Detection and Removal of Biofilms

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Goals of Today

- Background
- What is biofilm and why is it important?
- How is biofilm detected?
- How to remove/control biofilm?
Sterilex Background

Company Focus – developing innovative solutions for microbial control and detection

1989

Sterilex founders were funded by US Army to develop a universal decontamination technology for biological and chemical warfare agents

1991

First publication of studies conducted by the NFPA showing that the PerQuat® chemistry was effective in killing Listeria in biofilm and more effective than other routine sanitizers

1995

Sterilex Corporation is incorporated in the State of Maryland with the mission of developing specialty antimicrobial products for markets where biofilm control is an important factor

Present Day

- PerQuat based biofilm removal products – EPA approved
- EPA registered floor and mat sanitizers
- Indicon®: rapid biofilm detection technology
Environmental Pathogen
A pathogen capable of surviving and persisting within the manufacturing, processing, packing, or holding environment such that food may be contaminated and may result in foodborne illness if that food is consumed without treatment to significantly minimize the environmental pathogen.

Hazard Evaluation
...must include an evaluation of environmental pathogens whenever a ready-to-eat food is exposed to the environment prior to packaging and the packaged food does not receive a treatment or otherwise include a control measure (such as a formulation lethal to the pathogen) that would significantly minimize the pathogen.
What is Biofilm?

Biofilm is a grouping of cells that stick together, embedded in a self-produced matrix of extracellular polymeric substance composed of proteins, polysaccharides and other materials.
Pathogens Reside in Biofilm

- Natural habitat for microorganisms
  - Natural biofilms are multi-species
- Microorganisms in biofilm express resistance (1000 times the dose of sanitizers required)
- Conventional disinfectants have limited effectiveness against biofilm

![Image of biofilm and planktonic organisms]
Issues In The Control of Biofilm

- Biofilms more likely in wet, inaccessible areas and on corroded and soiled surfaces:
  - Underneath conveyor belts
  - Weld cracks
  - Drains
  - Complex grinder parts
  - Catheters
  - Jacuzzi water lines

- Not sufficient to kill the surface organisms – must remove the reservoir

- Failure to remove means repopulation in 48 hours – normally 168 hours to re-establish
Evidence of Biofilms

<table>
<thead>
<tr>
<th>Organoleptic signs</th>
<th>Analytical signs</th>
<th>Product signs</th>
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</thead>
<tbody>
<tr>
<td>Visual, rainbow appearance</td>
<td>Spike in generic microbiological counts/hot spots</td>
<td>Loss of shelf life</td>
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<tr>
<td>Slimy feel on equipment</td>
<td>Increase in environmental <em>Listeria</em> positives</td>
<td>Spoilage</td>
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<td>Sour or musty odor</td>
<td>Increased failures with ATP devices</td>
<td>Product micro failures</td>
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ATP Bioluminescence Testing

Most commonly used method

Advantages
- Quick and immediate results
- Measures level of sanitation by looking for levels of ATP common in food (and bacteria)
- Can track and trend results

Disadvantages
- Expensive
- Does not give you the levels of bacteria on surfaces, just food residues
- Can only pick up what the swab can reach
Microbiological Testing

- Results in 24–48 hrs.
- Inexpensive

Various methods
- Contact plates
- Dipsticks
- Petrifilm or pour plates
- Air testing

Establish internal microbiological standards (10, 50, 100 cfu/square cm)

Develop a testing program and establish a frequency of testing

Track and trend data

Critical for ready-to-eat processes (e.g., *Listeria monocytogenes*)
Sanitation Verification

- **Organoleptic (sight, smell, taste)**
- **Bioluminescence/ATP** (immediate results)
  - Aerobic plate count (results in 2–3 days)
  - Environmental monitoring (results in 3–5 days)
- **Recent trends**
  - Bio-Mapping
  - Rapid visual indication (< 2 mins.)
  - Identify likely biofilm harborage niches across a wide area

**Physical verification**

**Microbiological verification**

**Indicon® Gel Hygiene Indicator**
Recent Trends – Bio-Mapping

- Manually recording results vs. Electronic Data Management/Bio-mapping
- Predictive sampling
Recent Trends – Rapid Sanitation Verification

Rapid sanitation verification
- Cost-effective, nearly instantaneous hygiene monitoring tool
- Catch harborage niches before they become a problem

- Indicon® Gel (Sterilex)
- Listeria Right Now (Neogen)
Complete system for taking environmental *Listeria* tests with molecular-level accuracy that requires no enrichment and features a total time-to-result of under one hour.

Neogen’s *Listeria Right Now™*
Rapid Biofilm Detection Gel

- Rapid diagnostic to detect presence of biofilm
- Cost effective
- Training tool
- Pre-op QC tool
- “Seek and Destroy” applications

Blue colored gel that rapidly creates white bubbling action upon contact with biofilm (< 2 minutes)

Ready to use – no mixing

www.sterilex.com/indicongel
Indicon® Gel Reaction

Easily visible reaction when the product comes into contact with high concentrations of organisms located within a biofilm. Detect harborage niches in areas a swab can not reach!

Note: The lack of a positive reaction from Indicon Gel does not guarantee that the surface is free from microorganisms. Indicon Gel is not meant to take the place of routine microbial monitoring or organism specific diagnostic tests.
Indicon® Uses

Daily sanitation verification during pre-op

- Cover wide area to ensure elimination of harborage niches
- Microbial specific monitoring vs. ATP
- Positive = reclean, further testing

Seek and destroy

- Find harborage niche across wide area when positive found
- Reaches where a swab can not reach
Visually Identify Harborage Niches
Visually Identify Harborage Niches
## FEATURES

- Easy and safe to use
- Ready to use spray gel
- Allows for detection on large surface areas
- Micro-bubble reaction is readily visible on a variety of surfaces
- Not regulated for transport
- Freely rinseable
- Shelf Life: 12 Months +

## BENEFITS

- Rapidly detects the presence of invisible biofilm harborage niches
- Provides a cost-effective, quick visual indication of the presence of biofilm on a surface which may contain microorganisms such as Listeria, Escherichia coli (E. coli) or Salmonella
- Perfect for “seek and destroy” applications
- Reaches areas a swab cannot
How are Biofilms Removed/Treated?

**Hand Scrubbing**
- Impossible to scrub every niche
- Time consuming in certain areas like drains

**Burn Off Biofilm**
- Oxidizing chemistries such as chlorine bleach and PAA react with the outer layers of film first
- Can require up to 1000x the normal dose of sanitizer to remove

**Use specifically designed biofilm removal chemistry**
- Sterilex PerQuat Technology is the only EPA registered biofilm removal chemistry
- Effectively removes biofilm and kills pathogens within biofilm.
- The usual precautionary measures are to be adhered to when handling chemicals
Biofilm Treatment with Different Sanitizers

- Key: Access of disinfectant to target organisms

- Bacteria rapidly colonize dirty surfaces and residual biofilm

- Sterilex biocides are superior in biofilm-protected environments

- Sterilex biocides excel in removal of the biofilm matrix and accessing biofilm-protected microorganisms

**Sterilex completely removes biofilm**
Sterilex PerQuat® Technology

Disassociating \( \text{H}_2\text{O}_2 \) in an alkaline environment and pairing it with a positively charged Quat, forms intimate ion pair soluble in both lipid and aqueous phases.

Water Phase

\[ \text{H}_2\text{O}_2 + \text{Quat}^+X^- \xrightleftharpoons{\text{High pH}} [\text{Quat}^+ \text{OOH}] \]

Oxidative and hydrolytic OOH- anion transported by Phase Transfer Catalyst

Biofilm

\[ \text{OOH} + \text{Quat}^+X^- \xleftarrow{\text{Attack Pathogen}} [\text{Quat}^+ \text{OOH}] \]
Sterilex Food C&S Product Line

Sterilex Ultra Disinfectant Cleaner and Activator
EPA: 63761-8
- Can be used everywhere in a plant including on food contact surfaces and drains.
- Food pathogens including:
  - Listeria monocytogenes
  - Escherichia coli 0157:H7
  - Salmonella choleraesius
- Formulated with anti-foam
- CIP/HTST/Injector treatment

Sterilex Ultra CIP

Sterilex Ultra Soft Metal Safe Activator
- Replace Sterilex Ultra Activator
- Compatible with wide variety of aluminum surfaces
- Perfect for overheads, freezers, coils, etc.

Sterilex Ultra Step
EPA: 63761-10
- Same PerQuat® chemistry in dry form
- EPA registered solid floor sanitizer
- Less dusty
- Blue color

Indicon Gel – NEW!
- Biofilm Indicator
- Not to replace ATP swabs
- Rapid detection as quick as 30 seconds
Example Use Sites

**Food Contact Surfaces**
- Grinders
- Conveyors
- Dryers
- RTE/Clean Rooms
- Brine Injectors (CIP)
- Brine Chillers (CIP)

**Environmental Surfaces**
- Drains
- Walls
- Floors

**Other Harborage Areas**
- Chillers/HVAC (SM Safe)
- Coils (SM Safe)
- Freezers (SM Safe)
- Central Sanitizing Systems
- Lube Lines
THANK YOU!!

For additional information, feel free to contact me at tyler.mattson@sterilex.com; mobile: 443-886-4008

Questions?
www.sterilex.com