The Problem
Residual Spore-former Biofilm in Clean Processing Equipment

- Can cause a variety of production and quality issues
  - Out-of-spec spore count in powders
  - Shortened / inconsistent shelf-life
  - Acid accumulation and reduced pH
  - Non-sterility defects in UHT processed milk
  - Reduced membrane production flux and output
Today’s discussion

- Spore-forming bacteria and biofilms
- Presence and accumulation in dairy processing equipment
- New strategies for control and elimination
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
Review of literature
Surveys of spore-forming bacteria in dairy

**Most common aerobic thermophilic/thermoduric sporeformers in dairy processing & products**

- *Bacillus licheniformis*
- *Bacillus coagulans*
- *Bacillus cereus*
- *Bacillus pumilus*
- *Geobacillus* sp. (mainly *G. stearothermophilus*)
- *Anoxybacillus* sp.
- High heat resistant spores of mesophilic *Bacillus* sp. (e.g., *B. sporothermodurans*)
Bacteria Optimum Growth Temperature

- **Psychrophiles**
- **Mesophiles**
- **Thermophiles**

Temperature (°C)

Growth Rate

Thermal dairy processes
Where do the spores come from?

- **Soil** is a major reservoir of spore formers
- **Bedding materials** 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** (Anand, S., 2011, NCCIA)
Characteristics of bacterial spores

- Spores stick very tightly to steel and elastomers
- Once attached, they are very difficult to remove
- Exposure to caustic makes them even more so
Biofilm communities of sporeformers
Free-swimming cells alight on a surface and attach.

New genes are expressed to synthesize matrix polymers.

Cells coordinate by exchanging signaling molecules.
Bacteria reproduce and form microcolonies

Chemical gradients are established
Variety of environmental niches promotes coexistence of diverse species.

Biofilm affords protection from antimicrobial agents.
Critical Areas of Operations

Significant Sources of BIOFLIMS and THERMOPHILIC ENDOSPORES

MILK PROCESSING
- Balance tank
- Bactofugation or microfiltration (recommended)
- Separation/standardization
- HTST (or plate heat exchanger or pasteurizer)
- Homogenizer
- Product lines and pumps
- Pasteurized milk storage tanks

SWEETENED CONDENSED MILK PROCESSING
- Balance tank
- Homogenizer
- Cooling plate heat exchanger
- Slurry tank
- Mixing & crystallization tank

ULTRAFILTRATION
- Bactofugation
- UF membrane system

MILK EVAPORATION
- Balance tank
- Preheaters (plate or tube)
- Pasteurizer
- Distribution plate
- Calandria
- Vapor separator
- Concentrate pump
Spore populations in dairy powder production

- **Preheater Spore Contribution**
  - Thermophilic spores cfu/g vs. Hours
  - Preheater vs. Raw Silo

- **Separator Spore Contribution**
  - Thermophilic spores cfu/g vs. Hours
  - Whole vs. Baseline Skim

- **Evap Spore Contribution**
  - Thermophilic spores cfu/g vs. Hours
  - Skim Feed vs. Condensed

Ecolab Internal Data
Root Causes of High Spore Levels

- Plant issues
  - Dead legs
  - CIP design

- Residual soil - anywhere

- Equipment
  - De-sludge issues
  - Heat exchange surfaces

- Residual biofilm/spores
  - Gasket joints
  - Right angles
  - Cracked gaskets
  - Valves
Importance of gaskets
Spore-former biofilm control best practices

PM
- Replace old gaskets
- Inspect and maintain valve seats
- Correct any equipment issues

Optimize
- Verify CIP programs are running correctly
- Modify programming as required for an Intervention CIP program to be implemented

Execute
- Identify all units of operation in scope
- Design Enhanced CIP Program
- Baseline data and Implementation
Clean-In-Place and Biofilm Control

Root cause of many dairy quality problems is residual biofilm

- Traditional CIP programs leave small amounts of biofilm and spores in otherwise ‘clean’ equipment
  - Growth during production and subsequent survival of CIP is a selection pressure leading to the most fit organisms thriving

- Processing equipment inoculated at start up, exponential growth begins at 8-10 hours

- Enhanced CIP removes/inactivates biofilm and spores

- Bacterial population must start from scratch, exponential growth begins at 16-20 hours
Approaches to Clean-In-Place

**Conventional**
- Pre-rinse
- Caustic Wash
- Water Rinse
- Acid Wash
- Hot Water
- Sanitizer

**Enhanced**
- Pre-rinse
- Pre-treatment
- Built Caustic Wash
- Water Rinse
- Acid Wash
- Sanitizer
CHEMISTRY OF CLEANING

SOIL TYPE
- Fats & Oils
- Carbohydrates
- Proteins
- Minerals

EFFECT
- Dissolve
- Liquefy
- Hydrolyze
- Disperse
- Emulsify
- Rinse

CHEMISTRY
- Alkaline
- Acid
- Oxidizer
- Enzyme
- Solvent
- Surfactant
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Solution with pH >7
Dissolves or Disperses Organic Soils
Common Sources:
- Caustic soda NaOH
- Caustic potash KOH
- Silicates
Alkaline ingredients disperse or dissolve organic soil particles by charge repulsion.
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- Chemical that liberates oxygen to hydrolyze (break down) larger molecules → proteins
- Used as a booster in alkaline detergents: hypochlorite or peroxide
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- Compound that reduces surface tension (generally also called a detergent)
- Used to emulsify organic liquid soils (fats, oils, carbohydrates) in water
- Reduces temperature required to liquefy fats & oils
Improved cleaning with formulated caustic

Bremer et al. (2006) Intl J Food Microbiol
Adding mechanical force to chemistry

**Step 1**
- **Pre-treatment**: Circulates through the system prior to alkaline wash. Active ingredients penetrate biofilm.

**Step 2**
- **Over-riding**: Pre-treatment and alkaline detergent interact, triggering a reaction that ruptures the biofilm.

**Step 3**
- **Alkaline detergent addition**: Immediately following Step 1, an alkaline detergent is circulated through the system; the rise in pH and temperature triggers Step 3.

**Step 4**
- **Soil removal**: Fragmented biofilm is removed by the cleaning solution and planktonic spores are killed by the chemical sanitizer.
Laboratory Investigation: Biofilm Removal
Laboratory Investigation: Biofilm Removal

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SEM of SS Coupon

Control, SS Coupon, No Biofilm
SEM of Biofilm

Demonstration of biofilm layers on stainless steel control coupons

*Control, untreated SS disk imaged after growing biofilm (no chemical treatment)*

*Note biofilm layering, visible near edge of coupon*
SEM – Pre-treatment step

Control – condition before caustic step

Note smooth EPS coating with potential Bacillus spores present

Condition after pretreatment, before caustic step

Note exposed biofilm layers for more efficient further attack
SEM – Caustic step

After exposure to NaOH

Note uneven attack of biofilm structure

Conventional CIP

After pre-treatment and built caustic

Note even removal of biofilm structure down to base metal surface

Enhanced CIP
SEM – Acid step

After pre-treatment, built caustic, & built acid

Note reduced presence of microbial populations and better base metal surface resolution

After exposure to NaOH + HNO3

Note populations of microorganisms remaining on surface
SEM – Hot Water or Sanitizer step

**Conventional CIP**

Final Result from NaOH + HNO₃ + Hot Water

*Note very little additional removal of presumed spores vs. previous acid exposure, and significant remaining presence on surface*

**Enhanced CIP**

Final result from Enhanced CIP

*Note nearly complete removal of organic material observed after sanitizer step*
FIELD OBSERVATIONS
Cleaning observations

Evaporator

CONVENTIONAL CIP

ENHANCED CIP

Evaporator

CONVENTIONAL CIP

ENHANCED CIP
Spore control through Enhanced CIP

- Spore control program cleared up persistent infection
- Returned to low counts after ‘events’
Spore control in separators

Separator Sporeformer Growth

- Spore count cfu/ml
- Hours

Lines:
- Trial Skim
- Whole
- Skim

Graph showing the growth of sporeformers in separators.
Spore control for extended production

LARGE NONFAT DRY MILK PRODUCER

GOAL: <1000 cfu/g Thermophilic Spores in NFDM to meet export & infant formula demand

1. Powder out of target range within first few hours
2. Baseline showed high variability in day to day quality
3. Consistently <1000 cfu/g in first 12 hours
Controlling quality means controlling biofilms of spore-formers

1. Good practices on the farm & distribution
2. Correct sanitary design of equipment
3. Diligence in preventative maintenance
4. Enhanced CIP
Thank You

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