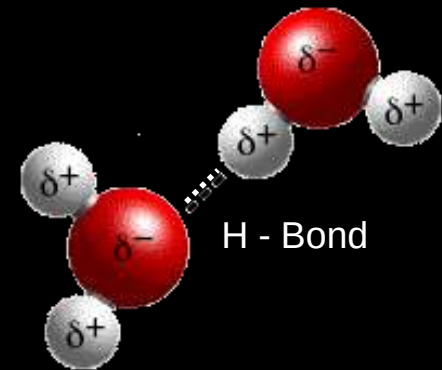
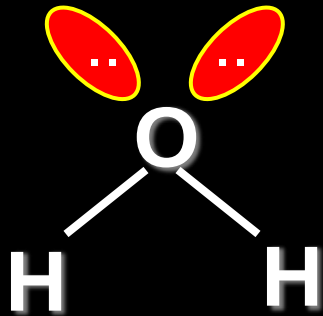
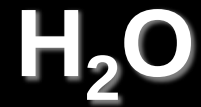
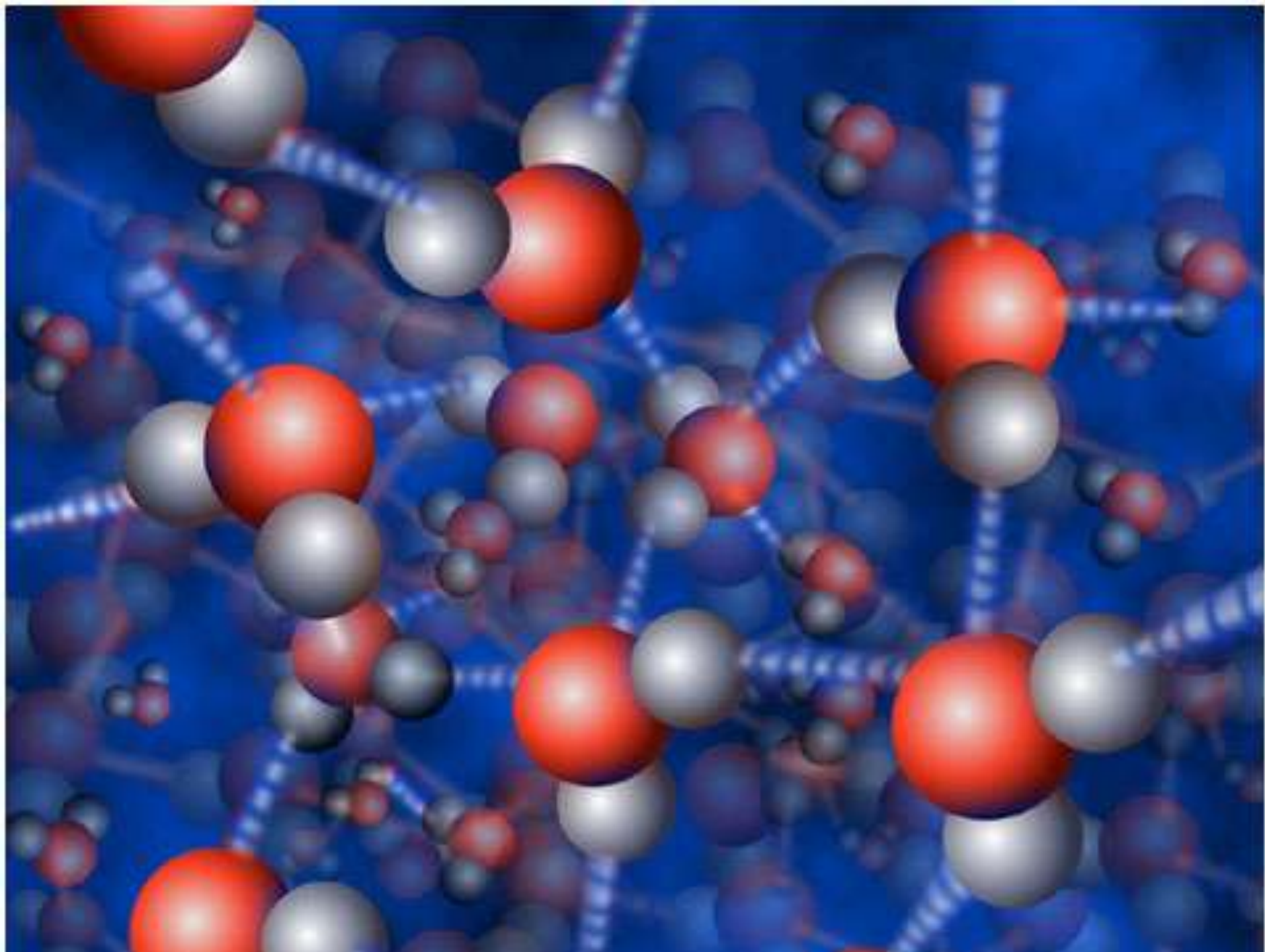


Antimicrobial Intervention for Airline Water Systems

Neeraj Khanna, Ph.D.
Bio-Cide International, Inc.

Water: Formula and Structure





Water: pH



Concentration	pH	Value
$[\text{H}^+] = [\text{OH}^-]$	Neutral	=7.0
$[\text{H}^+] > [\text{OH}^-]$	Acidic	<7.0
$[\text{H}^+] < [\text{OH}^-]$	Basic	>7.0

Water: Alkalinity

- Alkalinity is the measure of the ability of water to neutralize acids
 - Agents that contribute to alkalinity are: hydroxides, carbonates, silicates, borates, nitrates, etc.
 - High water alkalinity can impact the disinfection process
 - Alkalinity can be measured using a simple drop titration kit
-

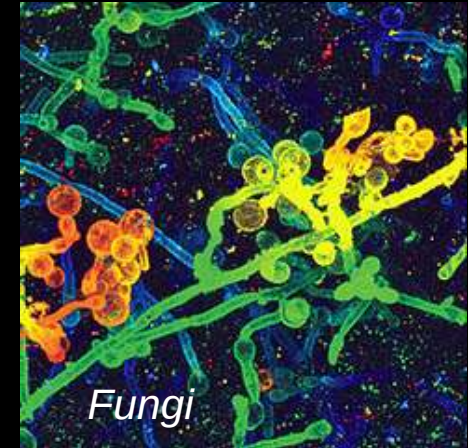
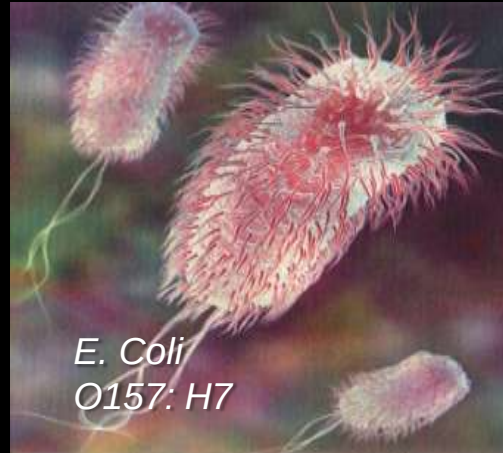
Water: Hardness

- Is a measure of minerals in water primarily calcium ion $[\text{Ca}^{2+}]$ and magnesium ion $[\text{Mg}^{2+}]$.
 - Water hardness causes scaling which can clog pipes, ruin water heaters, coats inside of coffee pots, and decrease life of toilet flushing units. Also, scaling causes challenges for disinfection of water.
 - Water hardness also provides minerals (nutrients) for microbial growth.
 - Water hardness can be measured on-site using simple drop titration kits.
-

Common Organisms of Concern In Drinking Water

- Bacteria: Coliform, *E.coli*, Fecal coliform, Legionella, etc.
 - Protozoa: *Cryptosporidium* (*C. parvum*), Giardia (*G. lamblia*)
 - Protozoa causes gastro intestinal illness with diarrhea. It is often an opportunistic pathogen in immunocompromised hosts and children.
 - The infective stage is oocyst which is highly resistant to traditional chemical disinfection (such as chlorine)
 - Viruses:
 - Cause high incidents of disease (Hep A, H1N1)
 - Difficult to monitor
 - Easy to kill
-

Waterborne Organisms

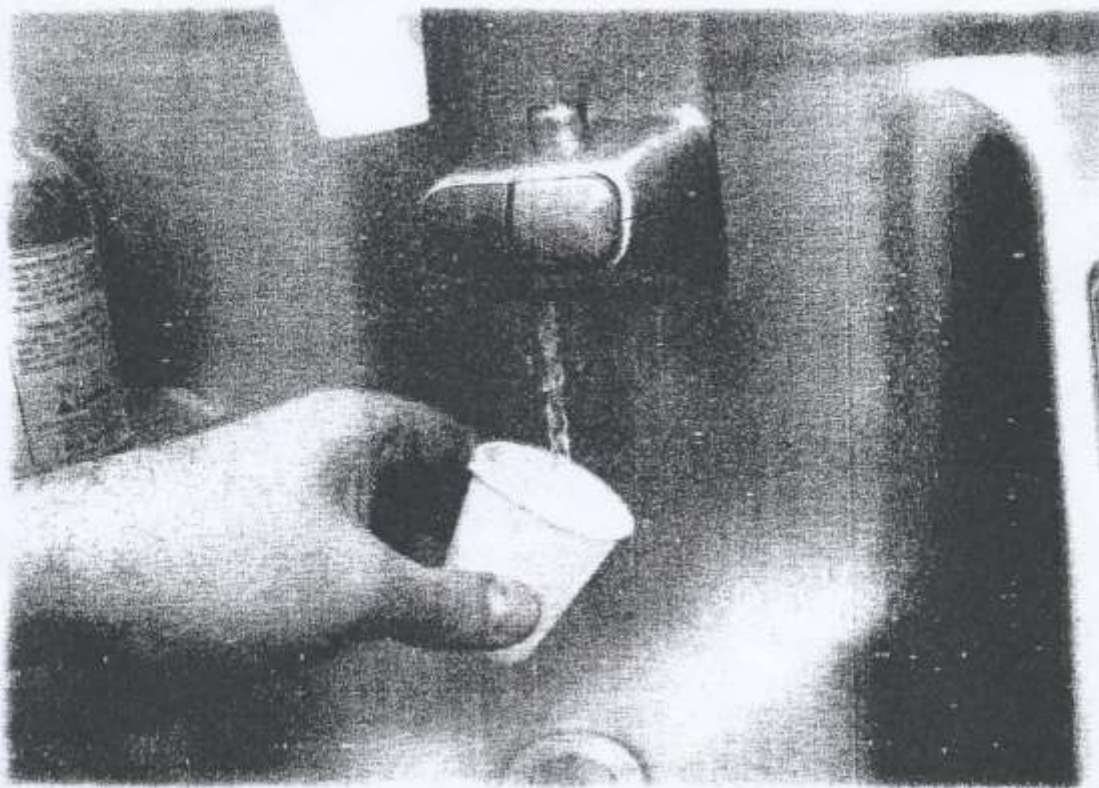


Microbiological Testing Standard Proposed by EPA for Drinking Water

- Total coliform
 - Not a health threat
 - An Indicator of potential gastroenteric pathogens in the water
 - If total coliform is positive, need to test for fecal coliform or *E.coli*
 - HPC (heterotrophic plate count)
 - Not a health threat
 - To evaluate the general population and variety of microorganisms in water
 - >500 CFU/ml suggests a high level of contamination and requires a corrective action
-

THE WALL STREET JOURNAL.

How Safe Is Airline Water?



Airlines say their galley and lavatory water is drinkable. But we did our own test in the skies—and found a host of bacteria and even insect eggs. Nancy Keates and Jane Costello report.

WHEN JUANITA GREEN needed to take some medication on a flight to Los Angeles, she washed down the pill with a cup of water from the plane's bathroom sink. It tasted funny, but she

results of our water-quality snapshot: a long list of microscopic life you don't want to drink, from *Salmonella* and *Staphylococcus* to tiny insect eggs. Worse, contamination was the rule, not the exception: Almost all of the bacteria levels were tens, sometimes hundreds, of times

Routine Sampling Requirement (ADWR)

- Frequency is specified based on routine disinfection and flushing schedule.
- Two, 100mL water samples taken from lavatory and galley, at least 72 hours after disinfection and flushing

Disinfection and Flushing Frequency

Quarterly

3 times per year

Semi-Annually

Annually

Coliform Sampling

Annually

Semi-Annually

Quarterly

Monthly

Repeat and Follow-up Sampling (ADWR)

- Repeat sampling:
 - If routine samples show positive for total coliform, but negative of fecal coliform/*E.coli*.
 - Three, 100mL water samples taken from lavatory, galley, and tap shown positive total coliform.
 - Must collect within 24 hr. of test results notification, or disinfect
 - Follow-up sampling:
 - Is required after corrective disinfection
 - Two, 100mL taken samples from routine sampling locations.
-

Sources of contamination on aircraft

- Water is already contaminated from the supplying resource
 - Cross contamination during handling and transportation (truck and hoses)
 - Breaking/leaking of water line
 - Biofilm build-up
 - Backflow prevention device failure
 - Improperly maintained portable water carts
 - Cross contamination of surfaces due to hand contact, splash-back, cleaning rags, aerosols
-

Sample Collection - Basics

1. Wash hands, wear loose sterile latex gloves
 2. Remove screen
 3. Flush for 2 minutes
 4. Clean faucet tip with alcohol swab
 5. Open sample bottle, secure cap
 6. Reduce flow to match diameter of a pencil
 7. Flush 1 minute, fill bottle , place cap
 8. Label bottle and place in a ziplock
 9. Ship it in cooler with ice bags.
-

Available Testing Methods for Coliform and *E.Coli*

- MPN method: Colilert and Quanti-Tray (www.idexx.com). *E.coli* colonies appear fluorescent.
- Petri-film (3M):
 - Isolation and enumeration
 - Simplicity of use and storage
 - *E.coli* colonies appear as blue color with gas bubble
 - Reliable result
- Culturing on MacConkey agar
- PCR with *E.coli* specific primers



Does your system suffer from any of these problems?

- Microbial count despite proper sanitation
- Plugged water injection points
- Clogged filters
- Water fouling and bad taste
- Unexplained corrosion

If so, you may be having a biofilm problem

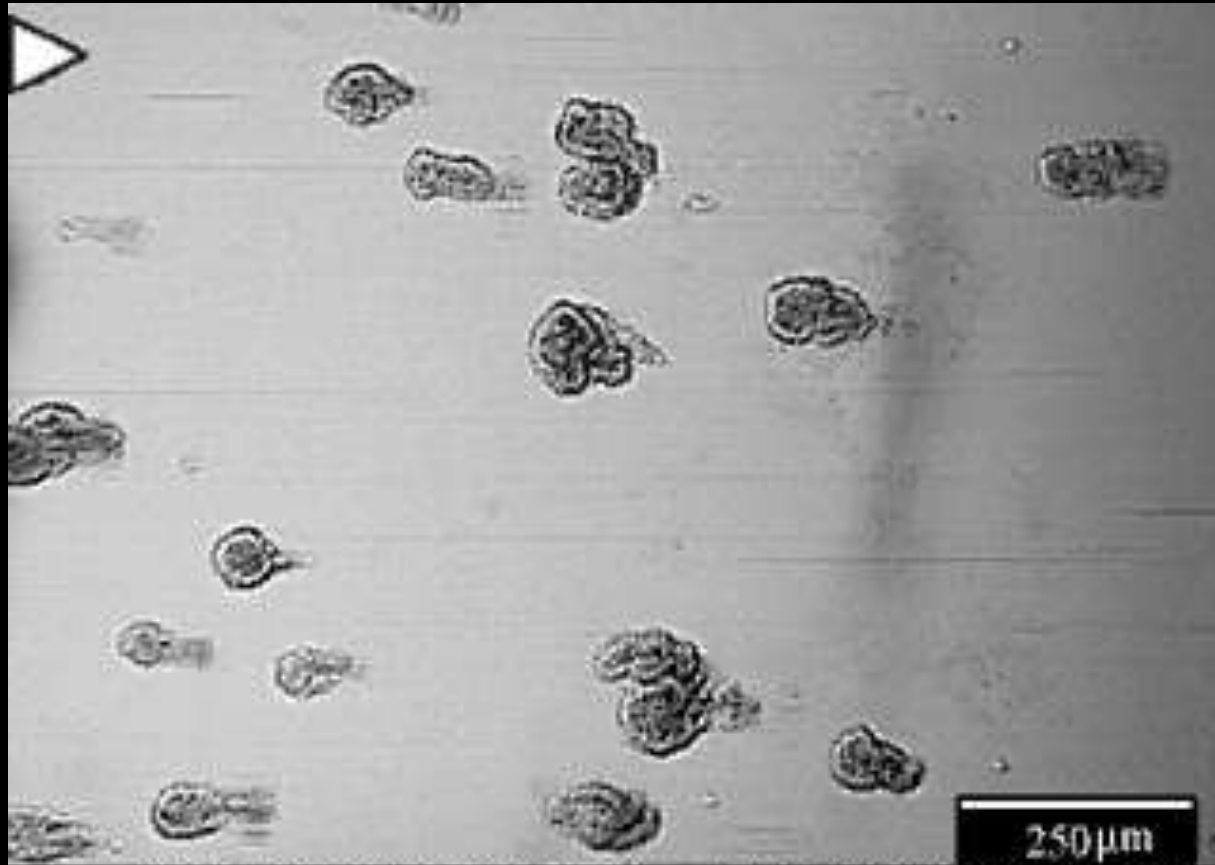
Bio-Film

- the hidden culprit

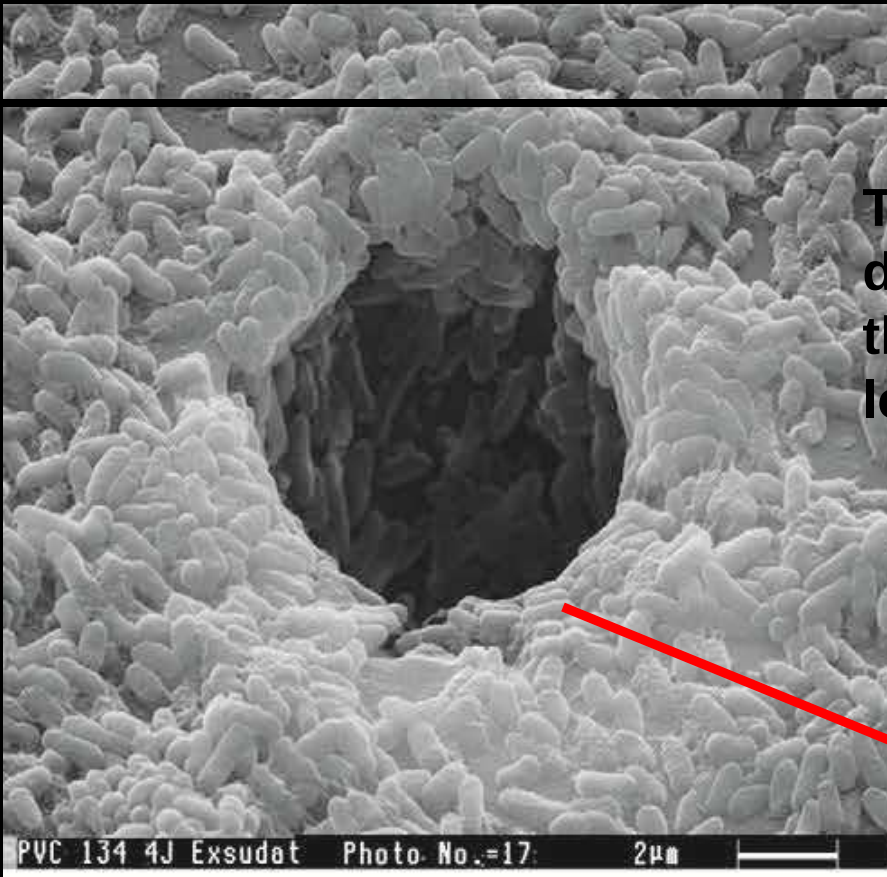
Bacterial Biofilm on Stainless Steel



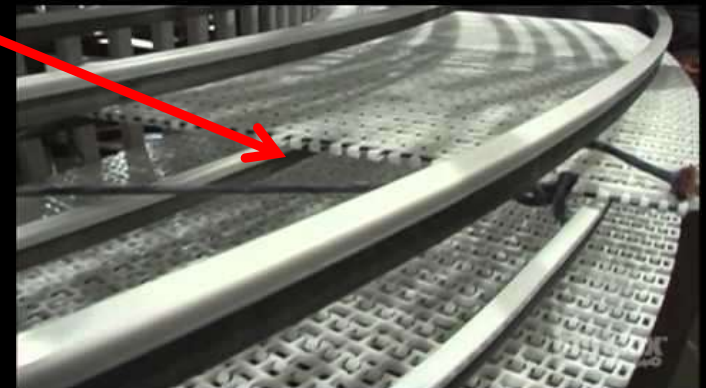
Bacterial Biofilm on Stainless Steel



Stoodley, P. & Lewandowski, Z.; ASM Biofilms Collection



Colonization by *Pseudomonas fluorescens* CCL 134 of a 6-micron hole in a PVC conveyor belt

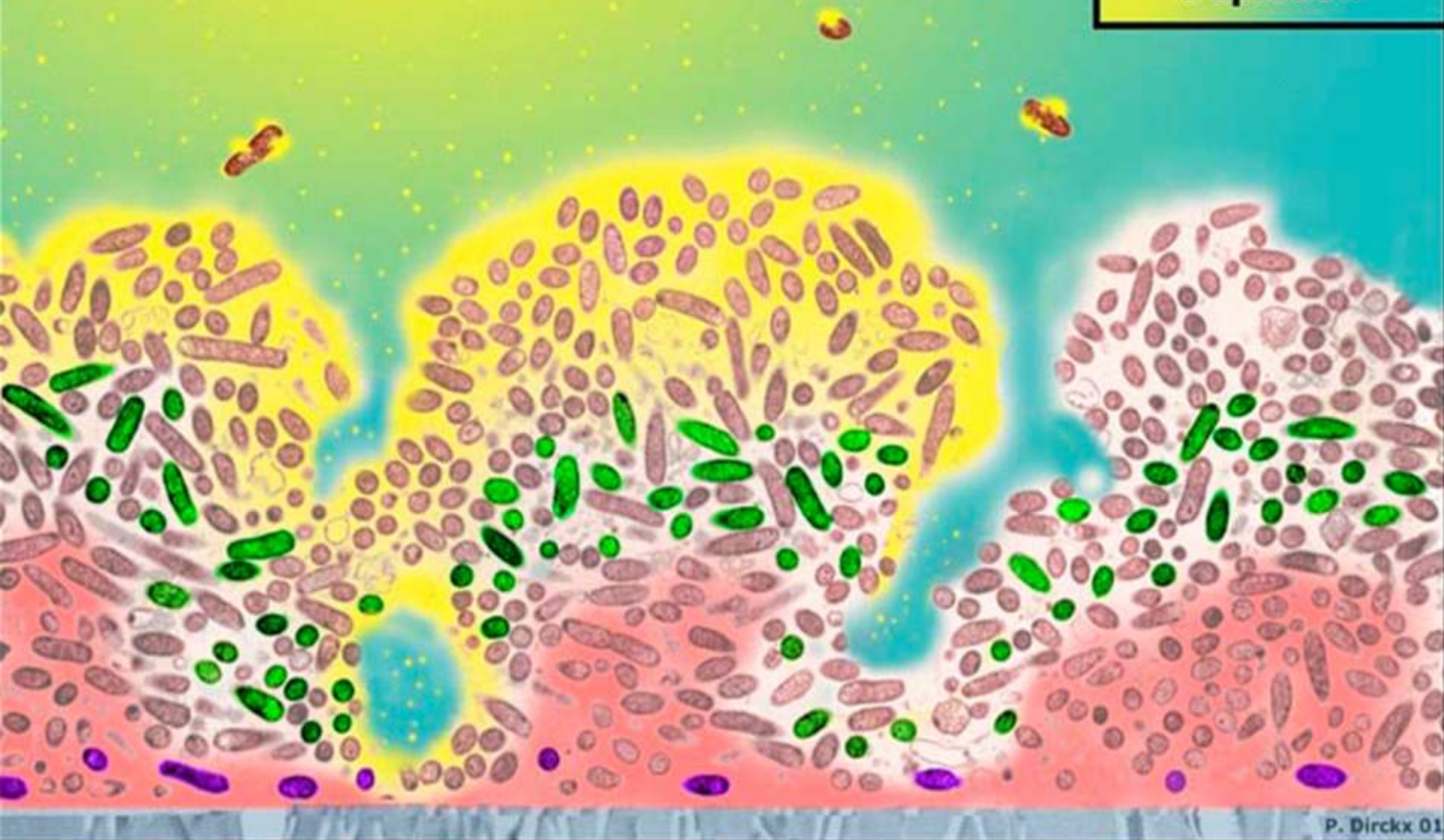


Components of Biofilm

- Extracellular polymers (polysaccharides and water). These components give the slimy texture of biofilm.
 - Microorganisms (homogenous/heterogenous population)
-

Mechanisms of Biofilm Tolerance

Antimicrobial
Depletion



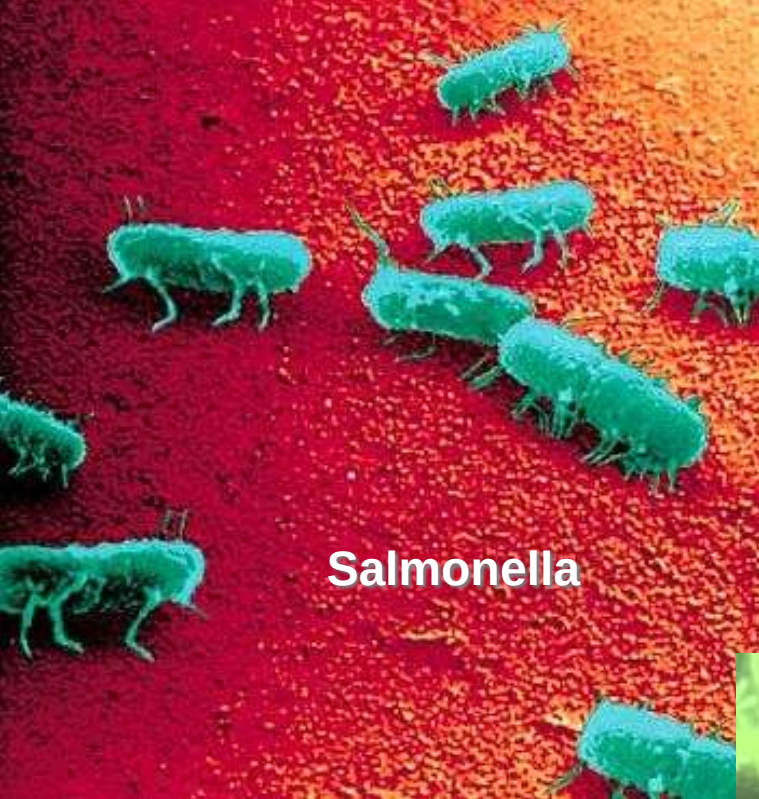
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**Slow
Penetration**

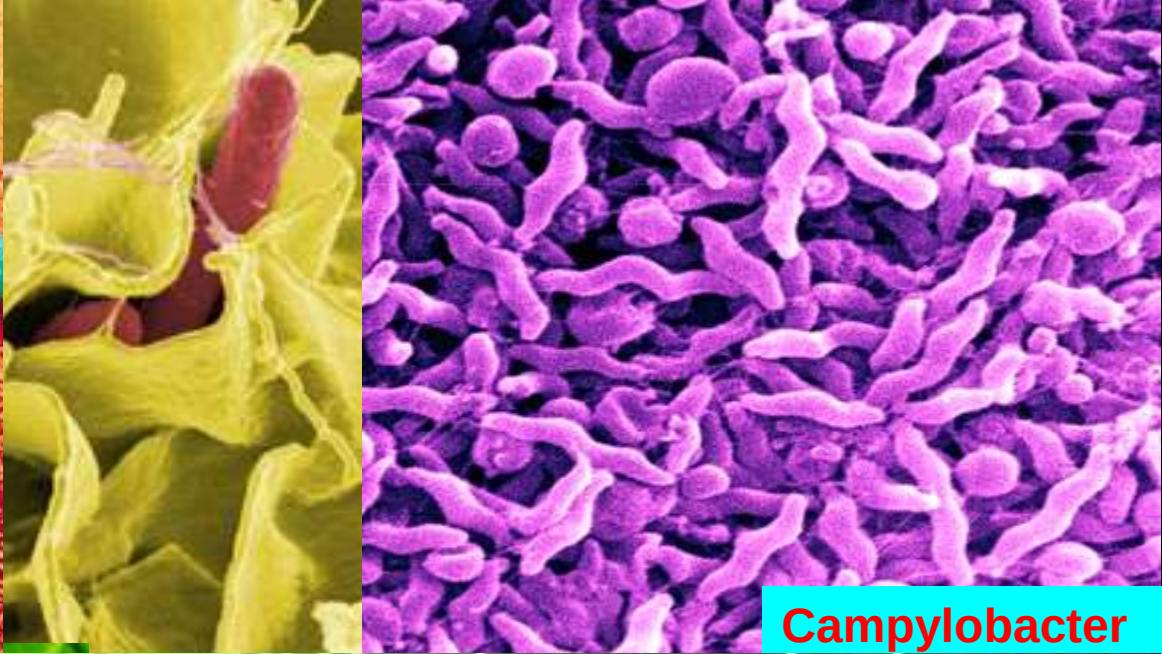
**Stress
Response**

**Altered
Microenvironment**

Persisters



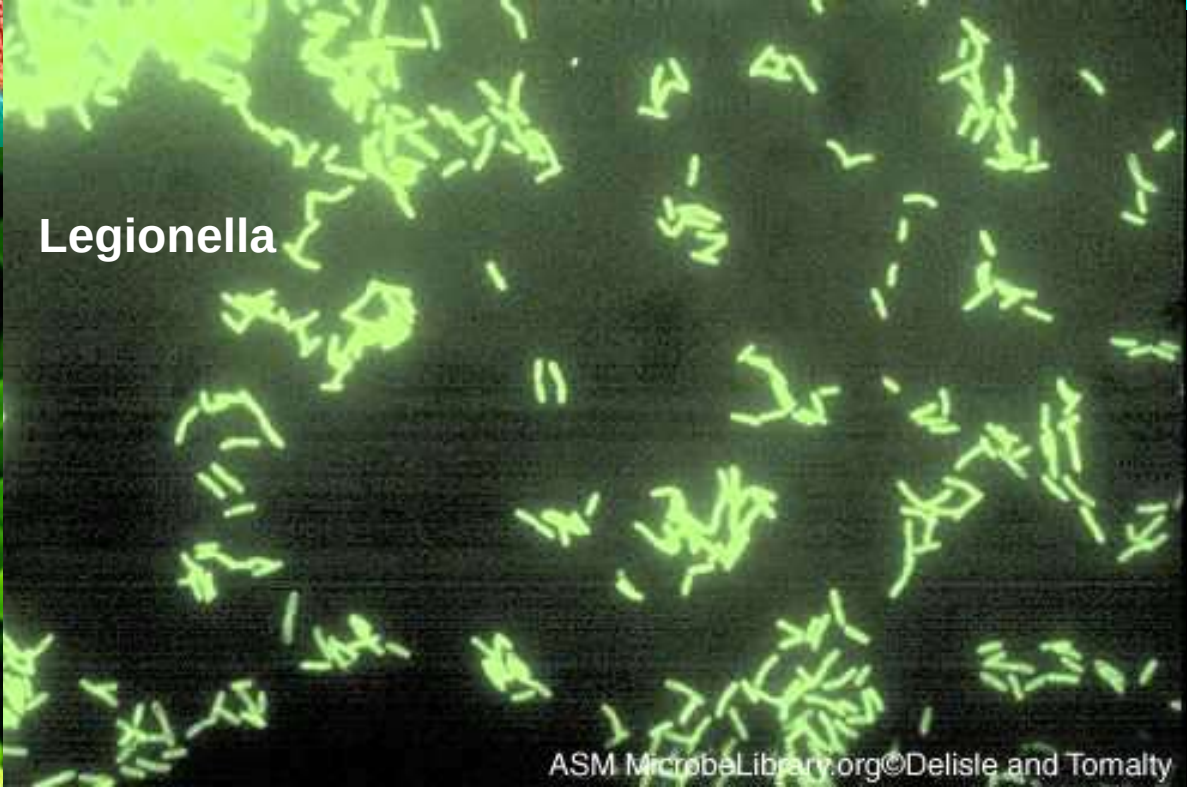
Salmonella



Campylobacter

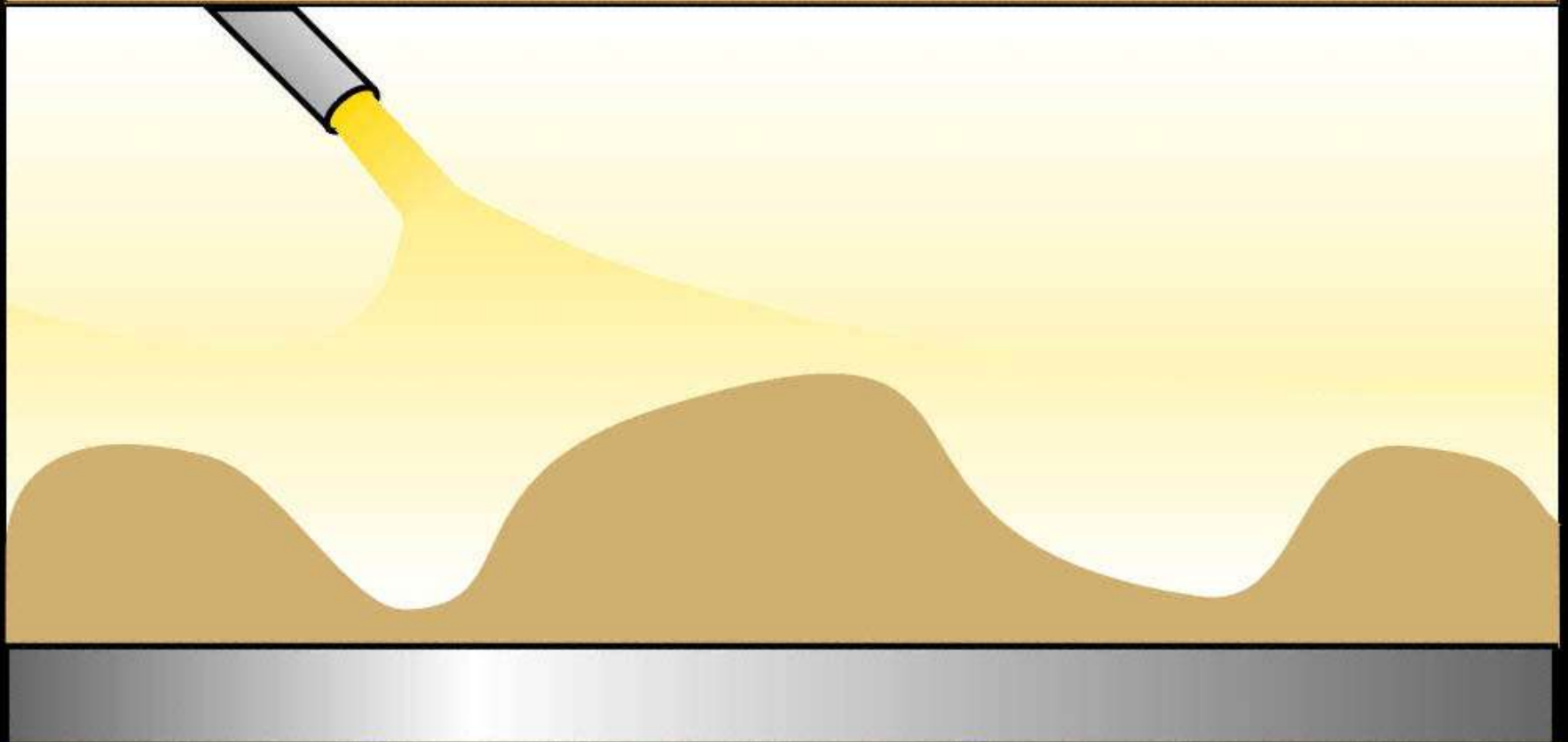


E.Coli O157: H7



Legionella

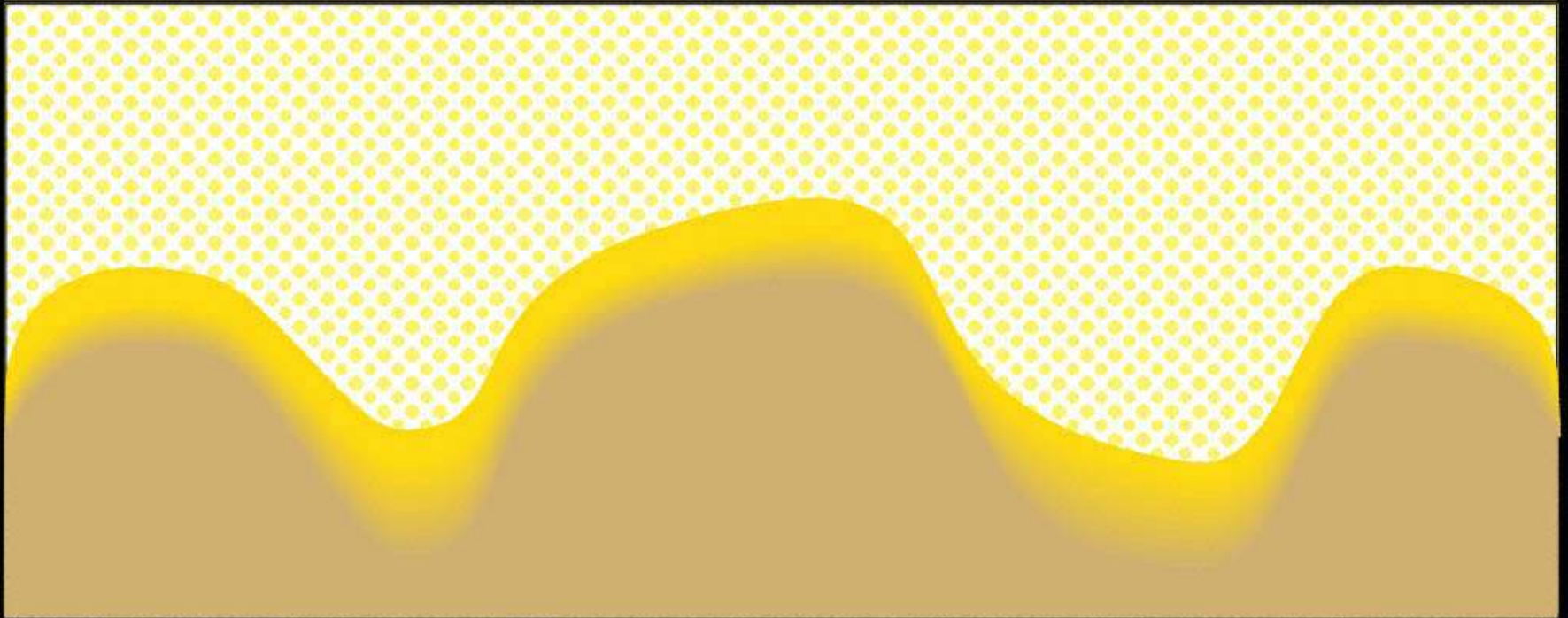
Biofilm Resistance to Antimicrobial Agents



Antimicrobial Neutralization

188197cs

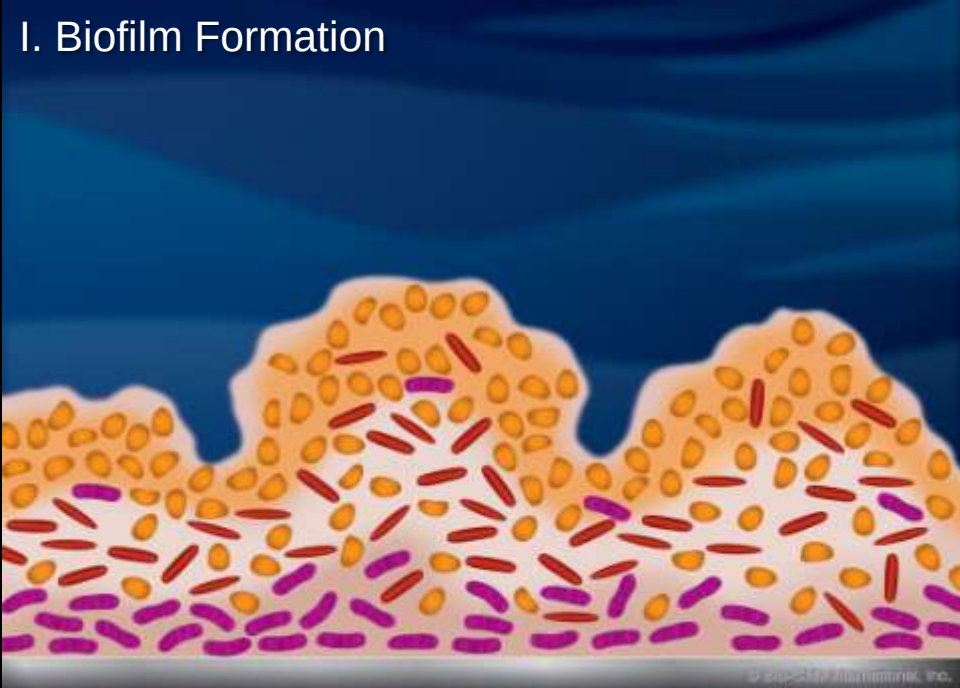
Biofilm Resistance to Antimicrobial Agents



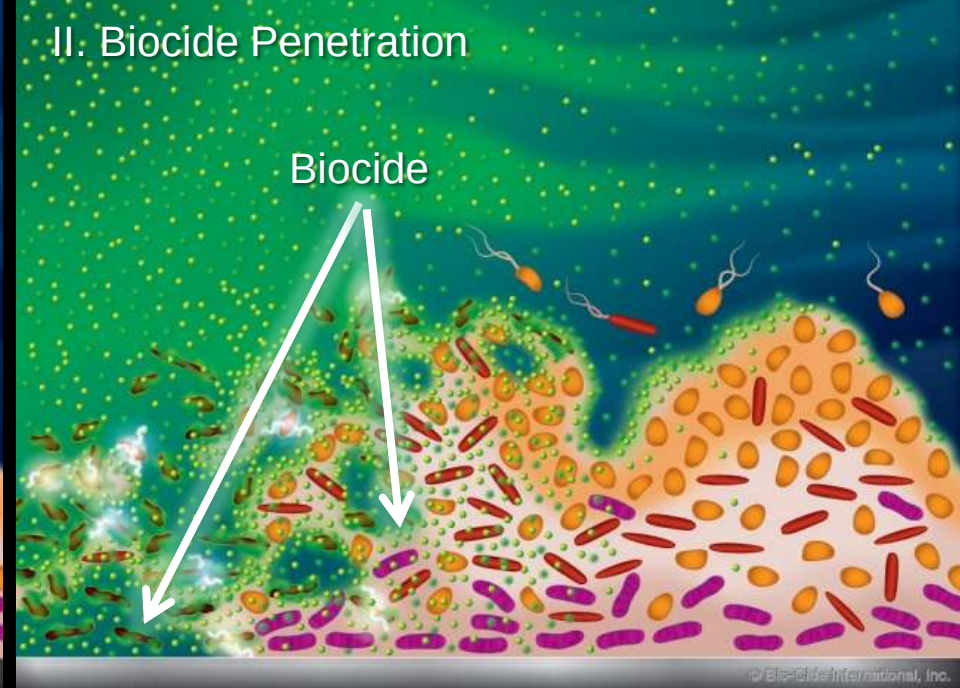
Penetration failure

188297cs

I. Biofilm Formation



II. Biocide Penetration



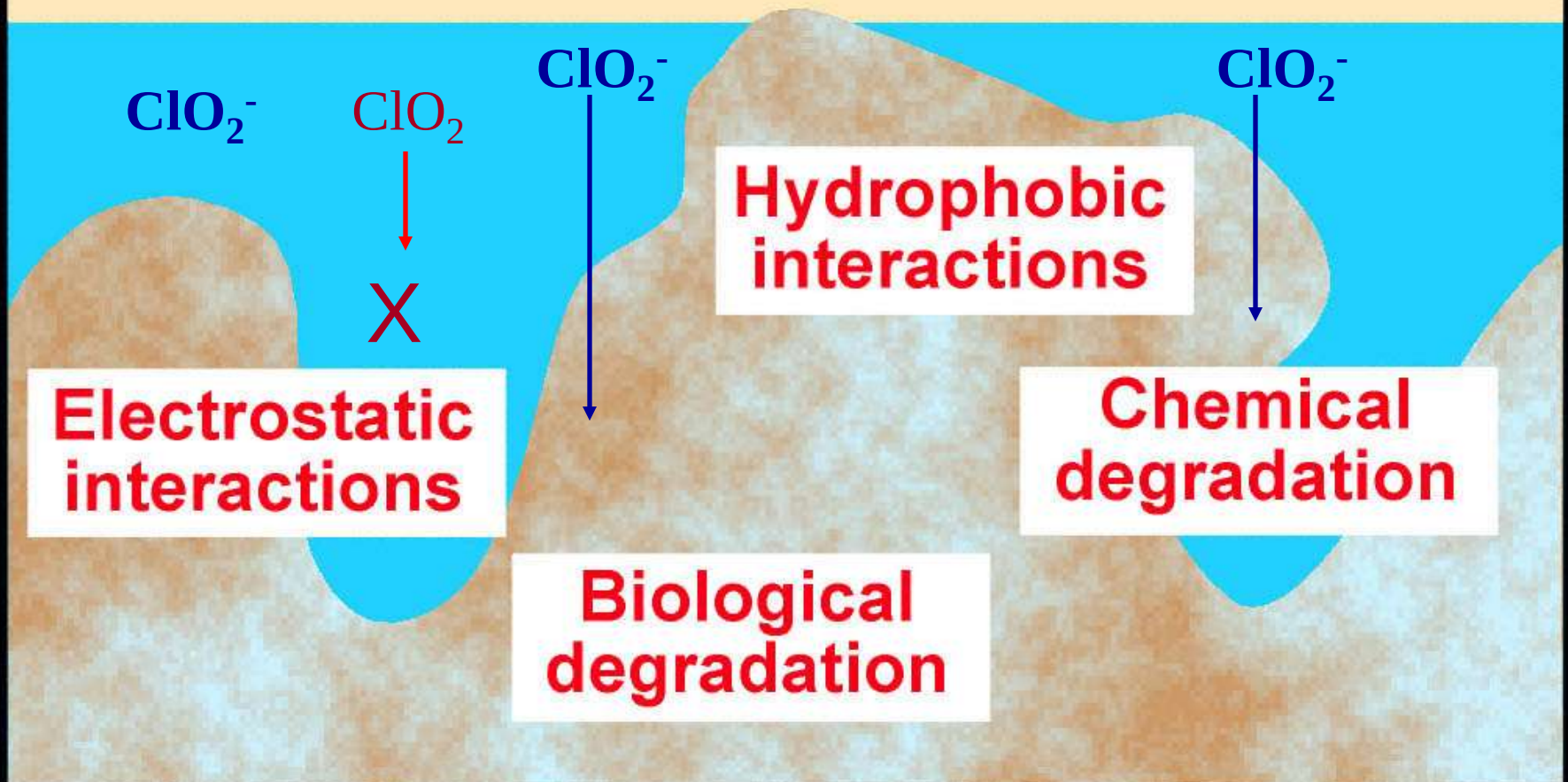
III. Biofilm Removal



IV. Clean System



Biofilm: Cohesive forces/detachment modes



Biofilm: Cohesive forces/detachment modes



196497cs

Biofilm Detection Method

- Swabbing and using ATP bioluminescence method. ATP value fluctuates day to day indicates the presence of biofilm.
 - Re-occurrence of high count with undetectable contamination source suggests the presence of biofilm in the system
 - Total plate count and total anaerobic plate count give an indication of bacteria contained in the biofilm
-

Variety of Sanitizers

- Chlorine (bleach & hypochlorites), Ozone, Chlorine dioxide, Hydrogen peroxide, Peracetic acid, Acid and acid blends, Quaternary ammonium chlorides (Quats), Surfactants, Antibiotics (triclosan), Alcohol, Organic blends, UV, IR, Radiation, Heat (steam), Filtration (RO)
- Choice is made based on: Suitability of application, Materials compatibility, Regulatory approvals, Other approvals (OEM), Cost

Typical Disinfectants

Disinfectant	Dosage	Contact time
Chlorine Dioxide	100 ppm	5 min
	50 ppm	1 Hr.
Ozone	1.5 ppm	20 min – 1.5 Hr.
Hydrogen Peroxide	400 ppm	1 Hr.
Hypochlorite	100 ppm	1Hr.
	50 ppm	4 Hr.

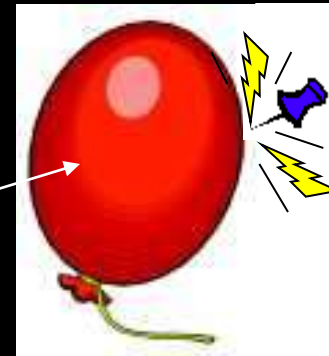
How do Sanitizers work...

Quats



Chemical “surrounds”
bacteria like a film on
a balloon.

Oxidizer

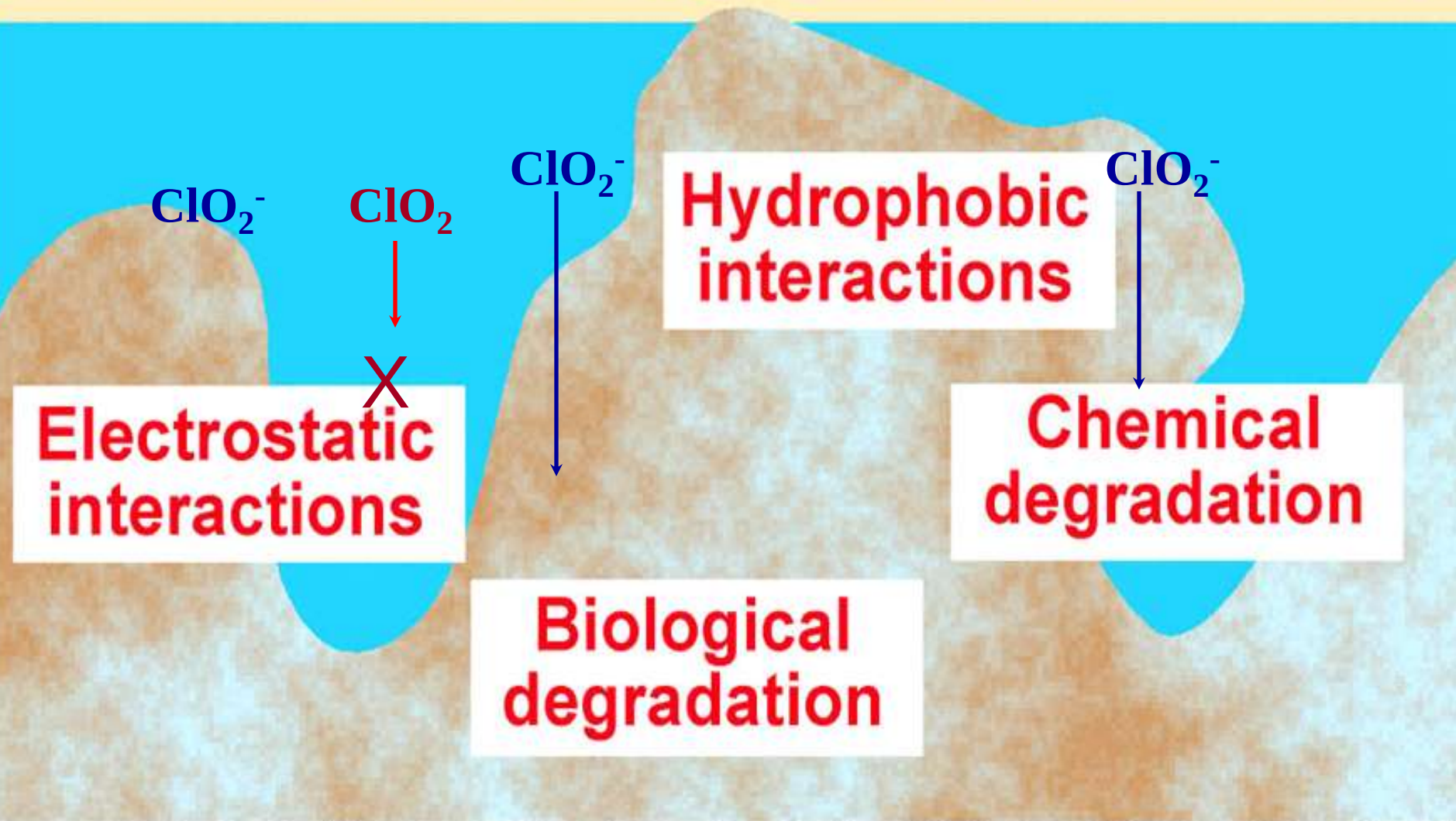


Oxidizers “pops”
bacteria like a pin
pops a balloon.

Biofilm control with ClO_2

- Oxidizers generally react on the surface of the biofilm to form an oxidized layer, like charring on wood.
 - ClO_2 penetrates bio-film by molecular diffusion.
 - Certain ClO_2 products is formulated to travel to the base of the film where it attacks microorganisms and loosens biofilm at its point of attachment.
-

Biofilm: Cohesive forces/detachment modes



Biofilm: Cohesive forces/detachment modes



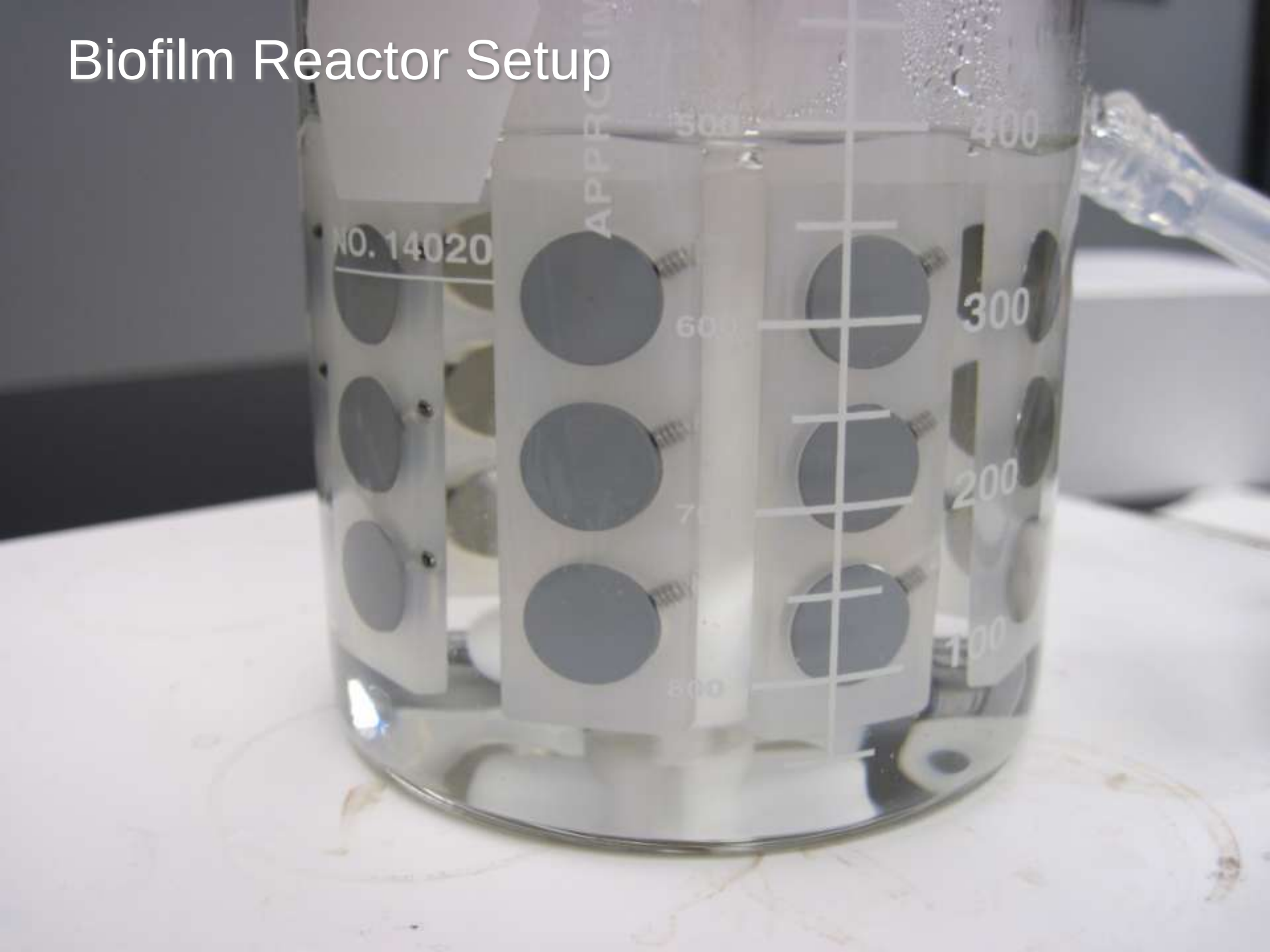
<http://watercenter.montana.edu/training/>

196497cs

Biofilm Reactor Setup



Biofilm Reactor Setup



Biofilm control with Biocides

Biocide	Concentration (ppm)	Population (log)
Control	NA	9.4
THPS	150	8.5
Glutaraldehyde Quaternary Amm.	135 67	Mixture 6.2
DBMPA	100	0.0
ClO ₂	25	0.0

THPS: Tetrakis hydromethylphosphonium sulfate

DBMPA: 2,2 Dibromo-3-nitrilopropionamide

Properties of ClO₂ based biocide

- A. Dual component
 - B. To activate – mix with powered citric wait (5 minutes for Purogene) and dilute
 - C. Light yellow solution
 - D. Must Be Generated at Use Site in a ventilated area
 - E. Active ingredient – aqueous gas
-

Sanitizer Properties to consider

ClO₂ with Chlorine

Property	Chlorine Dioxide	Chlorine (HOCl)
• Primary Chemical Reaction	Oxidation Only, No Chlorinated By-products.	Electrophilic substitution resulting in Cl-byproducts
• Corrosion potential	Low ($E_0 = 0.95V$)	High ($E_0 = 1.49V$)
• Taste & Odor	Negligible	Produces “chemical” taste
• Effective pH Range	1-10	6.8-7.6
• Reaction in Water	Does not hydrolyze	Hydrolyzes in water
• Reaction to Organic Load	Resists neutralization	Readily neutralized
• Reaction to Biofilms	Highly reactive	Largely ineffective
• Oxidative capacity	2.63	1

Other Features of Sanitizer Technology

- Broad spectrum antimicrobial efficacy
- Non-specific, oxidative mechanism
- Byproduct (table salt for some products)
- No adverse taste issues in water from residue
- Disposal requirements
- Corrosion potential
- EPA, FDA and OEM approved
- Cost
- Biofilm Removing Properties

Antimicrobial Effectiveness of Sanitizers

Concentration (ppm) of biocide required for > 5 log reduction in 60 seconds

Sanitizer	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. cerevisiae</i>	<i>E. Coli</i> (O157: H7)
Available Chlorine Dioxide	6	30	30	3
Sodium Hypochlorite	200	200	400	600
Peracetic Acid	30	60	300	20
Dodecylbenzenesulfonic Acid & Phosphoric Acid	40	80	600	90
Octanoic Acid, Decanoic Acid, Citric Acid & Phosphoric Acid	80	150	200	60

Source: Tanner, *et al.*; Dept. Of Microbiology, University of Oklahoma, Norman, OK & *J. Indst. Micro.* **1989**, 4, 145-154.

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Measuring Biocide Concentration

- **TITRATION**
- Iodometric Titration For Purogene (AWWA Method 4500)
- 1 to 500 PPM



- **DIPSTICKS**
- Indicator based Oxystix
- 0.5 to 100 PPM
- Dip time and wait time is critical



Equipment Configurations and Maintenance

- Wall Mounted
 - Stainless Steel Enclosure
 - Fiberglass Enclosure
- Portable
 - Optional Exterior/Interior Hose Controls and Hose Reel
- Full Cabinet
 - All Stainless Steel Construction
 - With Hose Reel & 200' Hose
- Retrofit Cabinets
 - Mounts to existing Water Cabinet



Equipment Suitability and Maintenance Requirements



Wall-Mounted System



Portable System

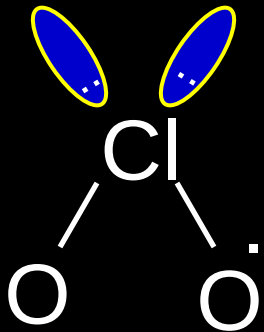


Full Water Cabinet PASS System



Questions?

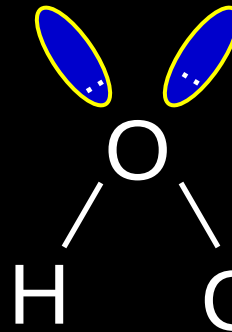
Structural Properties



Chlorine dioxide



Chlorine



Hypochlorous acid

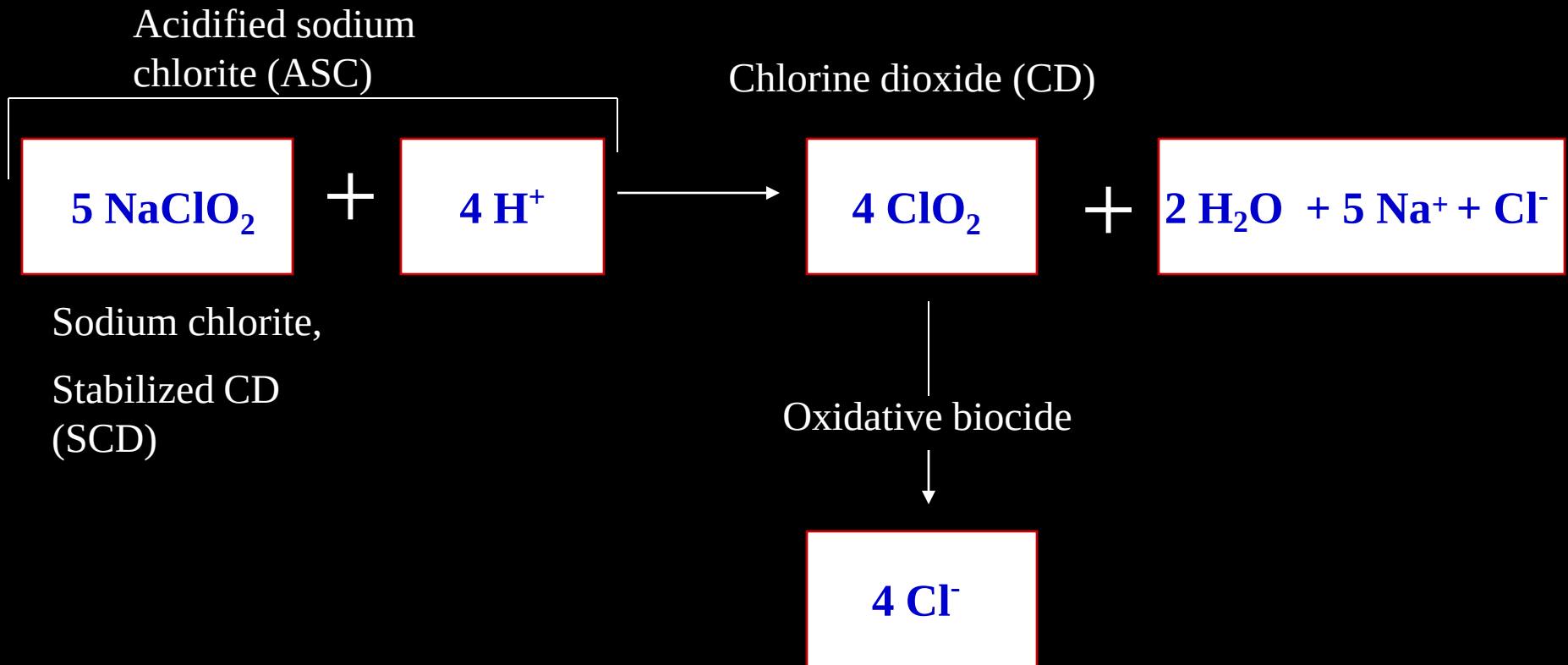


(Sodium hypochlorite)

Byproducts: Reactions of ClO_2 Vs. Chlorine

1. ClO_2 reacts primarily by oxidation reactions which produce few, if any, volatile or nonvolatile chlorinated organics.
 2. Breaks down into table salt
 3. Chlorine reacts by oxidation and electrophilic substitution which produces both volatile and non-volatile chlorinated organics. (e.g., THMs, dioxins, chlorinated phenols, trichloroacetic acid, chlorinated terpenes)
-

Generation of Chlorine Dioxide



- Ultimate breakdown product is sodium chloride

Antimicrobial Effectiveness of Sanitizers

Concentration (ppm) of biocide required for > log 5 reduction in 60 seconds

Sanitizer	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. cerevisiae</i>
Available Chlorine Dioxide	6	30	30
Acidified Quat	150	1,200	300
Phenolic Compound	1,500	380	190
Iodophor	440	440	450
Glutaraldehyde	1,600	2,200	18,000

Source: Tanner, *et al.*; Dept. Of Microbiology, University of Oklahoma, Norman, OK & *J. Indst. Micro.* **1989**, 4, 145-154.