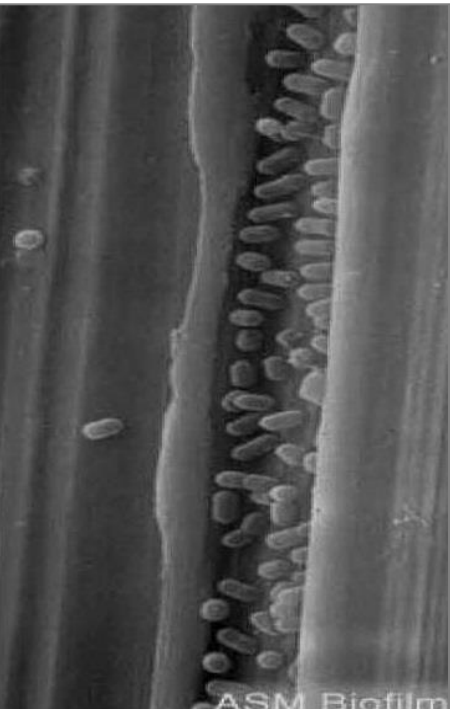
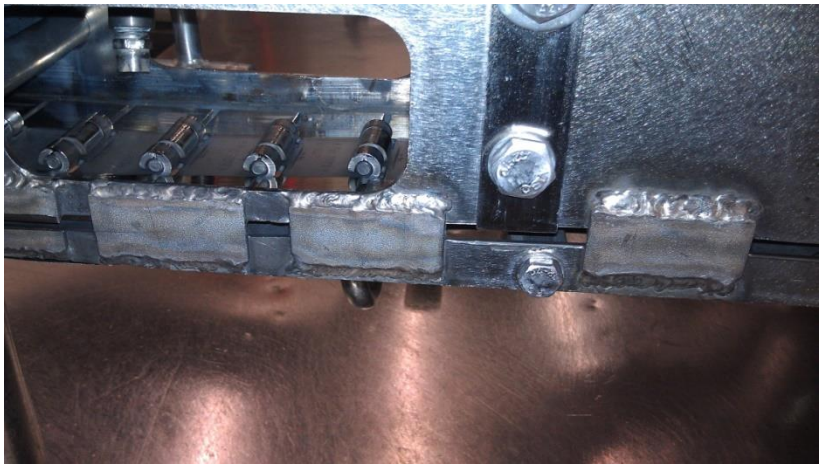
Eliminating Harborage Sites and Growth Niches with Chlorine Dioxide Gas





Kevin Lorcheim
Project Engineer

ClorDiSys

Principles of Decontamination

In order for any decontamination method to be effective, the following points must be satisfied

The decontamination method must:

- ✓ **Be able to kill the organism in question**
- ✓ **Achieve good and complete distribution**
- ✓ **Achieve thorough and total penetration**
- ✓ **Achieve sufficient contact time at the correct concentration**

Types of Antimicrobial Pesticides

Sterilizers (Sporicides): Used to destroy or eliminate all forms of microbial life including fungi, viruses, and all forms of bacteria and their spores. Spores are considered to be the most difficult form of microorganism to destroy. Therefore, EPA considers the term Sporicide to be synonymous with "Sterilizer."

Disinfectants: Used on hard inanimate surfaces and objects to destroy or irreversibly inactivate infectious fungi and bacteria but NOT necessarily their spores. Disinfectant products are divided into two major types: hospital and general use.

Sanitizers: Used to reduce, but not necessarily eliminate, microorganisms from the inanimate environment to levels considered safe as determined by public health codes or regulations.

Antiseptics and Germicides: Used to prevent infection and decay by inhibiting the growth of microorganisms. Because these products are used in or on living humans or animals, they are considered drugs and are thus approved and regulated by the Food and Drug Administration (FDA).

http://www.epa.gov/oppad001/ad_info.htm

Manual Spray/Mop/Wipe

Using a high level liquid disinfectant to manually spray and wipe all surfaces

- Decontaminating agent must be chosen based on level of decontamination required (germicide, sanitizer, disinfectant, sterilant)

- Requires keeping the surface wet or submersed per EPA approved label requirements (Bleach required 10+ hours)

Advantage:

- Easy to perform in-house

Disadvantage:

- Impossible for process to reach all surfaces including crevices and harborage sites

Fogging

Using a handheld or stationary fogging system to spray a liquid chemical onto surfaces

- Decontaminating agent must be chosen based on level of decontamination required (germicide, sanitizer, disinfectant, sterilant)
- Requires keeping the surface wet per EPA approved label requirements

Advantage:

- Easy to perform in-house

Disadvantage:

- Impossible for process to reach all surfaces especially crevices and harborage sites

Steam

Using a steam generator or in-house steam line to heat treat equipment and environments

- Steam is capable of eliminating all organisms
- Requires keeping the surfaces hot enough for the proper amount of time

Advantage:

- Easy to perform in-house
- No chemicals

Disadvantage:

- Can’t use on heat/liquid sensitive materials
- Potential for burns
- Difficult to attain and maintain high enough temperature for right amount of time within equipment
- Extremely difficult to steam treat large areas

Ozone Gas

Using a steam generator or in-house steam line to fumigate equipment and environments

- Ozone is capable of eliminating all organisms
- Requires keeping the surfaces dosed long enough to kill the organisms present

Advantage:

- True gas, distributes readily
- No residues

Disadvantage:

- Long treatment times (up to 36 hours)
- Breaks down quickly, 2.5 – 7 minute half life
- Makes it difficult to treat large areas
- Corrosive

Traditional Sanitation Methods

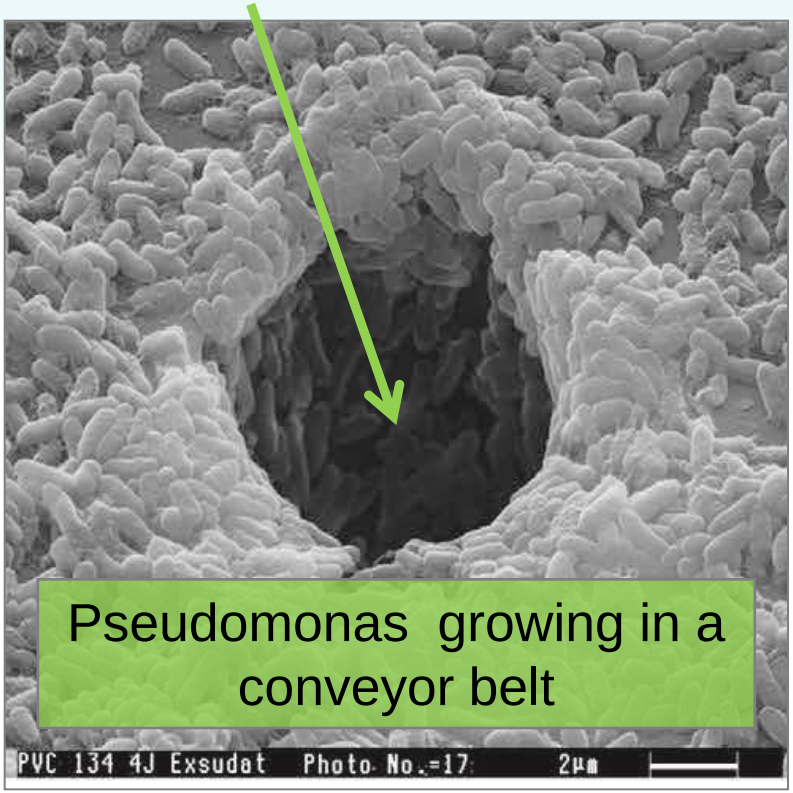
Traditional sanitation methods can have difficulty guaranteeing that all organisms have been contacted / contacted with the proper dosage

The decontamination method must:

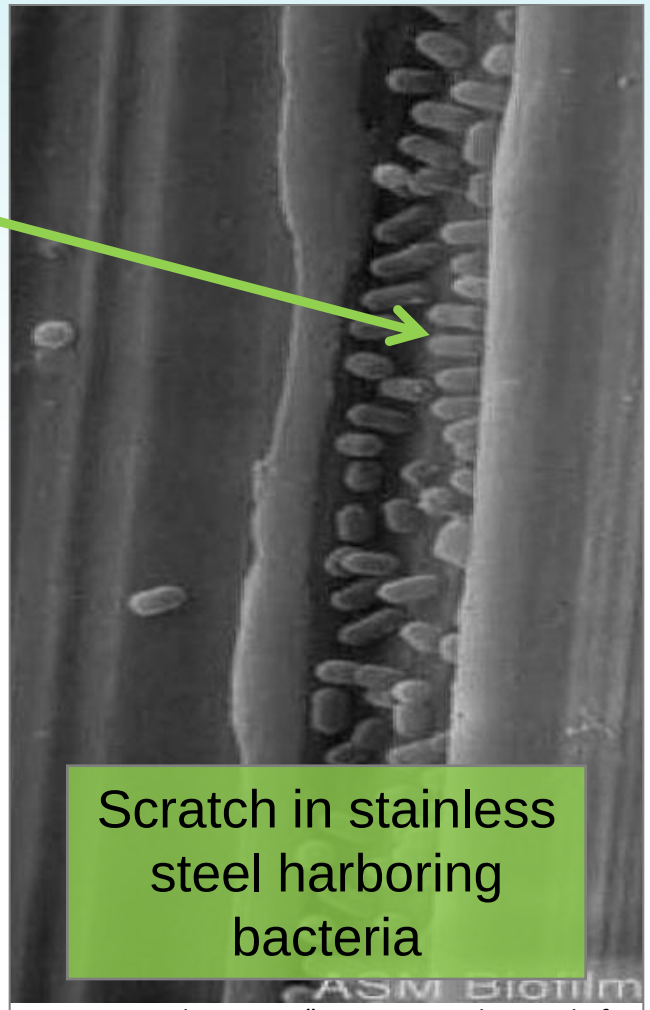
- ✓ **Be able to kill the organism in question**
- ? **Achieve good and complete distribution**
- ? **Achieve thorough and total penetration**
- ? **Achieve sufficient contact time at the correct concentration**

Problem Areas/Forming Niches

Scratches[2,3] - Crevices - Punctures[1]



Pseudomonas growing in a conveyor belt

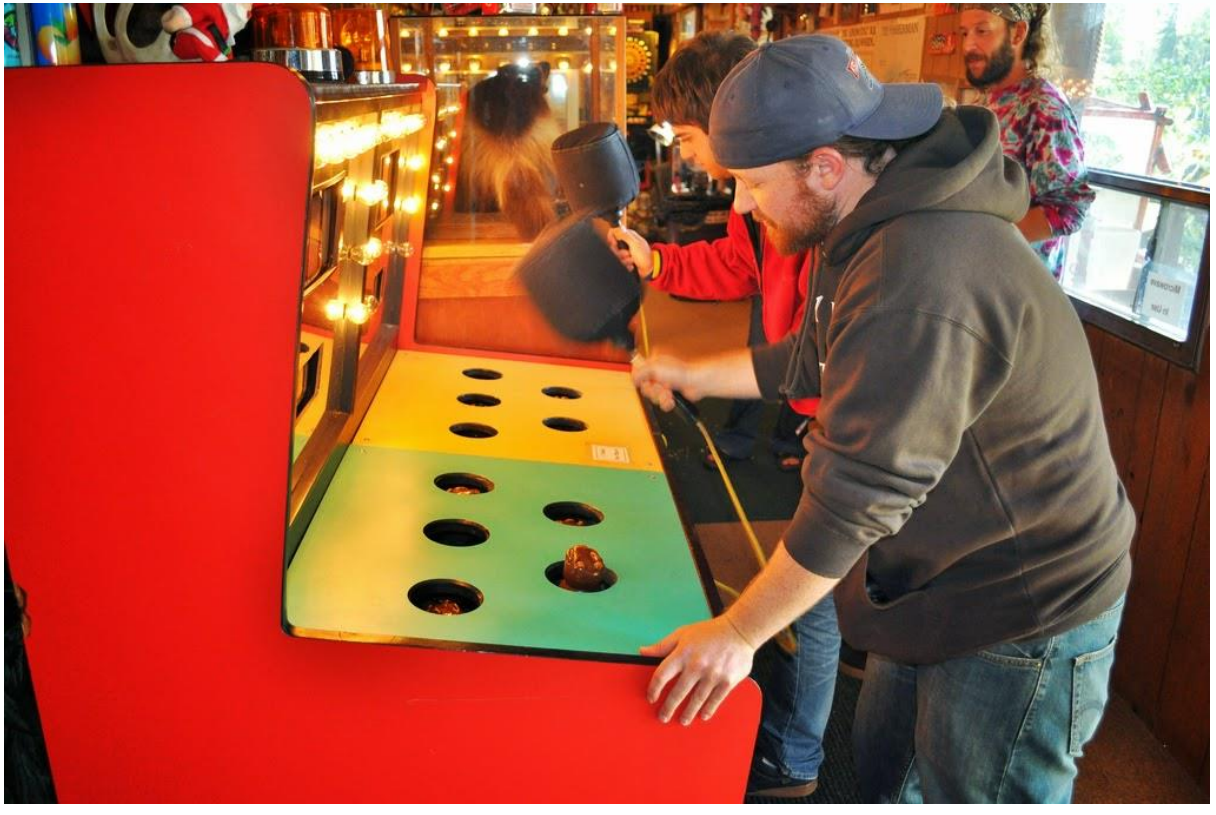


Scratch in stainless steel harboring bacteria

1. Carpentier B., Cerf O. "Review — Persistence of Listeria monocytogenes in food industry equipment and premises", International Journal of Food Microbiology 145 (2011) 1–8.
2. JENNY SCOTT, U.S. Food and Drug Administration-CFSAN, Washington, D.C., USA, "The Significance of Persistent Bacterial Strains in the Food Processing Environment", Presented at IAFP Session 21 (8-2-2011) Milwaukee.
3. Solioz, M, Biochimica - Copper kills bacteria: end of hospital-acquired infections? Scienza in rete, 18 April, 2011, Accessed on 6-26-2013 http://www.scienzainrete.it/en/content/article/copper-kills-bacteria-end-hospital-acquired-infections

Traditional Sanitation

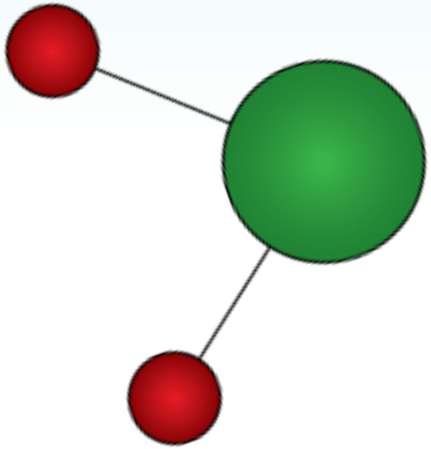
With the difficulty that traditional sanitation can have in reaching ALL of the organisms including the niches and harbor locations, you're left playing microbial whack-a-mole with persistent contaminations that keep popping back up.



Chlorine Dioxide Gas

Chemical Properties:

- Yellow-Green Gas [1]
- Water Soluble [2]
- Boiling Point 11^{o}C [3]

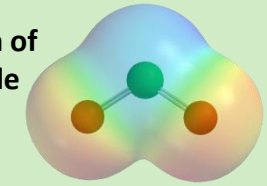


History of Chlorine Dioxide

A Brief Summary:

1912
Aqueous Germicide (Drinking Water Treatment Longest User)

1811
First Preparation of Chlorine Dioxide



1984
Chlorine Dioxide Recognized as a Gaseous Chemosterilizing Agent

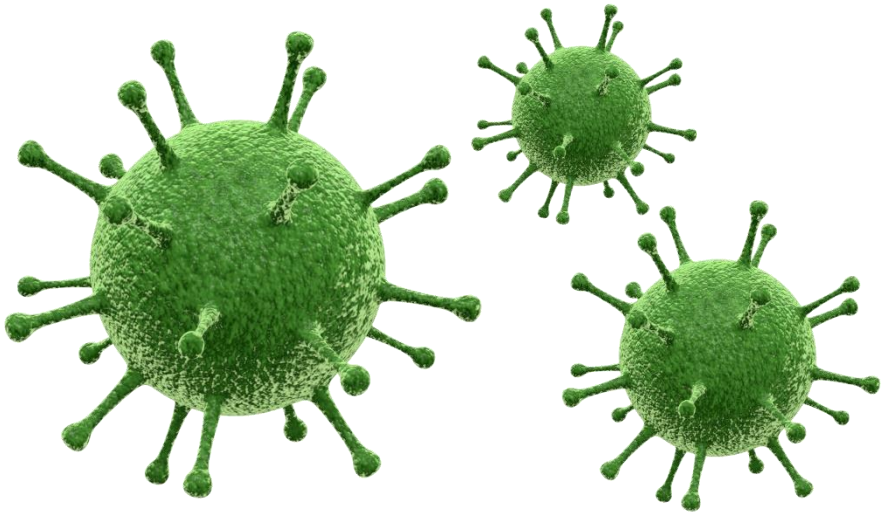
1940
Bleaching Agent (Pulp & Paper Industry Largest User)

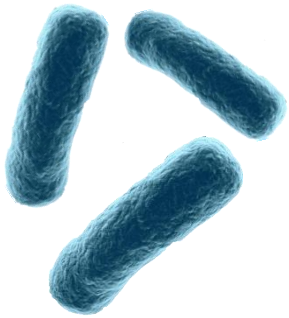
1988
First Registered with the US-EPA for use as a sterilant

Biocidal Effectiveness

	Chlorine Dioxide Gas
Registration	Sterilant

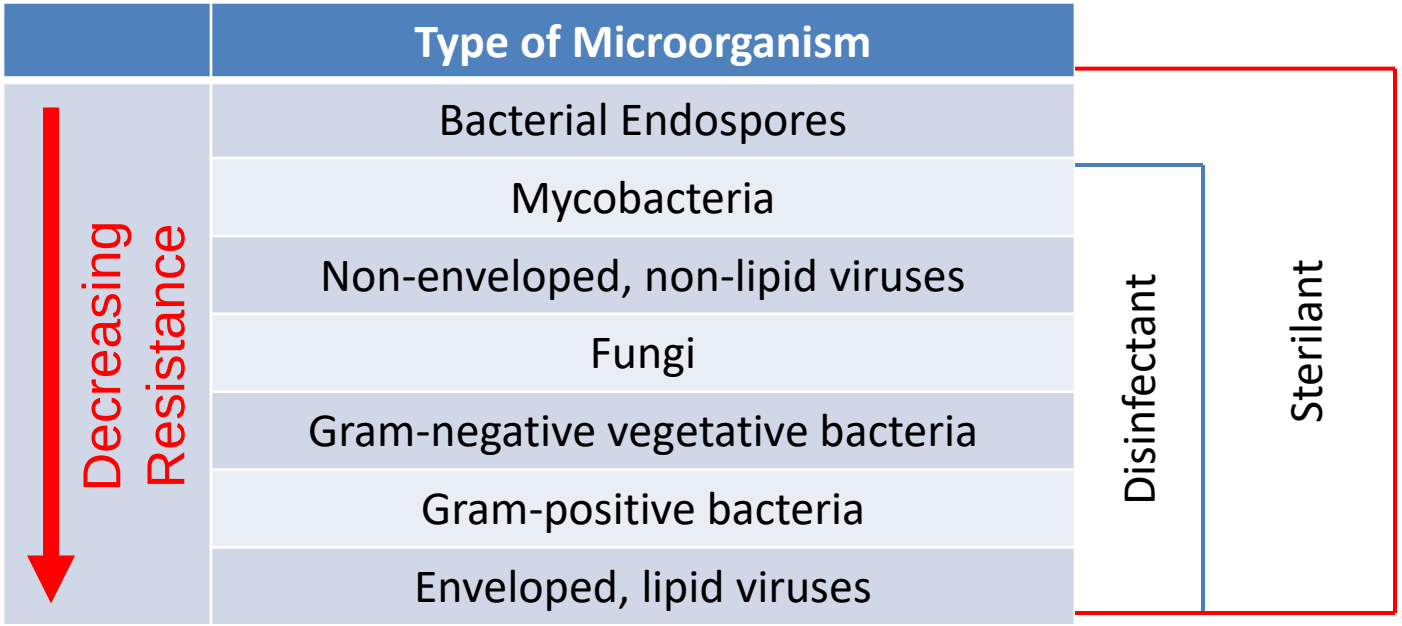
Certain chlorine dioxide gas products are registered as sterilants, which means they are capable of eliminating all viruses, bacteria, fungi and spores.





Spore Reduction

Spores are the most resistant microbial organisms. Spore reduction is aided by increased humidity (~65% RH) which causes spores to swell and crack, allowing the chemical to enter and inactivate the spore



Antimicrobial Efficacy

Target Organism	Dosage Required using CD Gas
Spores	~600 ppm-hrs for 6-log kill
Listeria	~300 ppm-hrs for a 5-log kill
Salmonella	~100 ppm-hrs for 6-log kill

Dosage measured in ppm-hours

600 ppm-hours can be attained by holding a:

300 ppm concentration for **2 hours** (300 x 2 = 600)

or

100 ppm concentration for **6 hour** (100 x 6 = 600)

or

Any equivalent combination of concentration and time*

*Ref. Kevin Lorcheim and Erik Melgaard. *Linearity of the Relationship Between Concentration and Contact Time for Sterilization with Chlorine Dioxide Gas*. ABSA 58th Annual Biological Safety Conference, 2015.

Efficacy and Humidity

Chlorine dioxide has been validated to be effective at lower relative humidity, but requires a higher dosage

RH	Dosage Required for 6-log Spore Reduction
65%	~600 ppm-hrs
55%	~1000 ppm-hrs
45%	~1550 ppm-hrs

*Ref. Mark A Czarneski. *Effects of Relative Humidity, Concentration, and Exposure Time on Chlorine Dioxide Gas Decontamination*. ABSA 54th Annual Biological Safety Conference, 2011.

Wet Areas

As Chlorine Dioxide Gas is water soluble, it is able to maintain its efficacy within water, so areas do not have to be completely dry for the process to work.

As wash downs and manual cleaning traditionally occur prior to decontamination, using chlorine dioxide gas means that surfaces do not need to be completely dry prior to decontaminating.

Distribution

A chemical can't kill what it can't reach.

	Chlorine Dioxide Gas
Boiling Point	**51°F**
Natural State at Room Temperature	**Gas**

Gasses fill the space they are contained within evenly and completely.

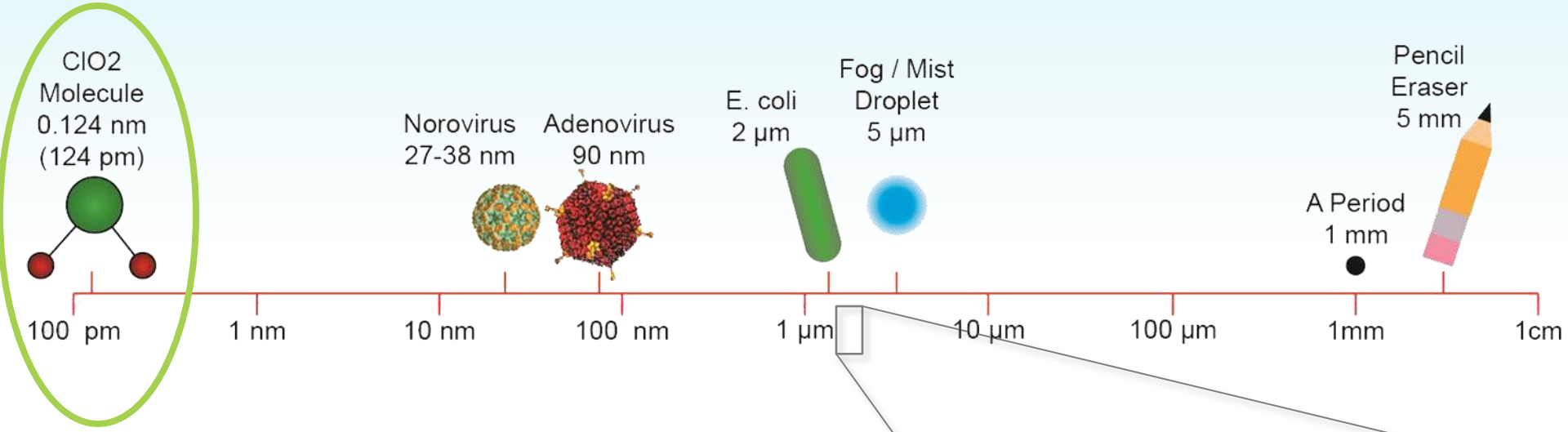
Chlorine Dioxide Gas is able to evenly fill the area it is decontaminating, no matter how large, tall or filled with equipment.

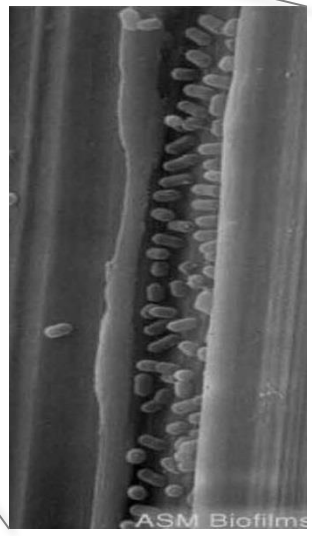
Distribution

Distribution

Penetration into Crevices

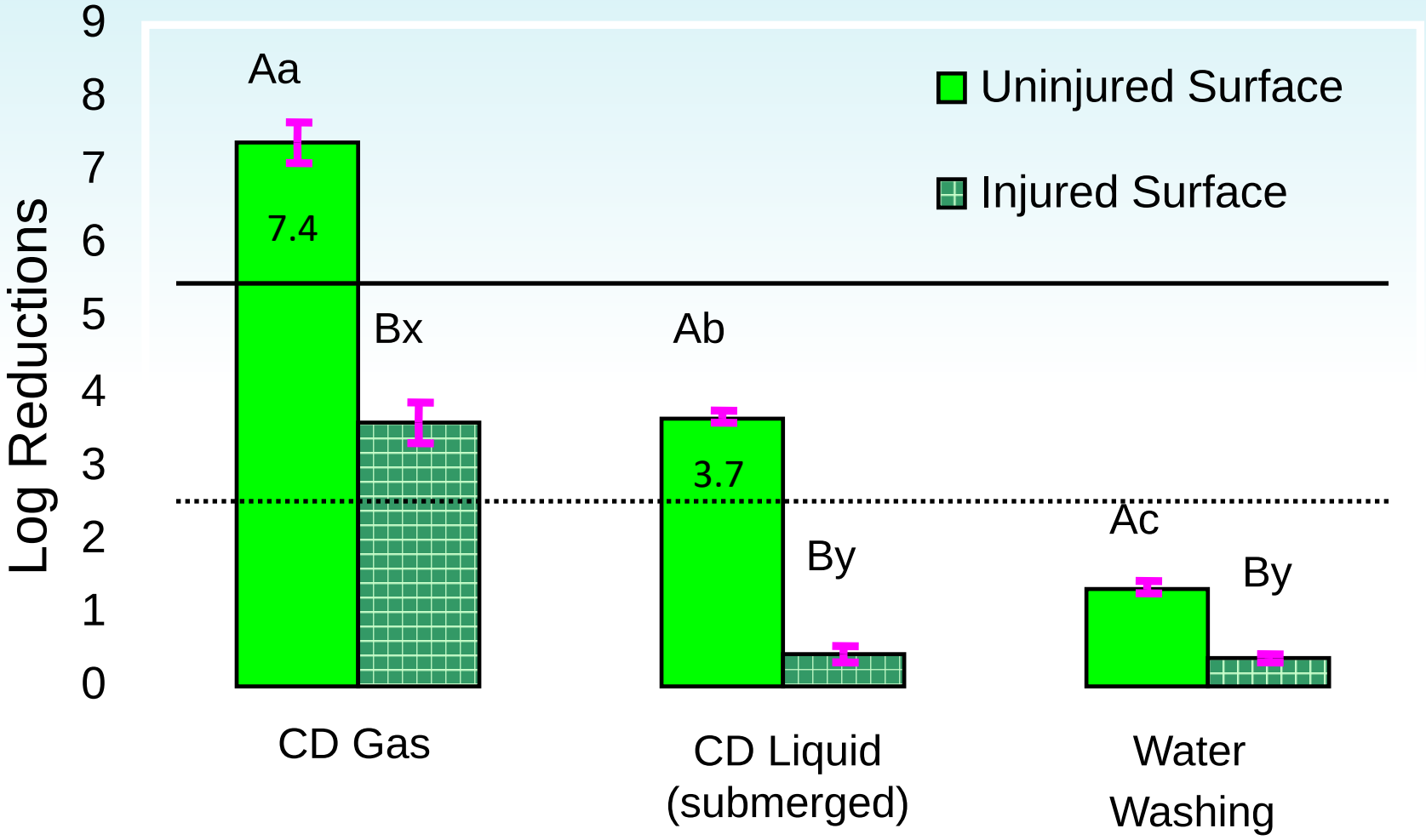
Organisms sizes vs ClO_2 molecule





Magnified scratch in stainless steel harboring bacteria

CD Gas vs. CD Liquid



Equal concentrations of CD Gas and Liquid used

All the treatments were for 10 min at 20°C

(Han, Y. et al, Reduction of Listeria monocytogenes on Green Peppers (Capsicum annuum L.) by Gaseous and Aqueoous Chlorine Dioxide and Water Washing and Its Growth at 7°C, Journal of *Food Protection, Vol 64, No 11, 2001 pages 1730-1738*)

Efficacy & Validation

QA Method	Description
Concentration Monitor	Verifies the concentration of CD gas during a decontamination and overall cycle dosage
Biological Indicators	Verifies that the decontamination cycle achieved 6-log sporicidal reduction
Swab Testing	Verifies that the organism itself is no longer present

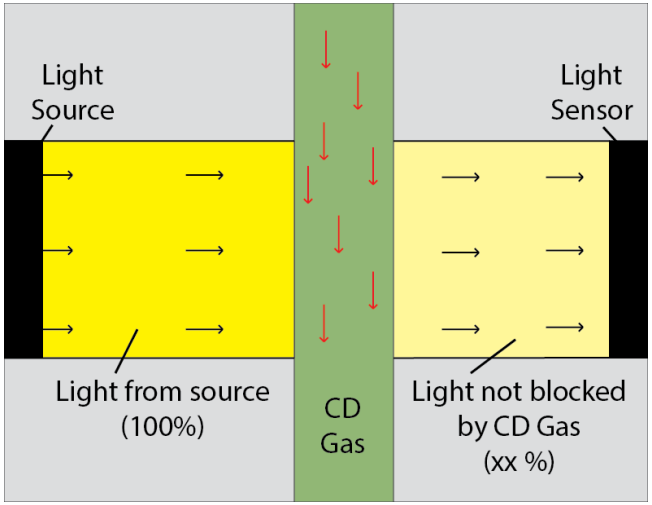
Concentration Monitoring

The concentration of chlorine dioxide gas can be monitored and logged during a decontamination.

2 main methods:

Chemical Sensor

uv-vis spectrophotometer



Concentration Monitoring

Chemical Sensor:

Advantages:

- Relatively Inexpensive

Disadvantages:

- Difficult to monitor multiple points within environment
- Less accurate due to saturation issues

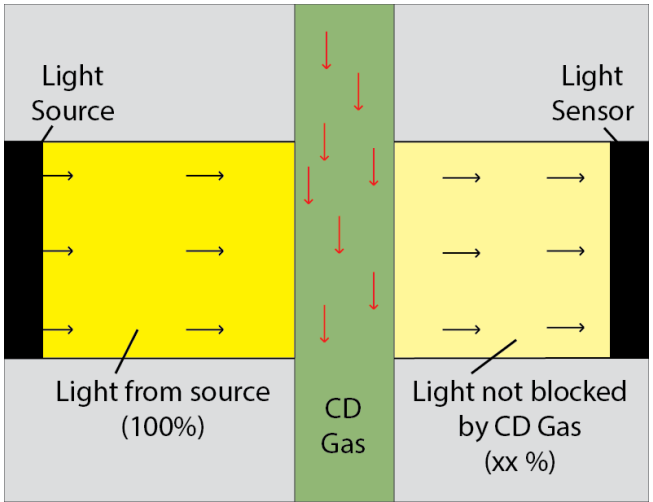
Uv-vis spectrophotometer:

Advantages:

- Highly accurate
- Able to monitor multiple points within environment

Disadvantages:

- More expensive

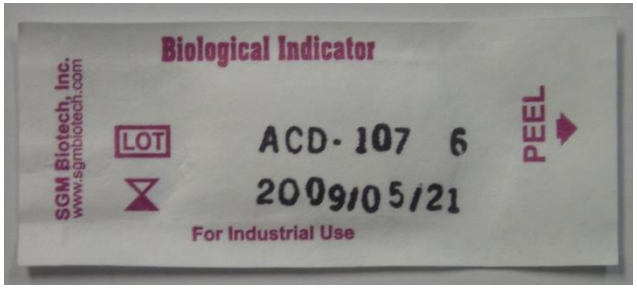


Decontamination Dosage

Ref. Mark A. Czarneski. *Microbial Decontamination of a New 65-Room Pharmaceutical Research Facility*. Applied Biosafety, Vol 13. No. 4, 2008

Biological Indicators

Biological indicators consist of a semi-permeable outer packaging and a interior carrier impregnated with bacterial spores. For sterilization, BI's contain over 1 million bacterial spores, providing the ability to prove a 6-log (99.9999%) sporicidal reduction.



Biological Indicators for chlorine dioxide gas use either *bacillus atrophaeus* or *geobacillus stearothermophilus* spores.

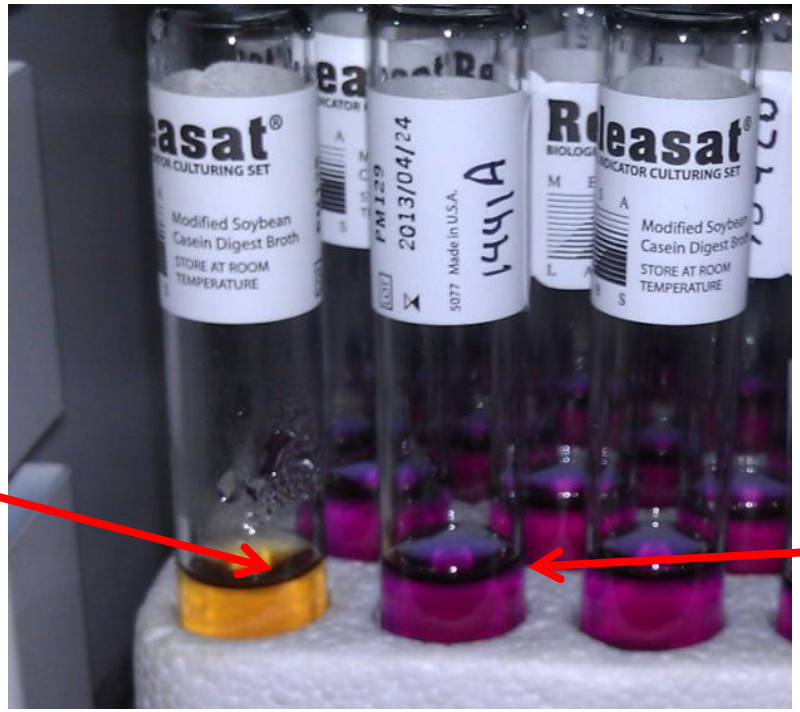
BI Analysis

After the decontamination process is complete, BI's are collected and aseptically dropped into growth media tubes and incubated. If even one spore was not killed, they will grow and the bacteria will multiply causing turbidity (cloudiness) or a color change within the media tube.

CD gas has a 36-48 hr incubation time depending on BI manufacturer.

Positive for growth

(Decon unsuccessful)



Negative for growth

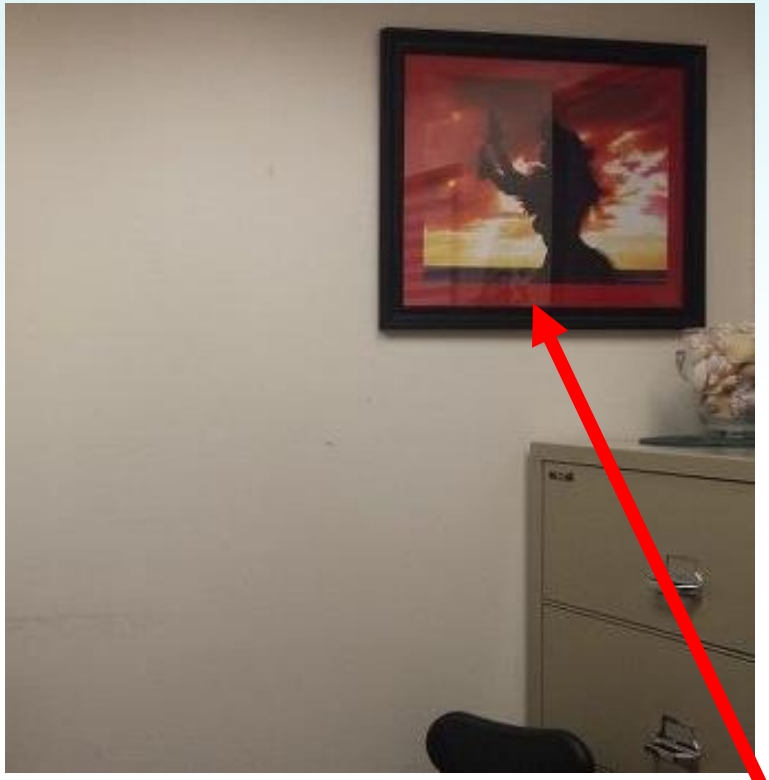
(Decon successful)

Penetration into Open and Closed Cabinets

Penetration Under Forklift Tire

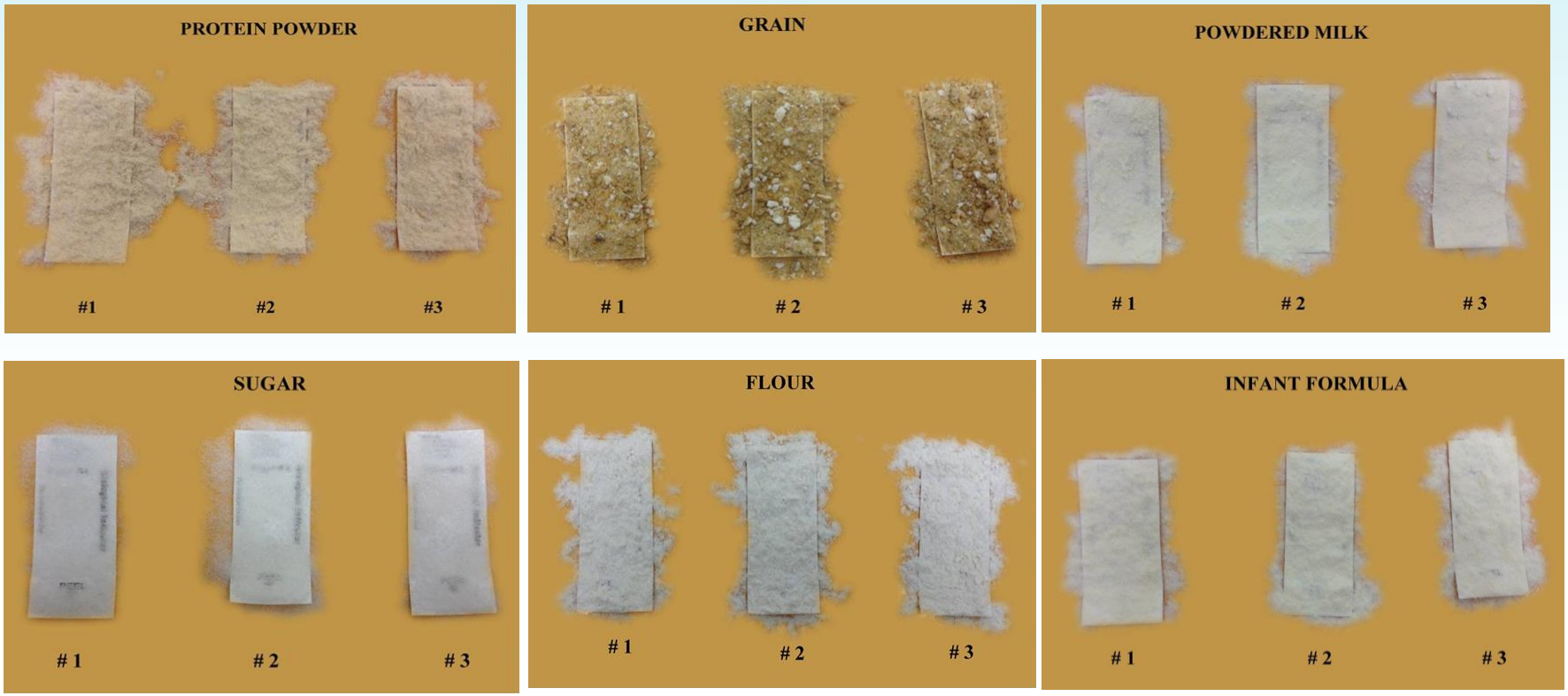
BI Killed

Penetration Behind Objects



BIs Killed

Penetration into Grain, Powdered Milk, Protein Powder, Sugar, Flour, Baby Formula



Biological indicators covered with up to ¼" of dry product were completely killed at a dosage of 720 ppm-hrs

Ref. Paul Lorcheim. *When is it Time to Clean for Facility Decontamination?*. Food Safety and Quality Magazine, April-May 2014

Cycle Flexibility

	Chlorine Dioxide Gas
Temperature	Not a factor above 52 F
Starting Relative Humidity	Not a factor
Equipment Within Space	Not a factor, gas gets everywhere
Room Shape / Size	Not a factor, gas gets everywhere

Principles of Decontamination

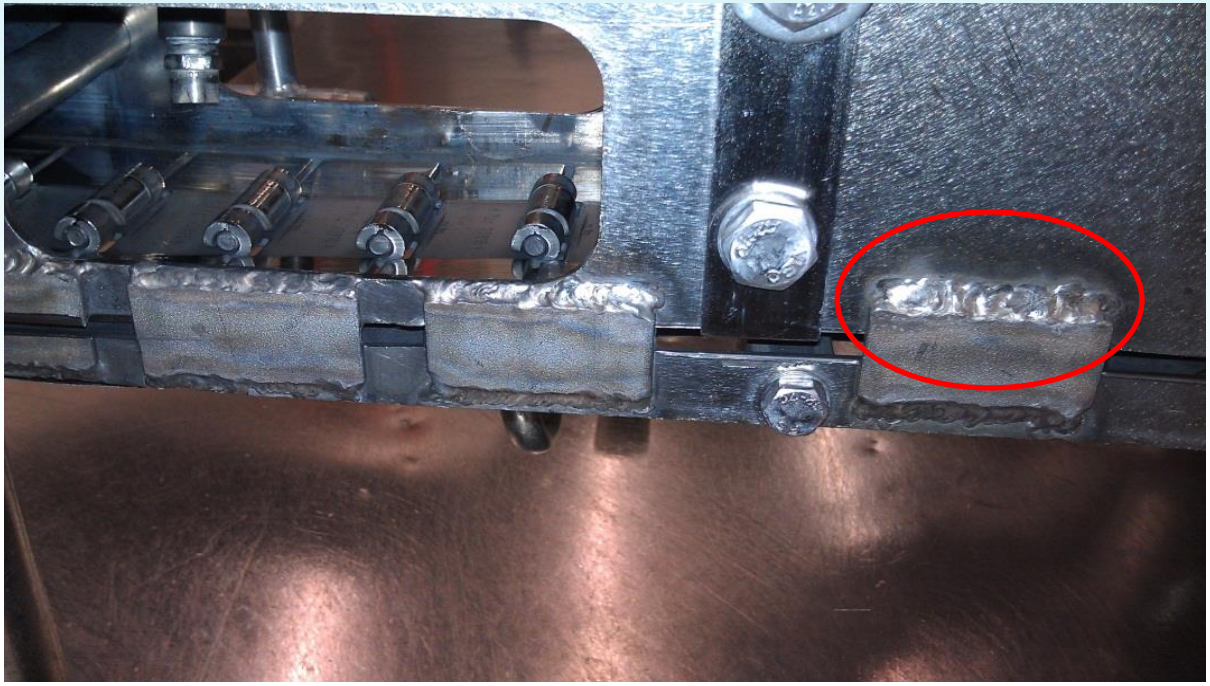
Chlorine Dioxide Gas is able to achieve these principles due to its chemical properties

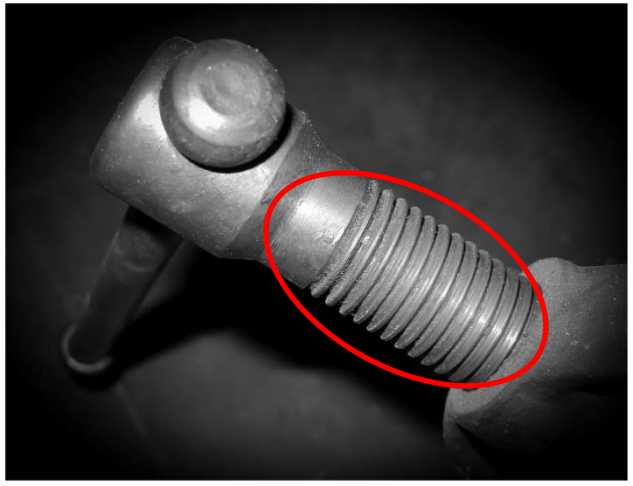
The decontamination method must:

- ✓ **Be able to kill the organism in question (Sterilant)**
- ✓ **Achieve good and complete distribution (True Gas)**
- ✓ **Achieve thorough and total penetration (True Gas & Small Molecule)**
- ✓ **Achieve sufficient contact time at the correct concentration (Accurate Concentration Monitoring)**

Hard to Clean Areas

Hard to Clean Areas

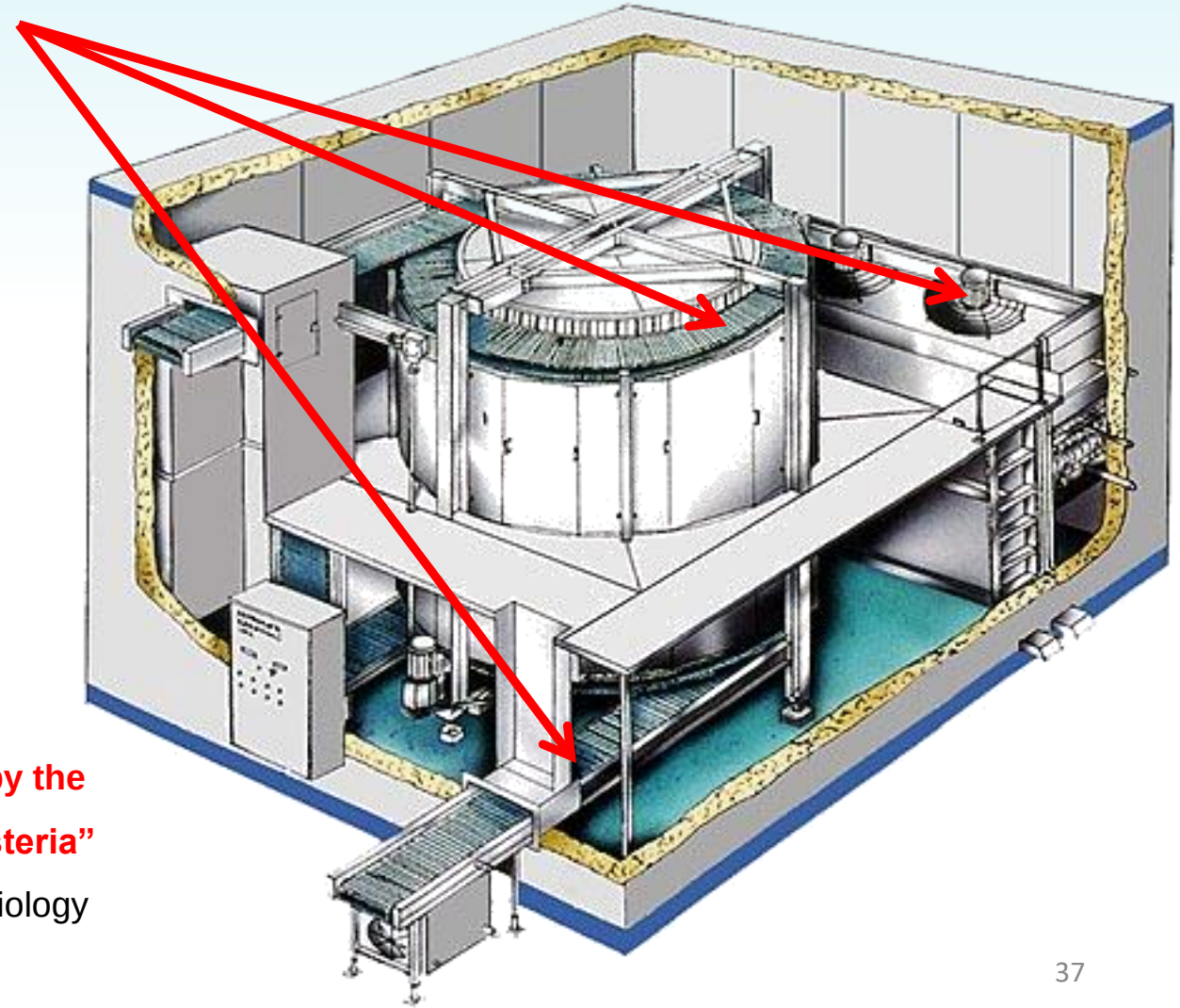




Spiral Freezers

Problem Areas

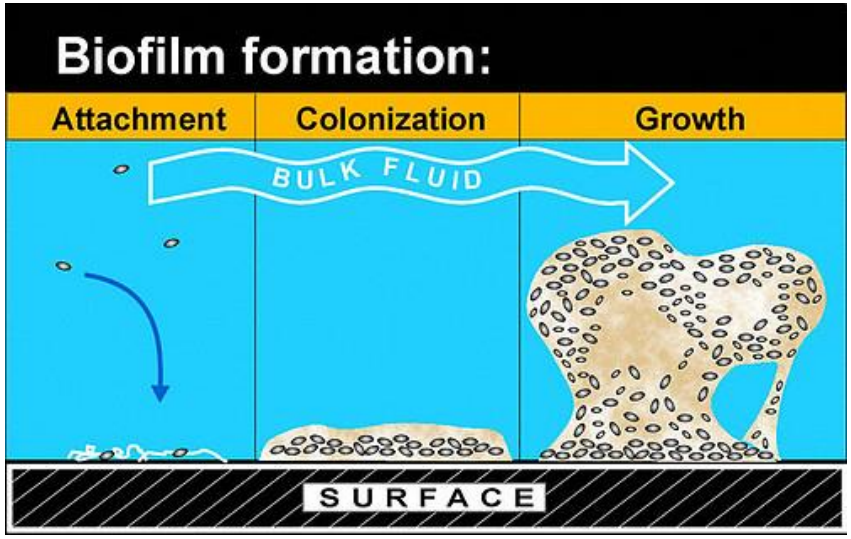
- Conveyor belt
- Penetrations into walls
- Wall seams
- Insulation behind wall panels
- Drains
- Cooling Coils
- Hard-to-reach areas



"Spiral freezers were designed by the devil to contaminate food with listeria"

-Dr. Jeff Kornacki, Kornacki Microbiology Solutions, Inc.

Biofilms



Chlorine Dioxide gas is effective against biofilms, which listeria monocytogenes can produce.

Ref. Valentina Trinetta, Richa Vaid, Qin Xu, Richard Linton, Mark Morgan. *Inactivation of Listeria monocytogenes on ready-to-eat food processing equipment by chlorine dioxide gas*. Food Control 26 (2012) 357-362.

Protection and Prevention

"The best defense, is a good offense"

The same principles that make chlorine dioxide gas effective against the most challenging locations, also make it the most effective at preventing contamination.

By eliminating harborage sites, food processing environments can stay cleaner for longer.

Preventive Decon – How Often?

The frequency of preventive decontamination depends on the application. Environmental sampling data should be used to determine the frequency of decontamination.

Process:

1. Establish baseline environmental sampling data under current sanitation method
2. Decontaminate with chlorine dioxide gas
3. Perform ongoing post-decon sampling to determine the preventive benefit of chlorine dioxide gas