Spore Wars

Robert Ellis

Technical Support Specialist

5 Nov 2015

ECOLAB®

Presentation topics

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

Dairy Spores and Biofilm

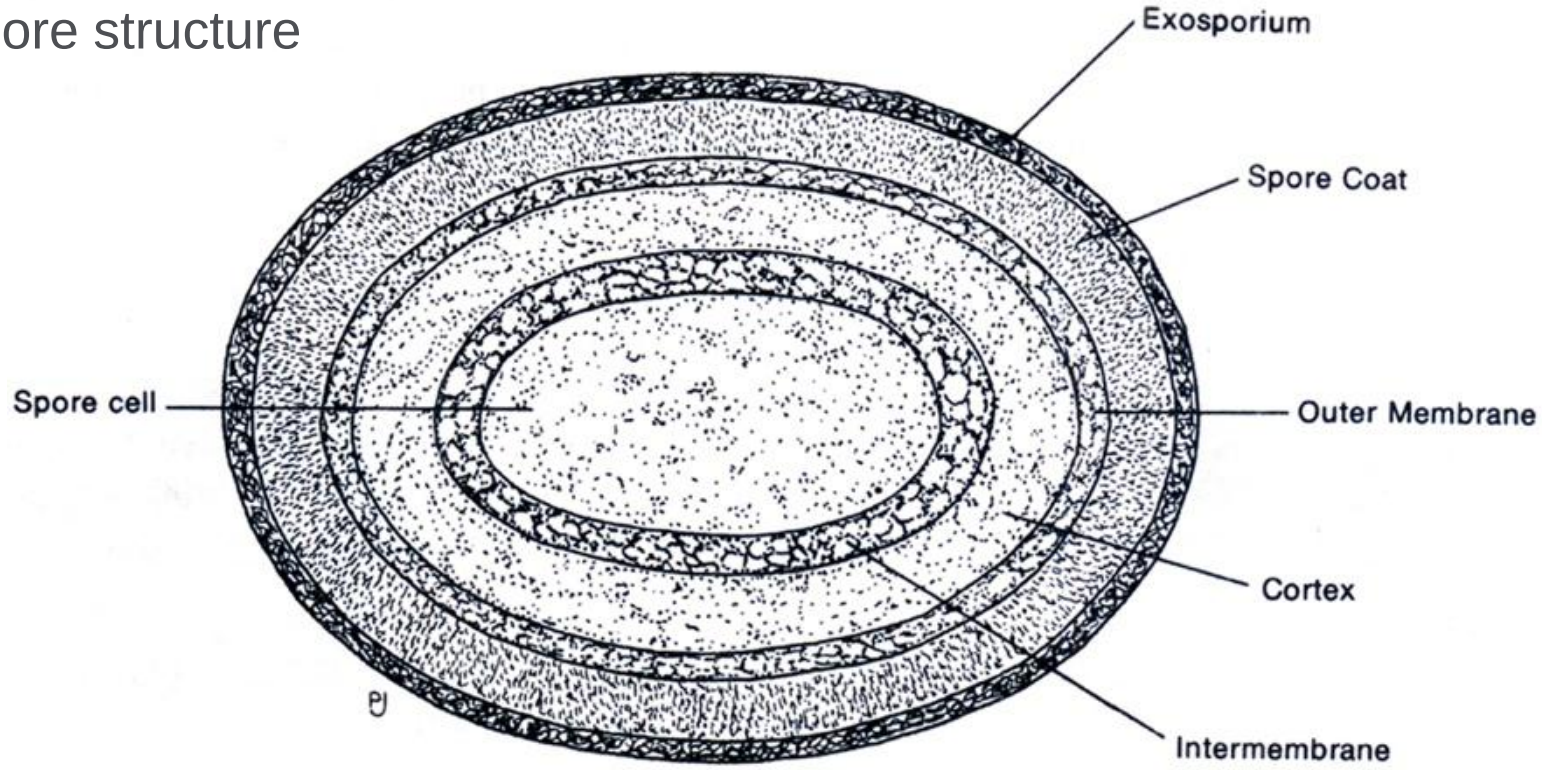
Spore formation in bacteria is not the principal method of reproduction but simply a method of surviving unfavourable conditions.

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

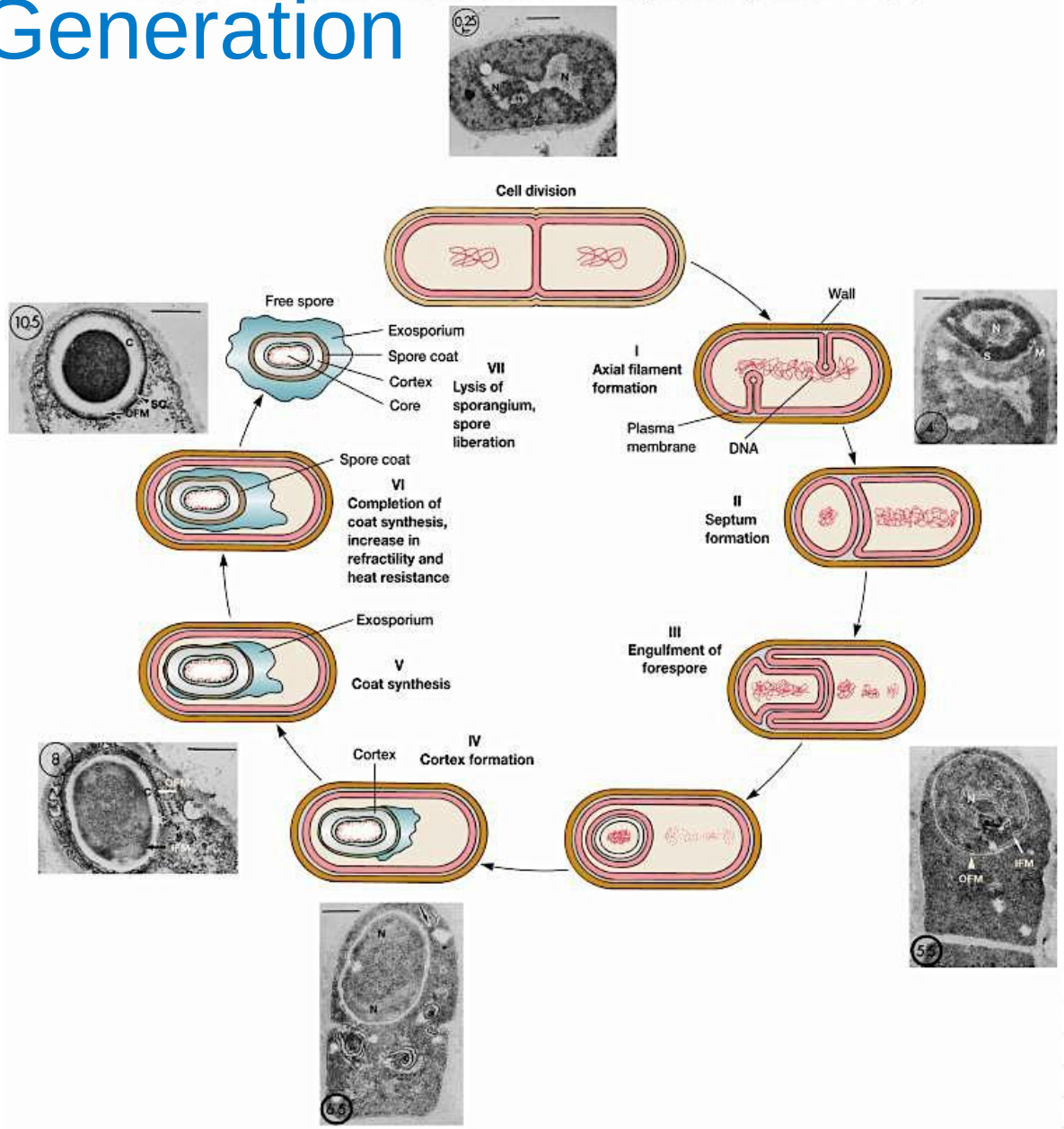
Spore structure



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

ECOLAB®

Spore Generation



ECOLAB®

Milk Microbiology

Milk in the cow is sterile.

Can become contaminated with all types of microorganisms.

Level of contamination is influenced by:

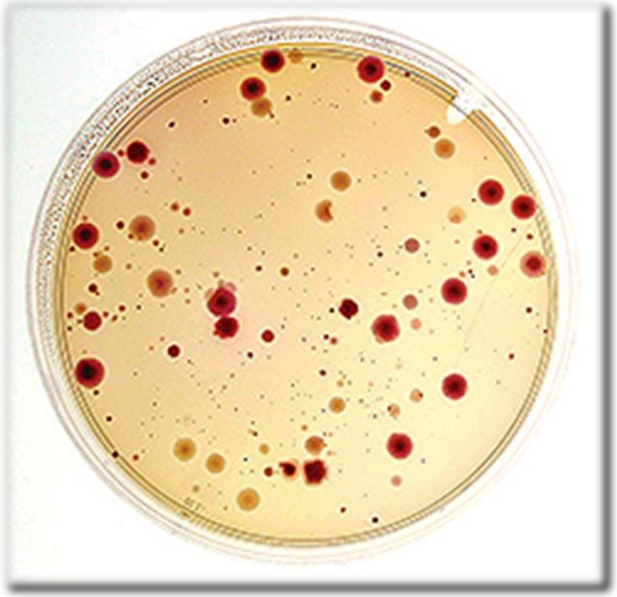
- health of the cow
- farm workers
- equipment
- environment
- holding temperature
- holding time

Three Microbial Causes of Milk Spoilage

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

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

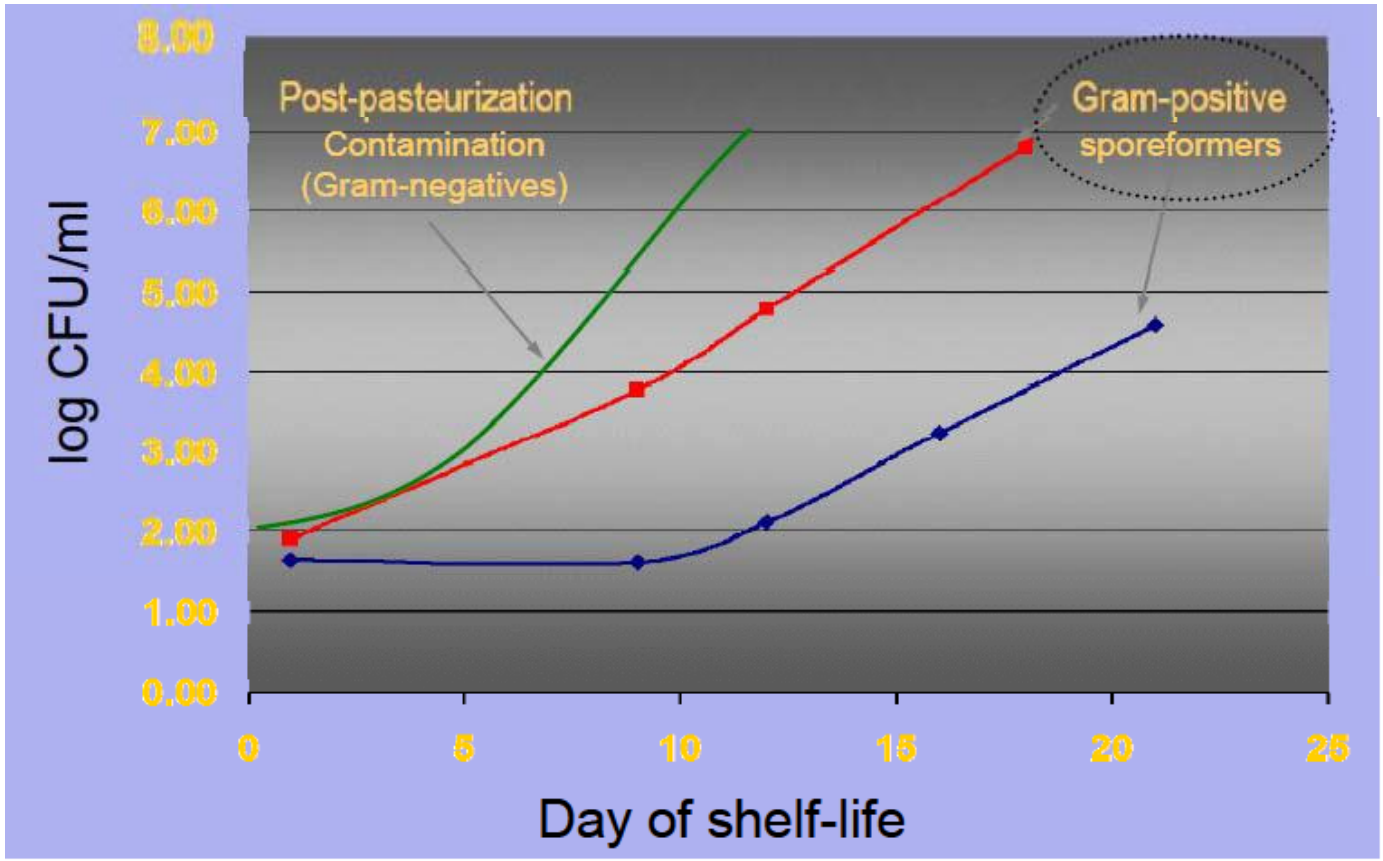
3. **Heat stable enzymes**
   - may come from raw milk



ECOLAB®

Bacterial growth trends in milk shelf-life:

Gram Positive organisms show up later than **Gram Negatives**



Slide Courtesy of Kathryn Boor
Cornell University

ECOLAB®

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

Understand your complete production sequence

ECOLAB®

Where do the spores come from or increase?

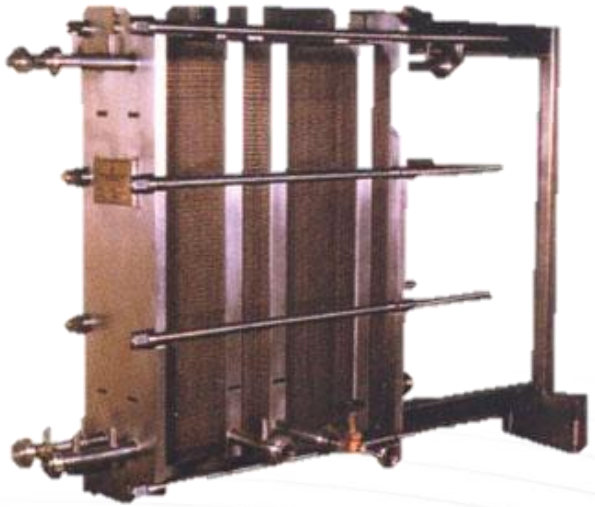
Farms

- 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 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 – 60C
- 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

ECOLAB®

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.

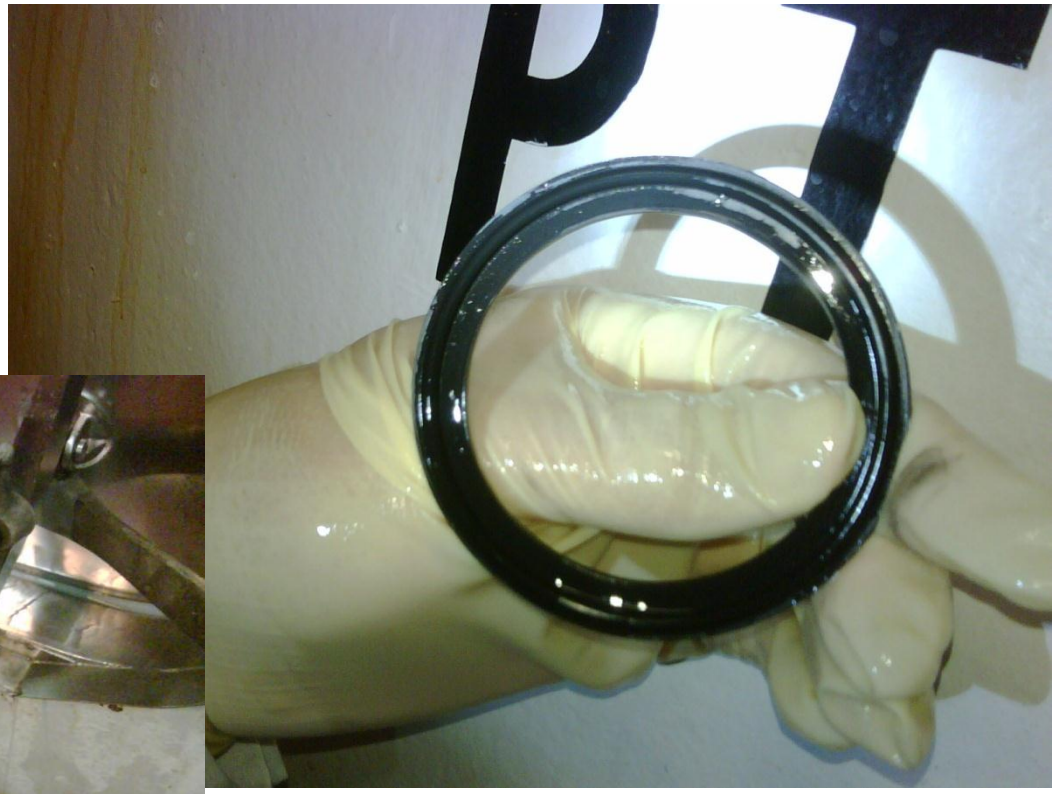
ECOLAB®

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

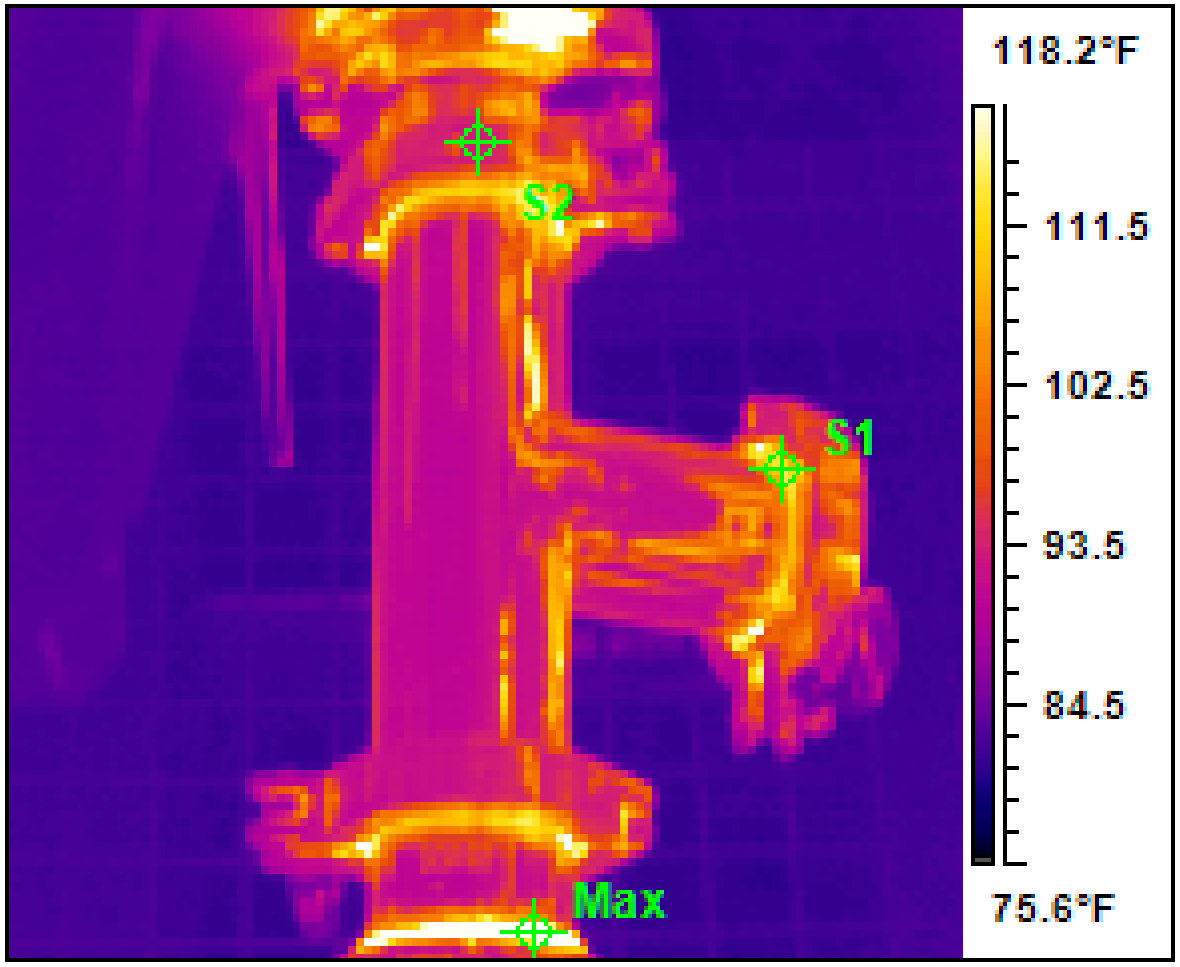
ECOLAB®

Difficult to Reach Biofilms



ECOLAB®

Difficult to Reach Biofilms



Temperatures

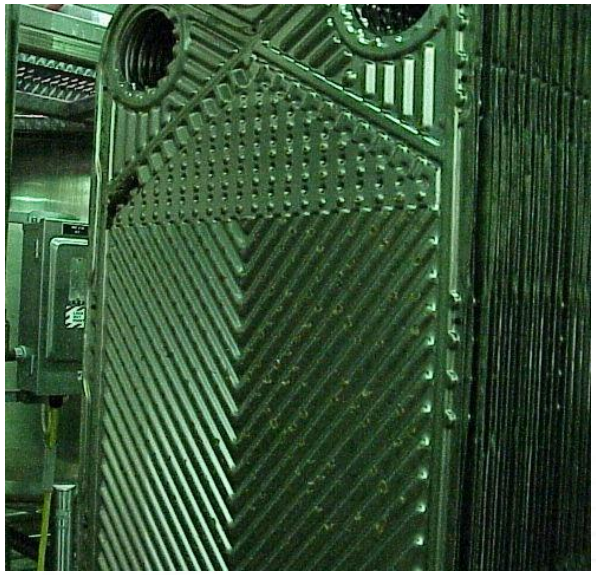
Max	62° C
S1	58° C
S2	**40° C**

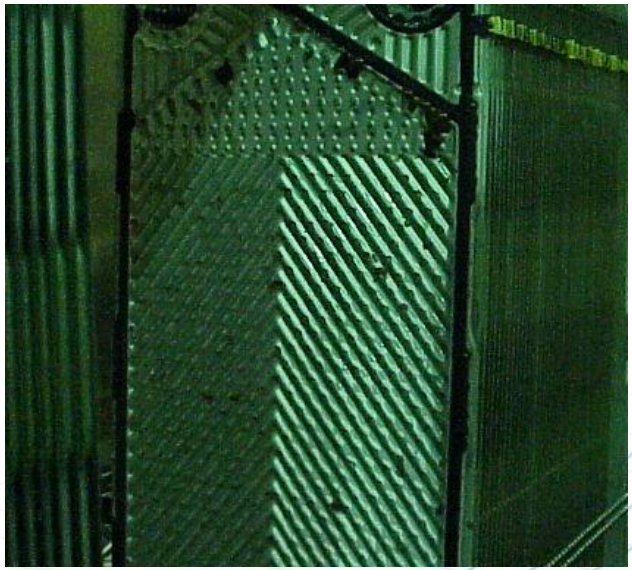
Thermal imaging shows areas with much lower temperature than bulk CIP solution

ECOLAB®

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





ECOLAB®

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 40-70ºC.
  - 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.

ECOLAB®

Minimising spores in product

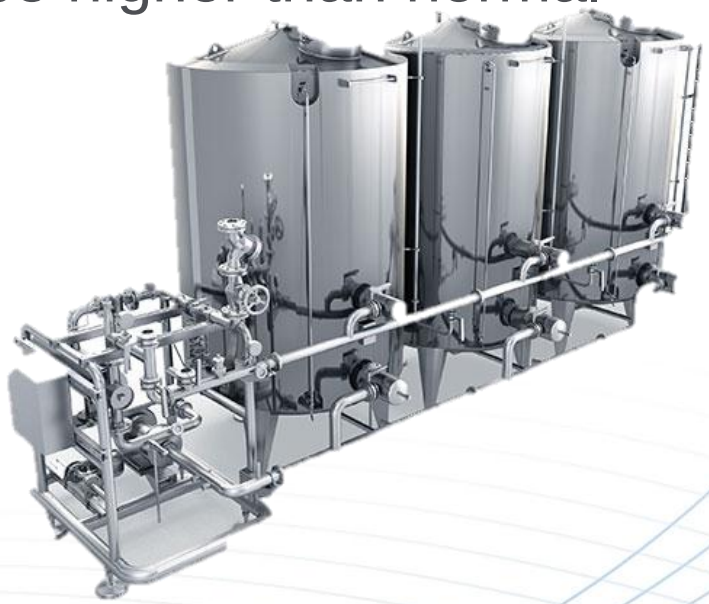
Fluid Milk Processing

- 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



ECOLAB®

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

ECOLAB®

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

ECOLAB®

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

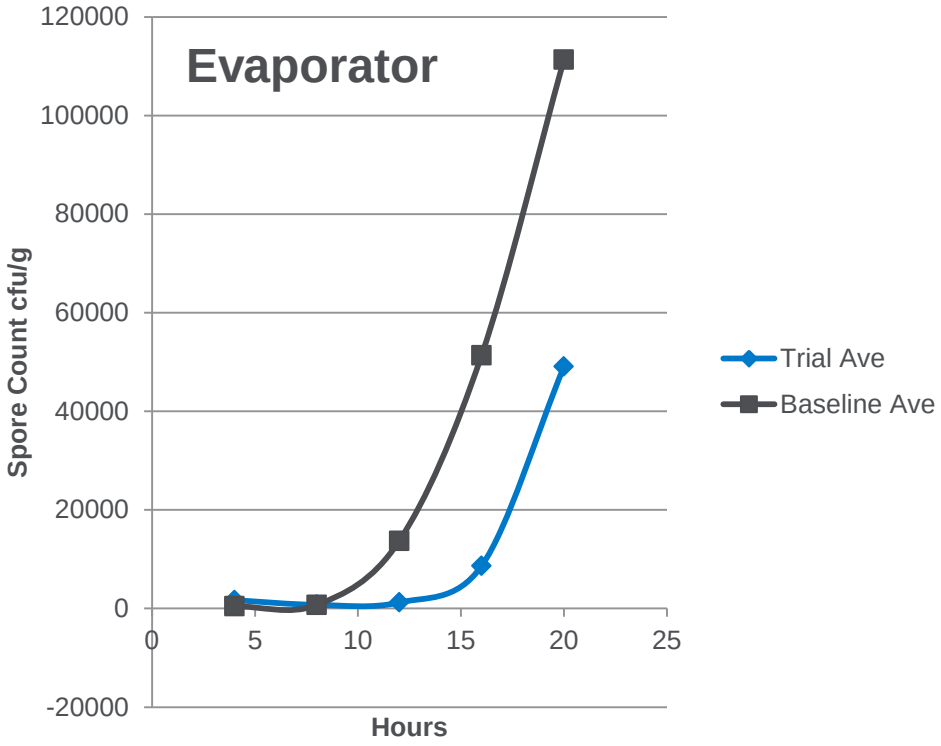
Limitations of oxidising pretreatment programs

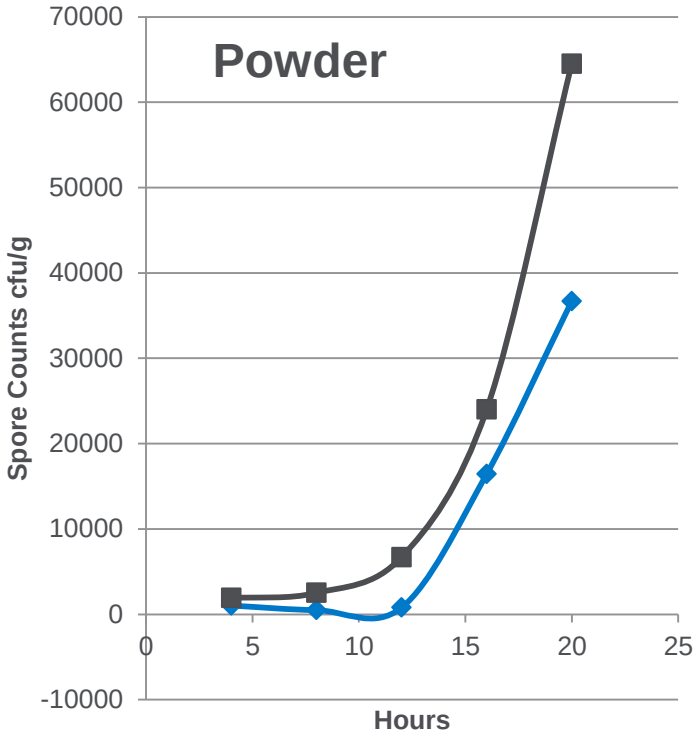
- Peracid pretreatment
  - Product recommendations need to be followed carefully
  - Not just any peracid product can be used
  - Temperature profiles are critical
  - Overall CIP times usually not increased
- Caustic product
  - Only certain products can be used
  - Do not use with EDTA based products
  - In some cases other additives may be required
- Sanitiser
  - Mixed peracids show superior activity against spores
  - Best when used at higher temperatures

ECOLAB®

Spore Reduction Trial

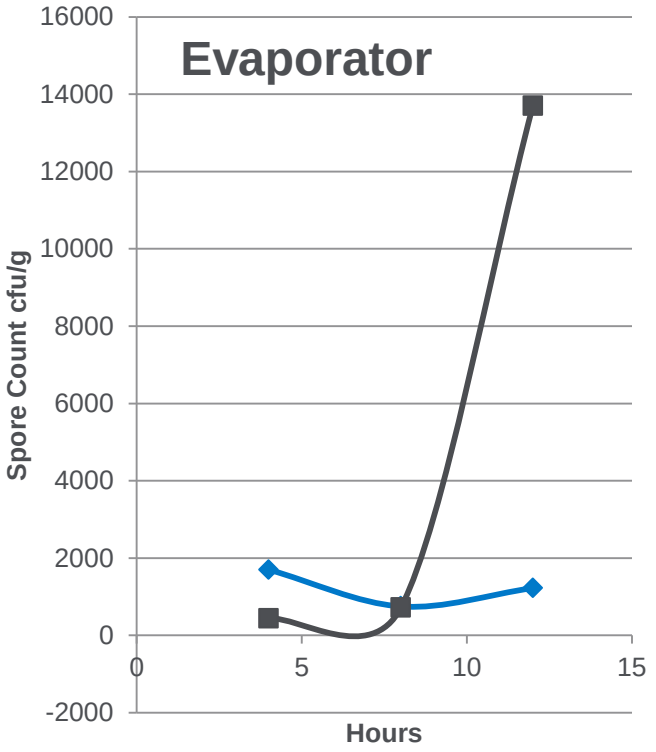
Results – Compendium Average

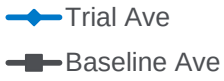


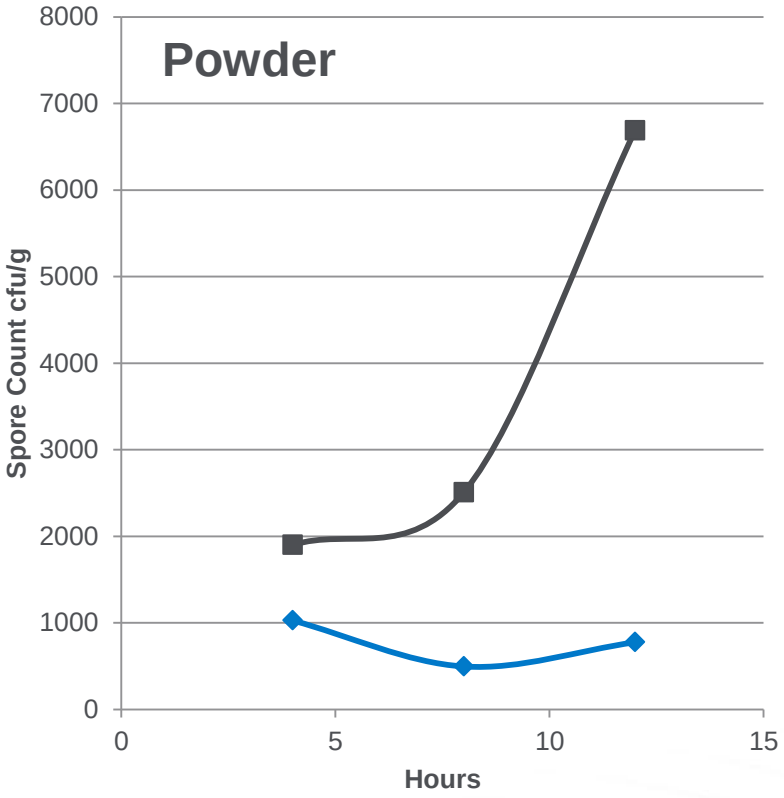


Spore Reduction Trial

Results – Compendium - 12 Hour Run







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

ECOLAB®

Interventions - Spores

Bacillus spp spore cocktail

Test Substance	Contact Time (min)	50° C Average Survivors (CFU/mL)	Average Log	Log Reduction
Mixed	2	2.2 x 104	4.3	1.8
Peracid	5	1.0 x 101	1	5.1
2600 ppm	10	<10	<1	>5.1
Peracid	2	<10	<1	>5.1
Cleaner	5	<10	<1	>5.1
5000 ppm	10	1.0 x101	1	5.1
Experimental	2	<10	<1	>5.1
Peracid	5	<10	<1	>5.1
2000 ppm	10	<10	<1	>5.1

Deep clean CIP

STEP	PRODUCT	CONCENTRATION	TEMP	TIME
Pre Rinse	water			
Pre-treatment	**Exelerate HS-I**	0.8 to 1.2% v/v	75-85C	10 to 20 mins
Alkali Wash **Note:** Alkaline cleaner is added to CIP solution as an **override**	**Glissen**	equivalent to 1.5 to 3% w/v free caustic	75-85C	20 to 40 mins
Post Alkali Rinse	water			
Acid Wash (if required**)**	**Super Stonekleen**	0.5%	50–60C	20 to30 mins
Post Acid Rinse (if required)	water			
Sanitise	**Oxysan ZS**	**0.1 to 0.15%**	Up to 80C	10 to 20 mins

ECOLAB®

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

Thank you

Spore Wars

Robert Ellis

Technical Support Specialist

5 Nov 2015

ECOLAB®