Spore Wars

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Presentation topics

- What are spores?
- Milk spoilage basics
- Where do spores come from?
- Biofilm issues
- Minimising spores
- CIP requirements
Dairy Spores and Biofilm

Spore formation in bacteria is not the principal method of reproduction but simply a method of surviving unfavourable conditions.

They have a number of features:

- They are very resilient to many sanitisers
- CIP cleaning steps do not affect them
- They can tolerate higher temperatures than live bacteria
- They can tolerate extreme dryness
- They can be found in biofilm and soil deposits
Dairy Spores and Biofilm

Spore structure

**Exosporium** - A thin delicate covering made of protein.

**Spore coats** - Composed of layers of spore specific proteins.

**Cortex** - Composed of loosely linked peptidoglycan and contains dipicolinic acid (DPA), which is particular to all bacterial endospores.
Spore Generation
Milk Microbiology

Milk in the cow is sterile.
Can become contaminated with all types of microorganisms. 

Level of contamination is influenced by:

- health of the cow
- farm workers
- equipment
- environment
- holding temperature
- holding time

ECOLAB®
Three Microbial Causes of Milk Spoilage

1. **Gram Negative**
   - post-pasteurization contamination

2. **Gram Positive** spore formers
   - Psychrotrophic Thermodurics
     - may come from raw milk

3. **Heat stable enzymes**
   - may come from raw milk
Bacterial growth trends in milk shelf-life:

**Gram Positive** organisms show up later than **Gram Negatives**
Factors influencing shelf-life of dairy products

- Microbiological quality of raw milk
- Pasteurization/thermal process temperature & time
- Post-pasteurization/ post thermal process contamination
- Sanitary Design
- Sanitation
- GMP’s
- Packaging
- Storage & Distribution – temperature, time, cleanliness
Where do the spores come from or increase?

Farms

- Somatic cell count has no correlation with spore count
- Soil is a major habitat for spore formers
- Feed and environmental conditions can contaminate udder and teats
- Milk cross contamination from silage
  - Total spore formers in silage $10^2$-$10^6 \text{ cfu/g}$ (Giffel 2002)
- Dirty and poorly maintained milking equipment and practices
- Spore counts are almost random in some cases
Where do the spores come from or increase?

Dairy Plants

- Processing steps operating at 45 – 60°C
- Regeneration sections of pasteurisers
- Preheater and evaporator after 9 – 12 hr. runs
- Extended raw milk storage
  - Raw tank turn around
- Separators
- Holding tanks
  - Turn around time
Sources of Post-Pasteurization Contamination

Dairy Plants

Filler 50%

Piping 30%

Pasteurisation 20%

**CAUSE:** Inadequate Cleaning and sanitizing

Most noticeable after non-production days
Microbial Spoilage of Fluid Milk

Post-Pasteurization Contamination:
- Spoilage 10 - 14 days
- **Gram Negative** Bacteria
  - (e.g. Pseudomonas sp.)
- Stress Test: Growth
  - (using Gram Negative media)
- Coliform Test: Out of Spec.

Thermoduric Psychrotrophs:
- Spoilage 18+ days
- **Gram Positive** Bacteria (Spore forming)
  - (e.g. *Bacillus* and *Panebacillus* sp)
- Stress Test: No Growth
  - (using Gram Negative media)
- Coliform Test: Within Spec.
Biofilm issues

Traditional biofilms generally not an issue
- Stainless steel pipes and tanks are clean and sanitized
  - Confirmed by ATP swabs and micro sampling

Residual soil acts as hybrid biofilm/soil matrix
- Non-traditional biofilms

Nooks and Crannies
- Gasketed joints
- Valves
- Plate heat exchangers
- Evaporators

Low flow areas

Biofilms newly generated during long runs
Difficult to Reach Biofilms

Location Sampled
Difficult to Reach Biofilms

Thermal imaging shows areas with much lower temperature than bulk CIP solution

Temperatures
Max 62°C
S1 58°C
S2 40°C
Heat Exchanger Biofilm Issues

- Plate heat exchangers are difficult to fully clean
- Very difficult to confirm a proper clean
- Buildup of soil in low flow regions
- Optimal growth conditions for thermophiles
- Long runs lead to high counts later in run
Heat Exchanger Biofilm Issues

- Evaporators difficult to clean properly
- Low flow areas often not fully cleaned
Minimising spores in product

Minimizing spore ingress at the farm

- Monitor spore levels in incoming milk
- Segregate low count milk for high quality products

Keeping the levels as low as possible during processing

- Plant and equipment designed to help streamline the process.
- Minimizing milk residence time at temperatures that favour thermophilic spore-former growth 40-70°C.
- Prevention of fouling and biofilm formation. Minimizing surface areas where biofilm can build up and subsequently release bacteria in the product.

Implementing an effective cleaning system

- to remove residual product, fouling and microbes, including spore-formers. This will minimize re-contamination from run to run.
Minimising spores in product

Fluid Milk Processing

- Keep processing times between CIP down to 9 to 12 hrs
  - this significantly limits productivity
- For long production times isolate first 9 to 12 hrs as low spore content product
- Do not store product in holding tanks for long periods
- Don’t run pasteurisers for extended periods
- Consider intermediate CIPs
- Install bactifuges
  - secondary microbial intervention
Minimising spores in product

CIP programs

- Ensure all CIP parameters are correct and maintained over length of CIP
- Use built products and additives not NaOH alone
- Set points for CIP may need to be higher than normal
- Consider more frequent CIPs
- Consider intermediate CIPs
- Use peracid sanitisers for all equipment
Aggressive CIP Interventions Required

Global Best Practices from Low Spore Count Producers

- Premium cleaning chemistry
  - Suitable additives for caustic
  - Peracid override programs
  - Validated time between CIPs
- Peracid sanitizing under hot conditions
  - Where it can be accurately controlled
- Sanitizer use in all areas, including
  - Membranes
  - Evaporators
  - Pasteurisers
The CIP Program

• Existing products used in new ways
• Targeting the spores

- Oxidising detergent pretreatment
  - For all critical equipment
  - Helps remove biofilm

- Caustic override and caustic wash
  - Significantly improves removal of soils

- Standard acid wash if applicable
  - Need to eliminate any scale build up

- Peracid sanitiser recirculated warm
  - Additional sanitising effect
The CIP Program

Existing premium products used in new ways

Full wash program used on
- Separators, preheaters, pasteurisers, bactifuges
- Evaporators, high heat treatment sections

How is this different?
- Pretreatment programs not usually used on non heat exchange equipment
- Sanitisers not usually used on pasteurisers and evaporators
- Peracid sanitiser recirculated at warm to hot temperatures
  - Not single pass cold sanitising
  - Longer contact times may also be required
The CIP Program

Limitations of oxidising pretreatment programs

Peracid pretreatment
- Product recommendations need to be followed carefully
- Not just any peracid product can be used
- Temperature profiles are critical
- Overall CIP times usually not increased

Caustic product
- Only certain products can be used
- Do not use with EDTA based products
- In some cases other additives may be required

Sanitiser
- Mixed peracids show superior activity against spores
- Best when used at higher temperatures
Spore Reduction Trial
Results – Compendium Average

Evaporator

Powder

Proprietary Information of Ecolab, Inc.
Spore Reduction Trial
Results – Compendium - 12 Hour Run

Evaporator

Powder

Spore Count cfu/g

Hours

Trial Ave
Baseline Ave

Proprietary Information of Ecolab, Inc.
Spore Reduction Trial

Results

- High heat section cleaned with pretreatment program
- Large amounts of heavy soil removed
- These soils were residual from normal CIP process
## Interventions - Spores

Bacillus spp spore cocktail

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Contact Time (min)</th>
<th>50° C Average Survivors (CFU/mL)</th>
<th>Average Log</th>
<th>Log Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed</td>
<td>2</td>
<td>2.2 x 10^4</td>
<td>4.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Peracid 5000 ppm</td>
<td>10</td>
<td>&lt;10</td>
<td>&lt;1</td>
<td>&gt;5.1</td>
</tr>
<tr>
<td>Peracid 2600 ppm</td>
<td>10</td>
<td>1.0 x 10^1</td>
<td>1</td>
<td>5.1</td>
</tr>
<tr>
<td>Peracid 2</td>
<td>&lt;10</td>
<td></td>
<td>&lt;1</td>
<td>&gt;5.1</td>
</tr>
<tr>
<td>Cleaner 5000 ppm</td>
<td>10</td>
<td>1.0 x 10^1</td>
<td>1</td>
<td>5.1</td>
</tr>
<tr>
<td>Experimental 2</td>
<td>&lt;10</td>
<td></td>
<td>&lt;1</td>
<td>&gt;5.1</td>
</tr>
<tr>
<td>Peracid 2000 ppm</td>
<td>10</td>
<td>&lt;10</td>
<td>&lt;1</td>
<td>&gt;5.1</td>
</tr>
</tbody>
</table>

*Proprietary Information of Ecolab, Inc.*
# Deep clean CIP

<table>
<thead>
<tr>
<th>STEP</th>
<th>PRODUCT</th>
<th>CONCENTRATION</th>
<th>TEMP</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Rinse</td>
<td>water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>Exelerate HS-I</td>
<td>0.8 to 1.2% v/v</td>
<td>75-85C</td>
<td>10 to 20 mins</td>
</tr>
<tr>
<td>Alkali Wash</td>
<td>Glissen</td>
<td>equivalent to 1.5 to 3% w/v free caustic</td>
<td>75-85C</td>
<td>20 to 40 mins</td>
</tr>
<tr>
<td>Note: Alkaline cleaner is added to CIP solution as an override</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Alkali Rinse</td>
<td>water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid Wash (if required)</td>
<td>Super Stonekleen</td>
<td>0.5%</td>
<td>50–60C</td>
<td>20 to 30 mins</td>
</tr>
<tr>
<td>Post Acid Rinse (if required)</td>
<td>water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sanitise</td>
<td>Oxysan ZS</td>
<td>0.1 to 0.15%</td>
<td>Up to 80C</td>
<td>10 to 20 mins</td>
</tr>
</tbody>
</table>
Eliminating Spores

Summary

- Know your incoming product quality
- Limit long production times
- Retrace the process if spores are present in your product
  - Check all heated processes
  - Look for biofilms or soil residues
- Maintain very high levels of CIPs
  - Use quality cleaning chemicals (not commodity)
  - Use deep clean CIP process like caustic override
  - Use peracid sanitisers