NEW STRATEGIES FOR BIOFILM ELIMINATION

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May 2, 2017
3-A Equipment & Beyond

Everywhere It Matters.™

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

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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

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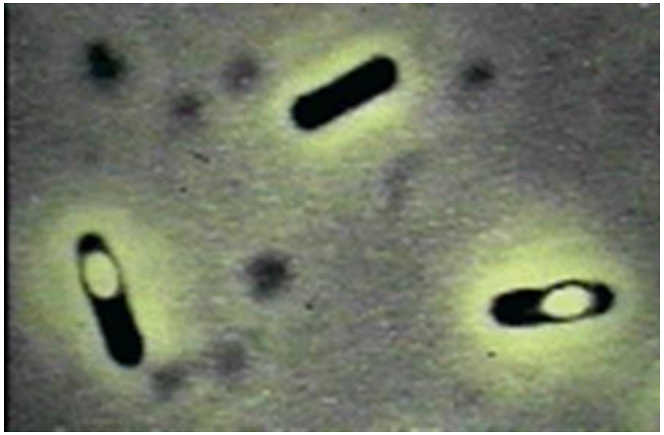
Review of literature

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*)



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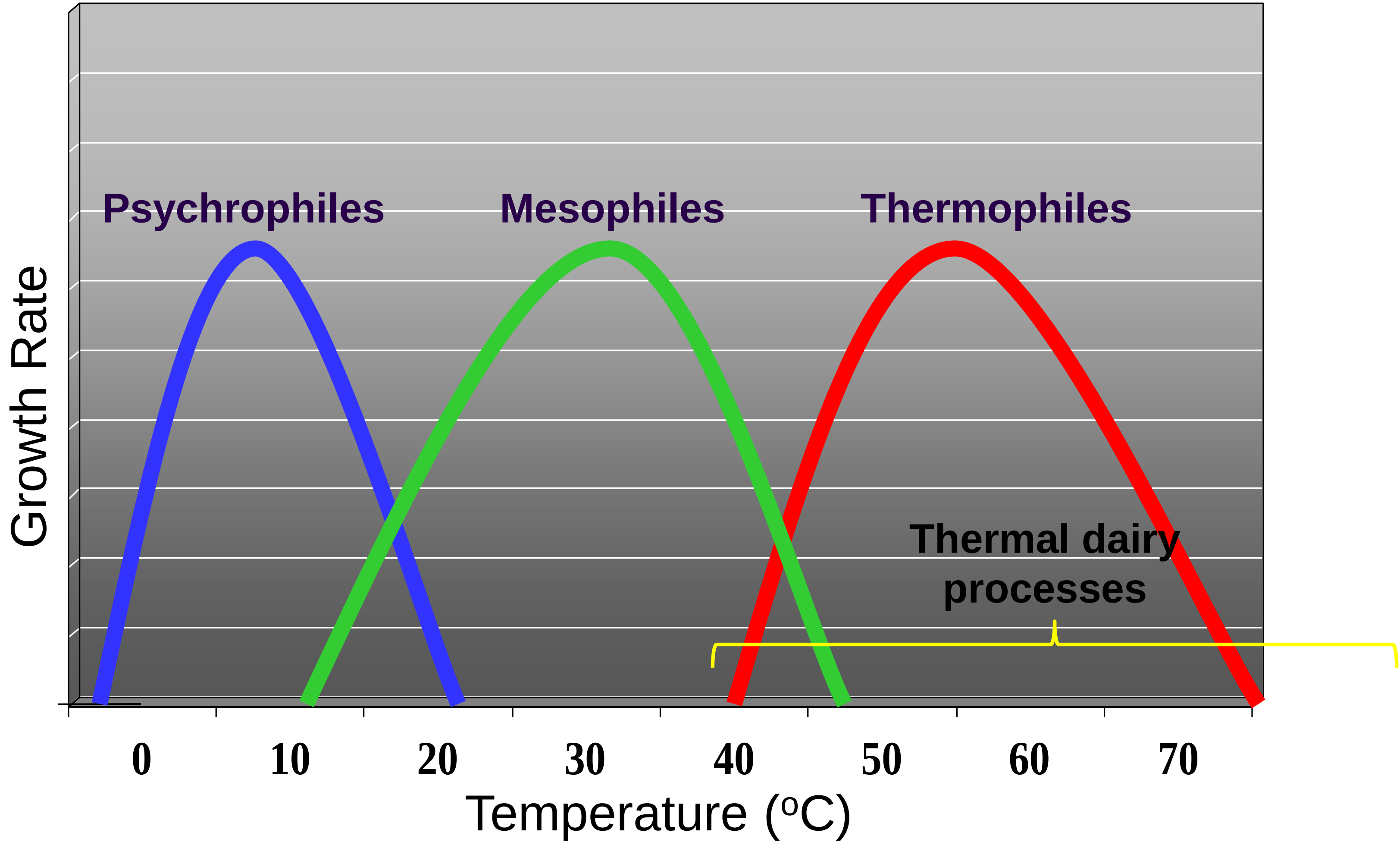
Bacteria Optimum Growth Temperature

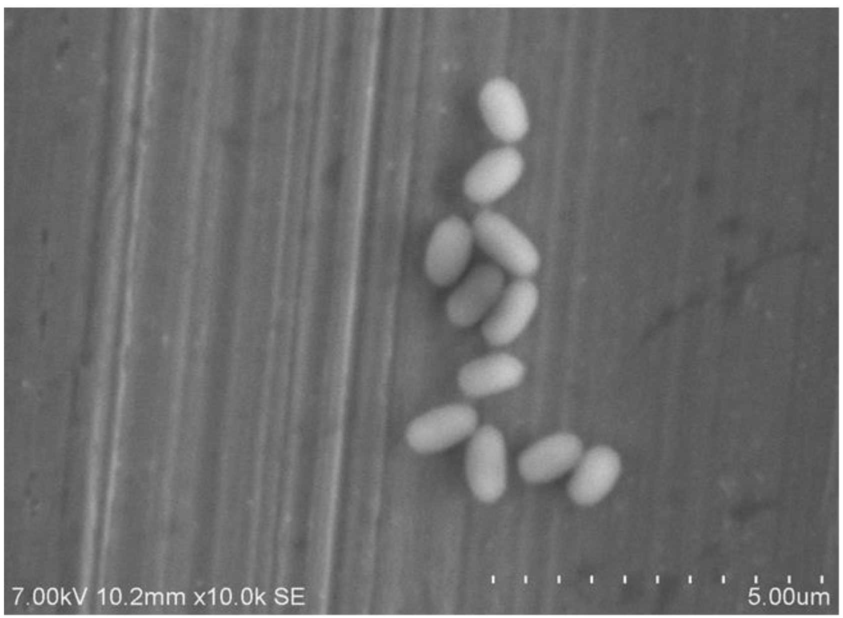


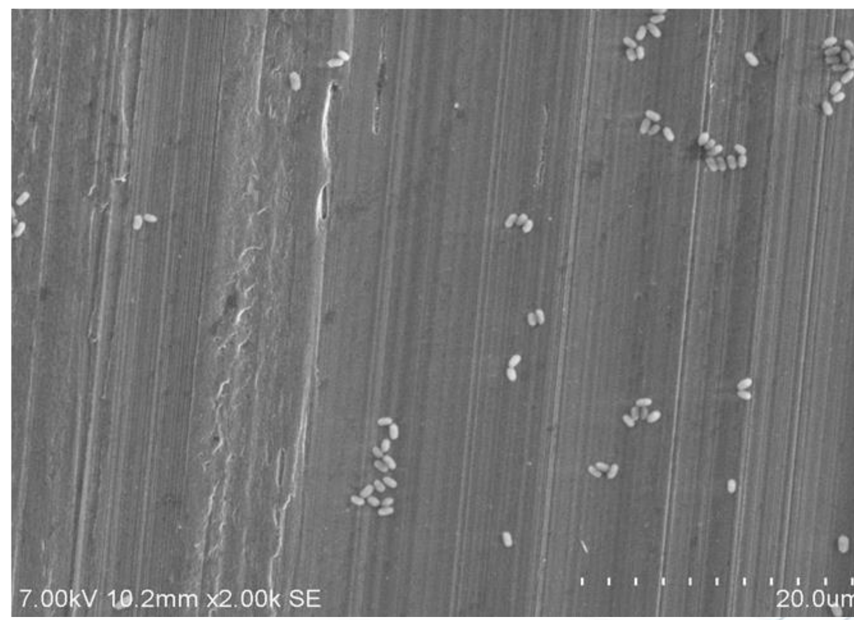
Where do the spores come from?

- 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





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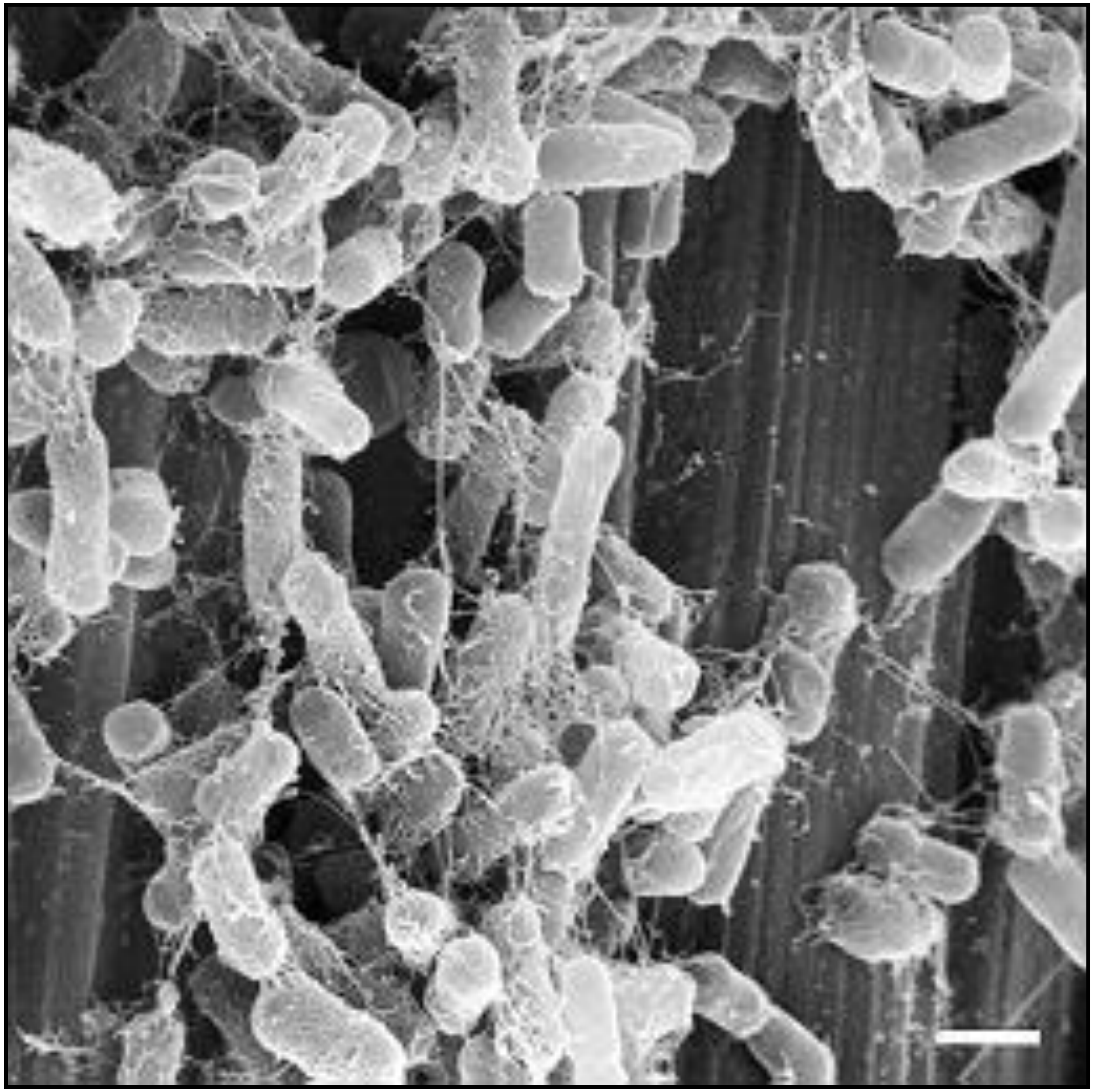
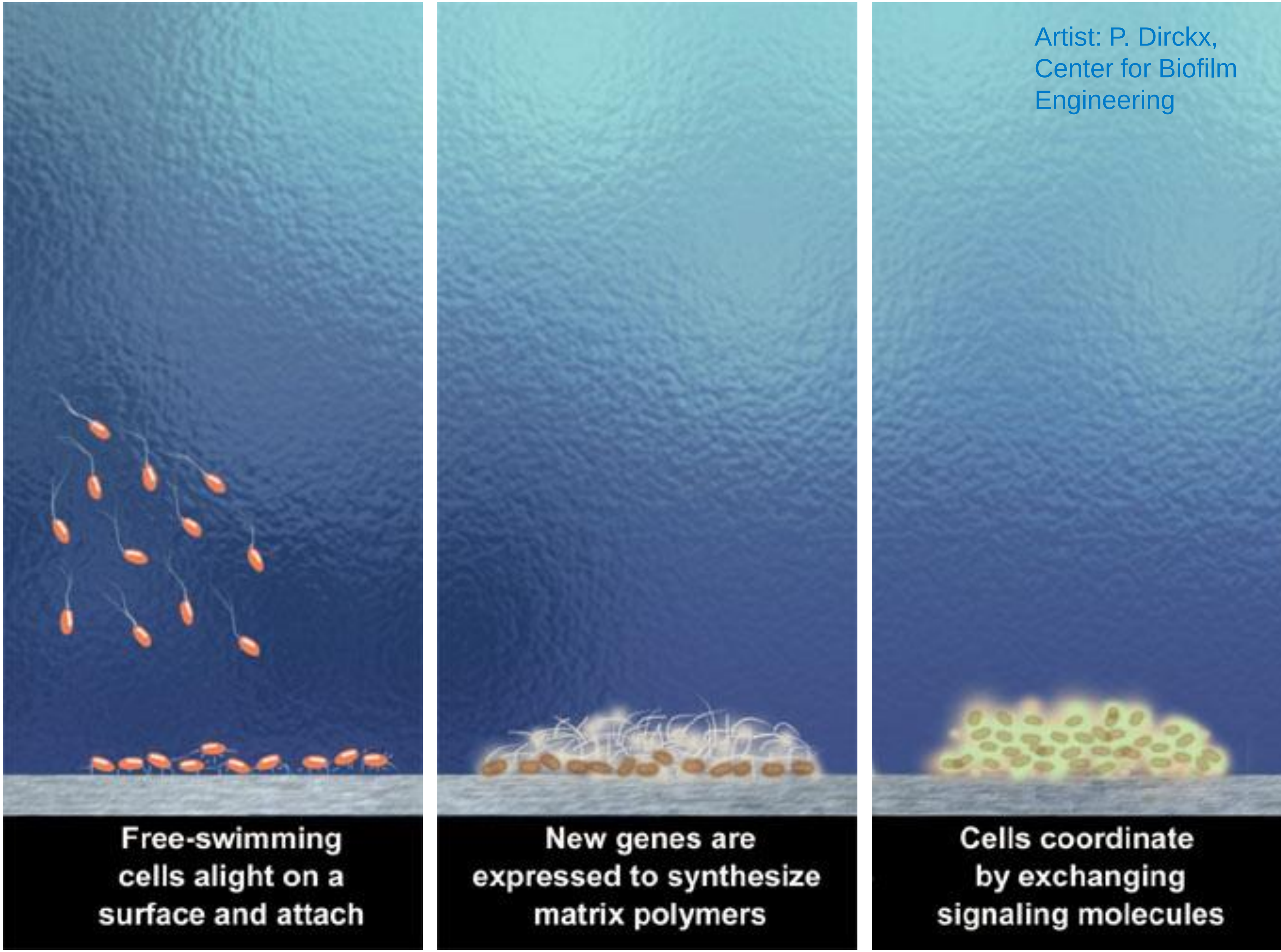
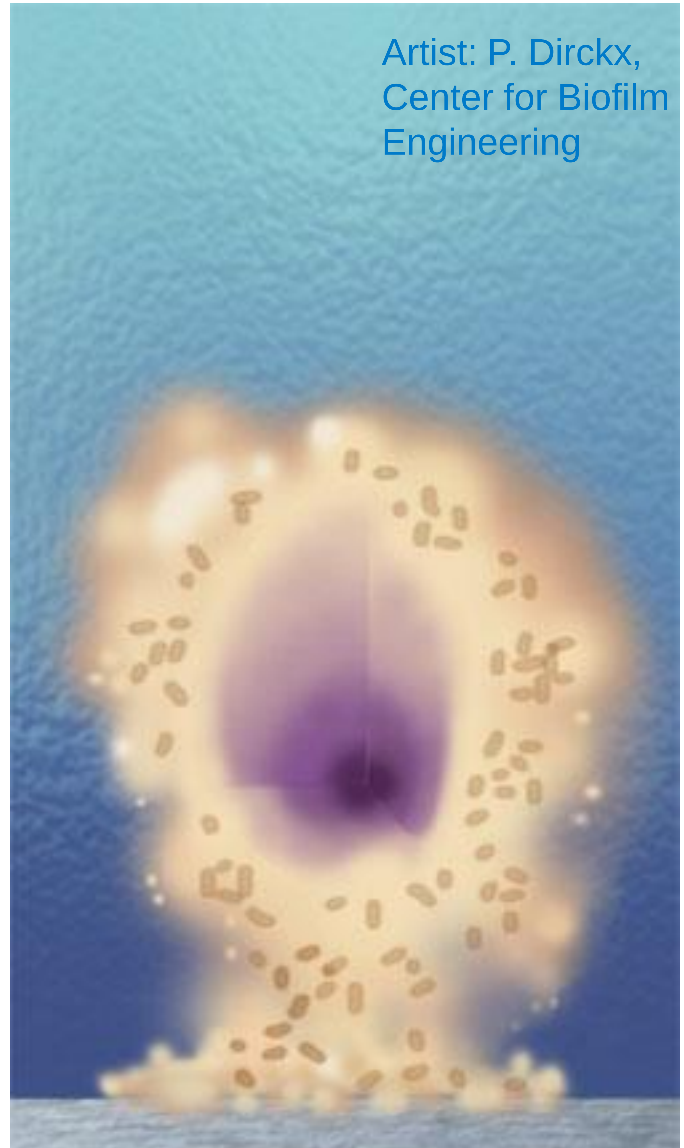
Biofilm communities of sporeformers

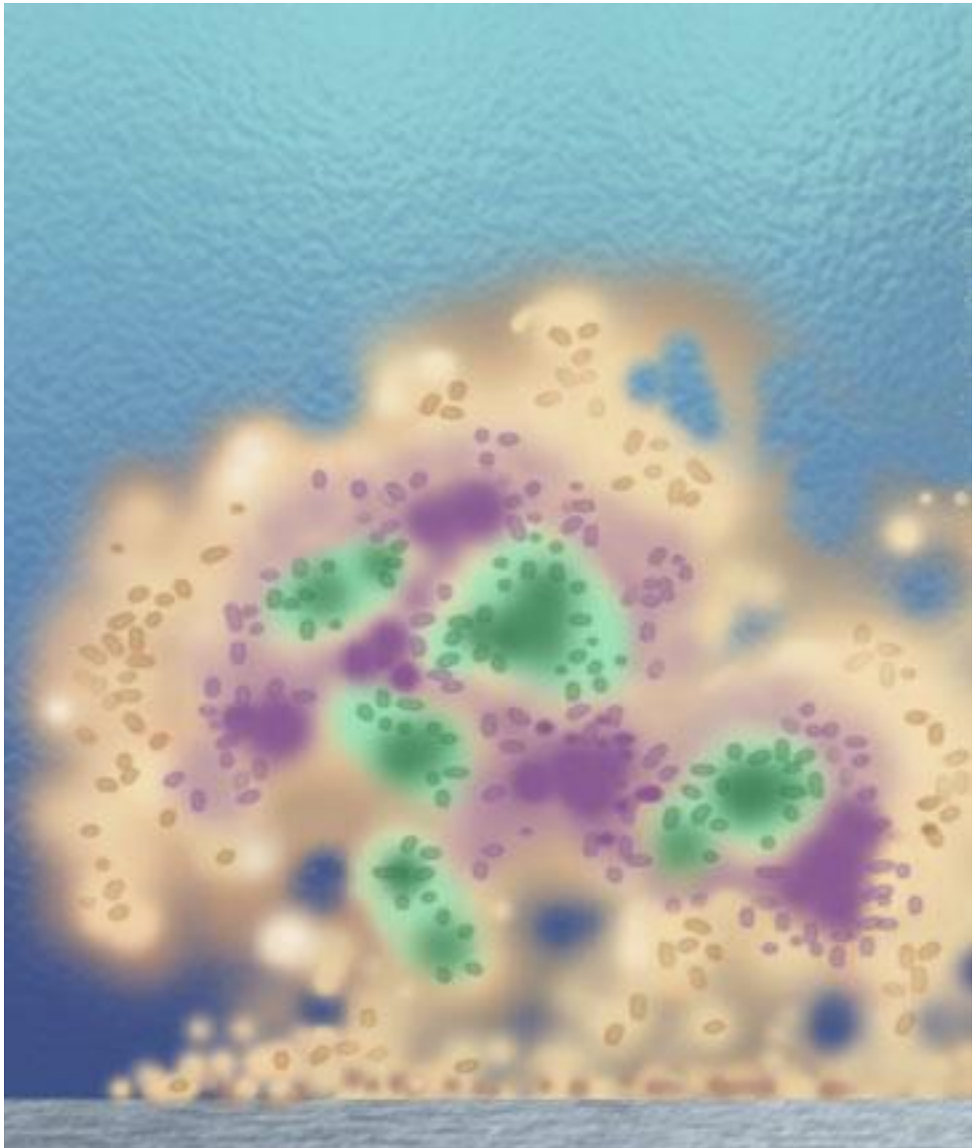



Artist: P. Dirckx,
Center for Biofilm
Engineering
Free-swimming
cells alight on a
surface and attach
New genes are
expressed to synthesize
matrix polymers
Cells coordinate
by exchanging
signaling molecules

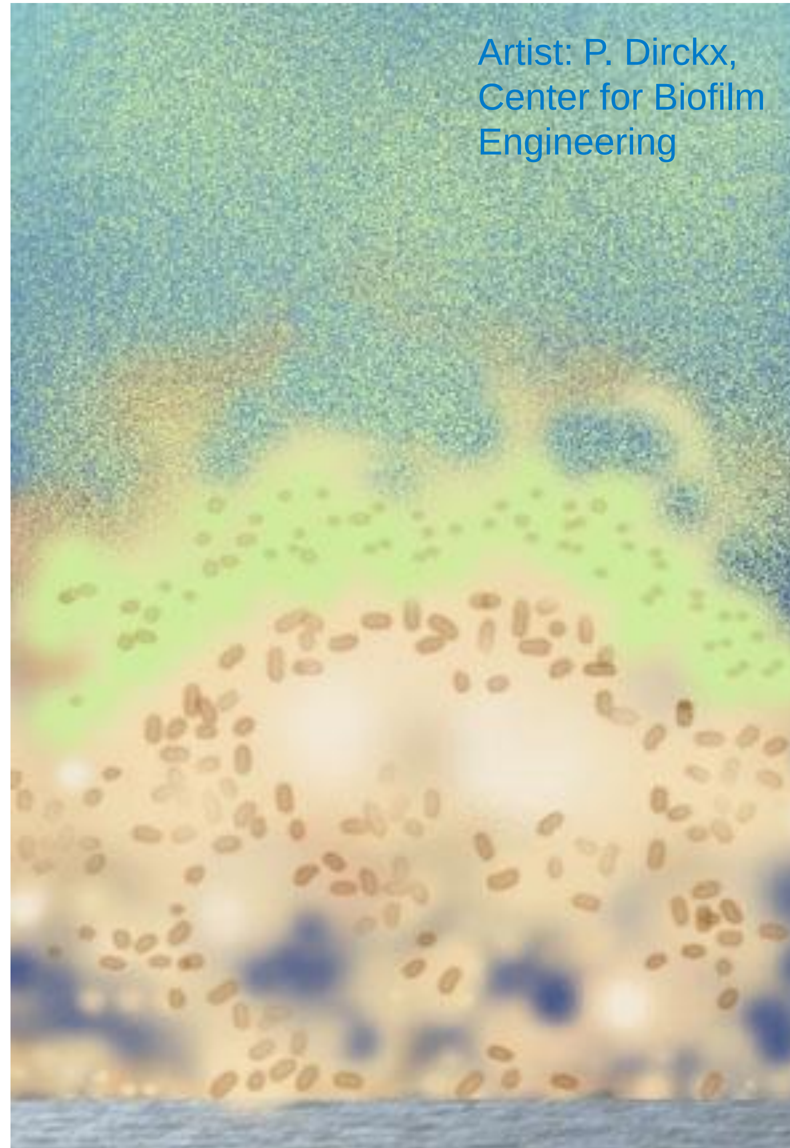
Bacteria reproduce and form microcolonies



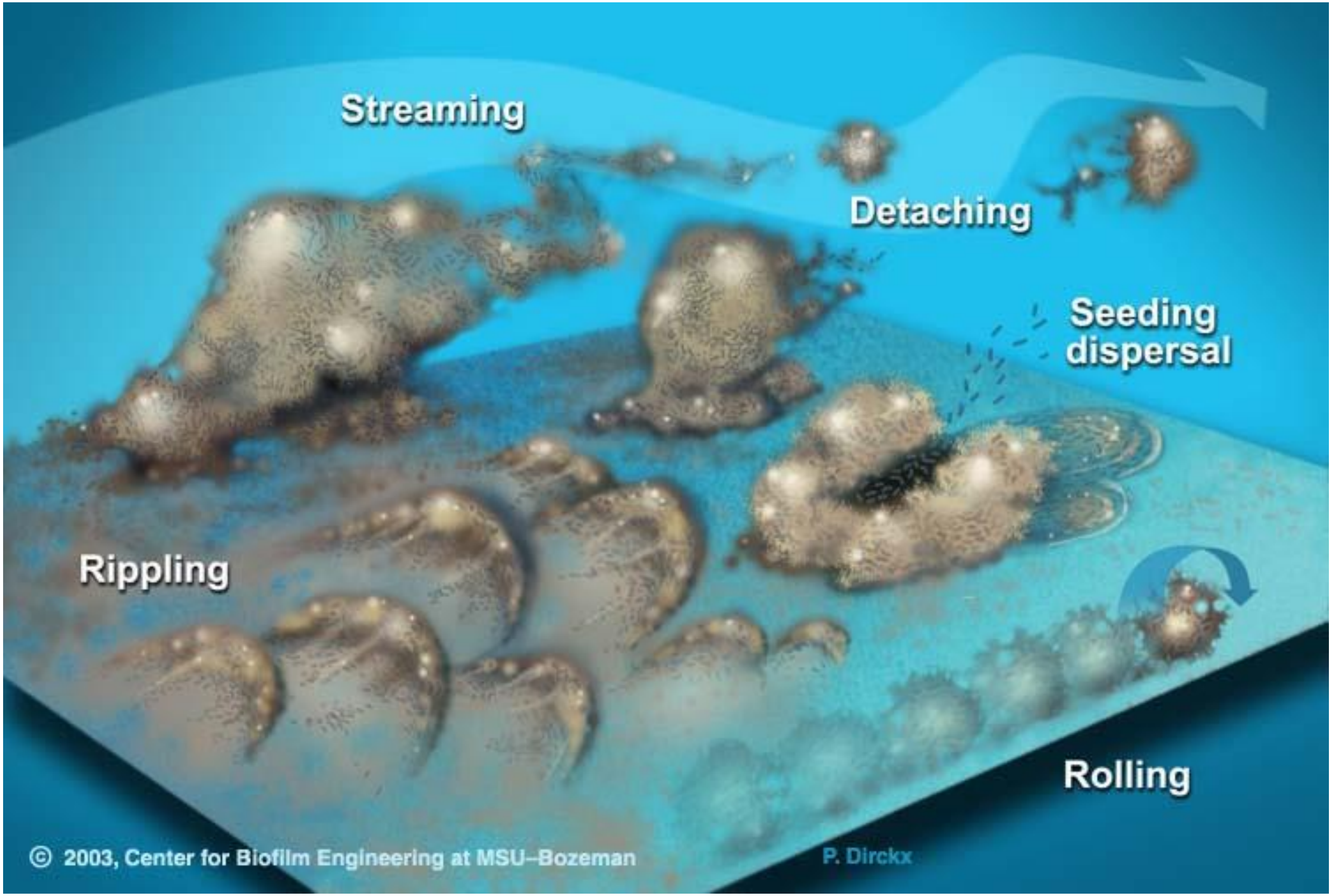
Chemical gradients are established



Variety of environmental niches promotes coexistence of diverse species



Biofilm affords protection from antimicrobial agents


Streaming
Detaching
Seeding
dispersal
Rippling
Rolling
© 2003, Center for Biofilm Engineering at MSU–Bozeman
P. Dirckx

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

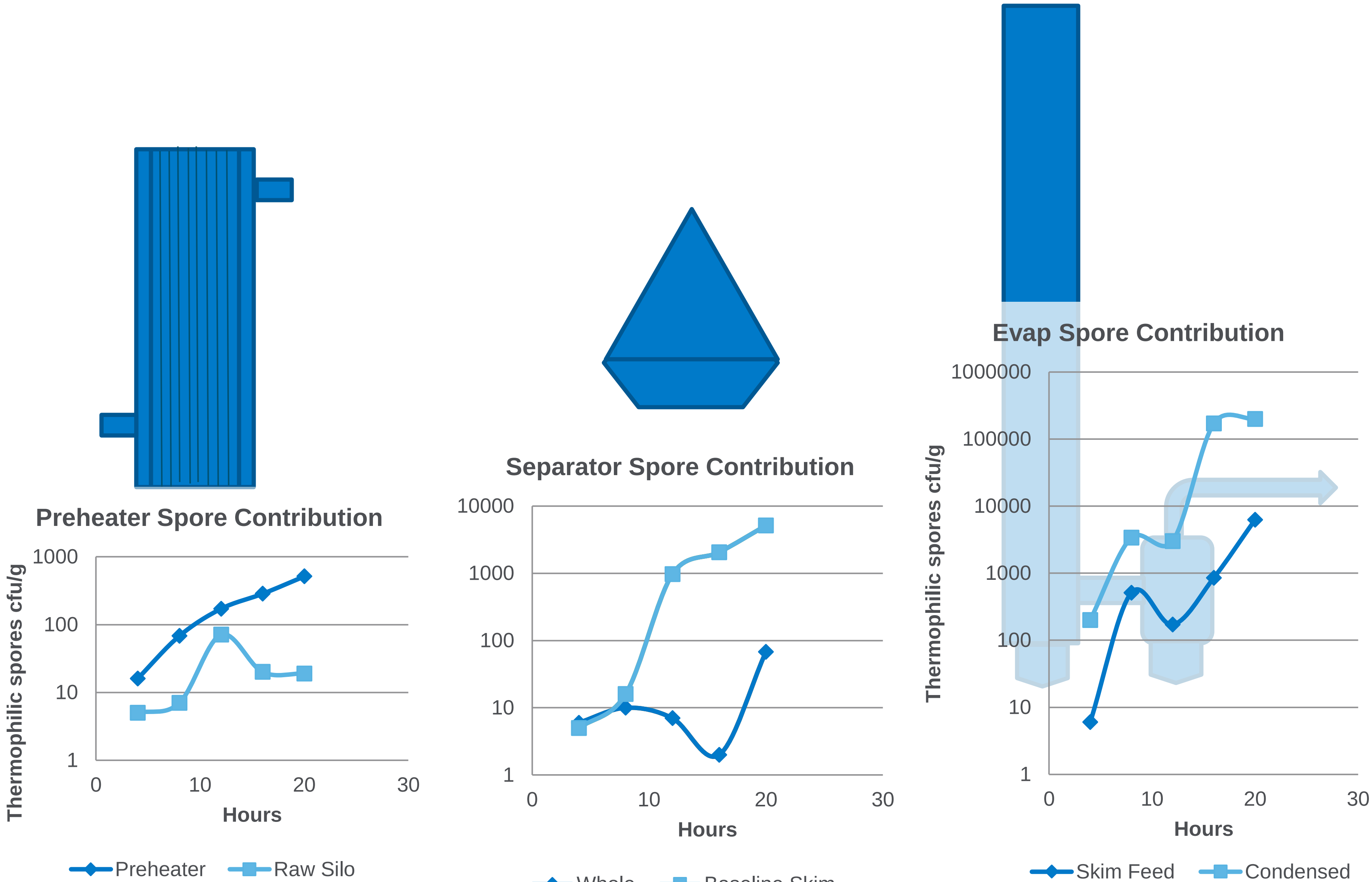
ULTRAFILTRATION

- 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



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

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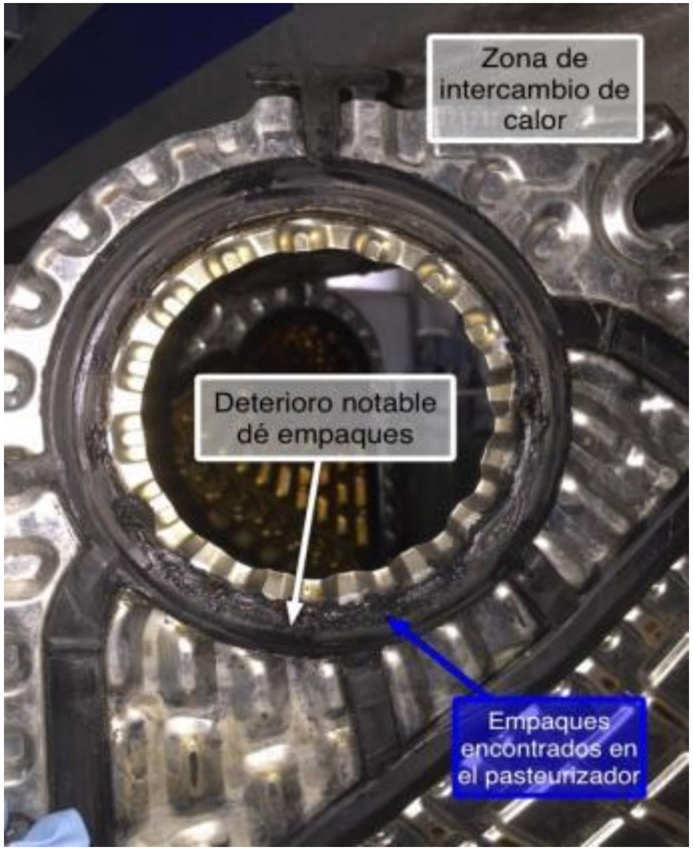
Importance of gaskets



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

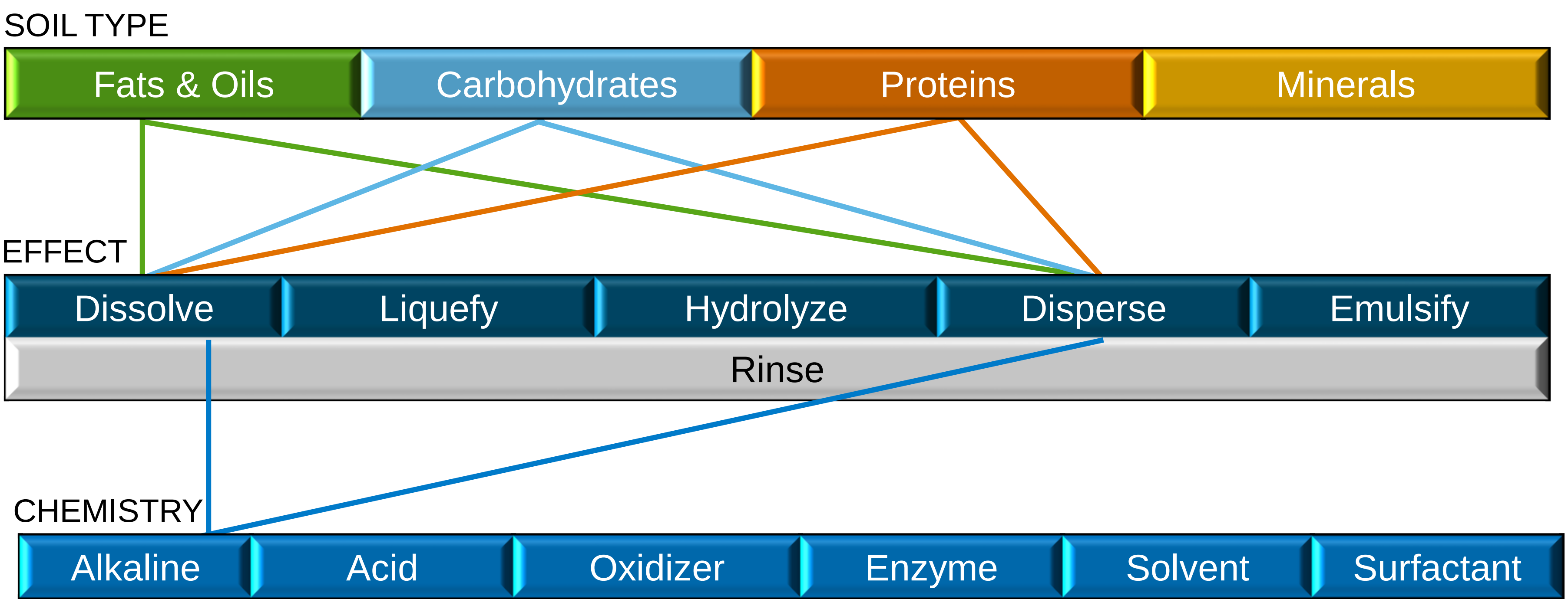
EFFECT

Dissolve	Liquefy	Hydrolyze	Disperse	Emulsify
Rinse				

CHEMISTRY

Alkaline	Acid	Oxidizer	Enzyme	Solvent	Surfactant

CHEMISTRY OF CLEANING



- Solution with pH >7
- Dissolves or Disperses Organic Soils
- Common Sources:
  - Caustic soda NaOH
  - Caustic potash KOH
  - Silicates

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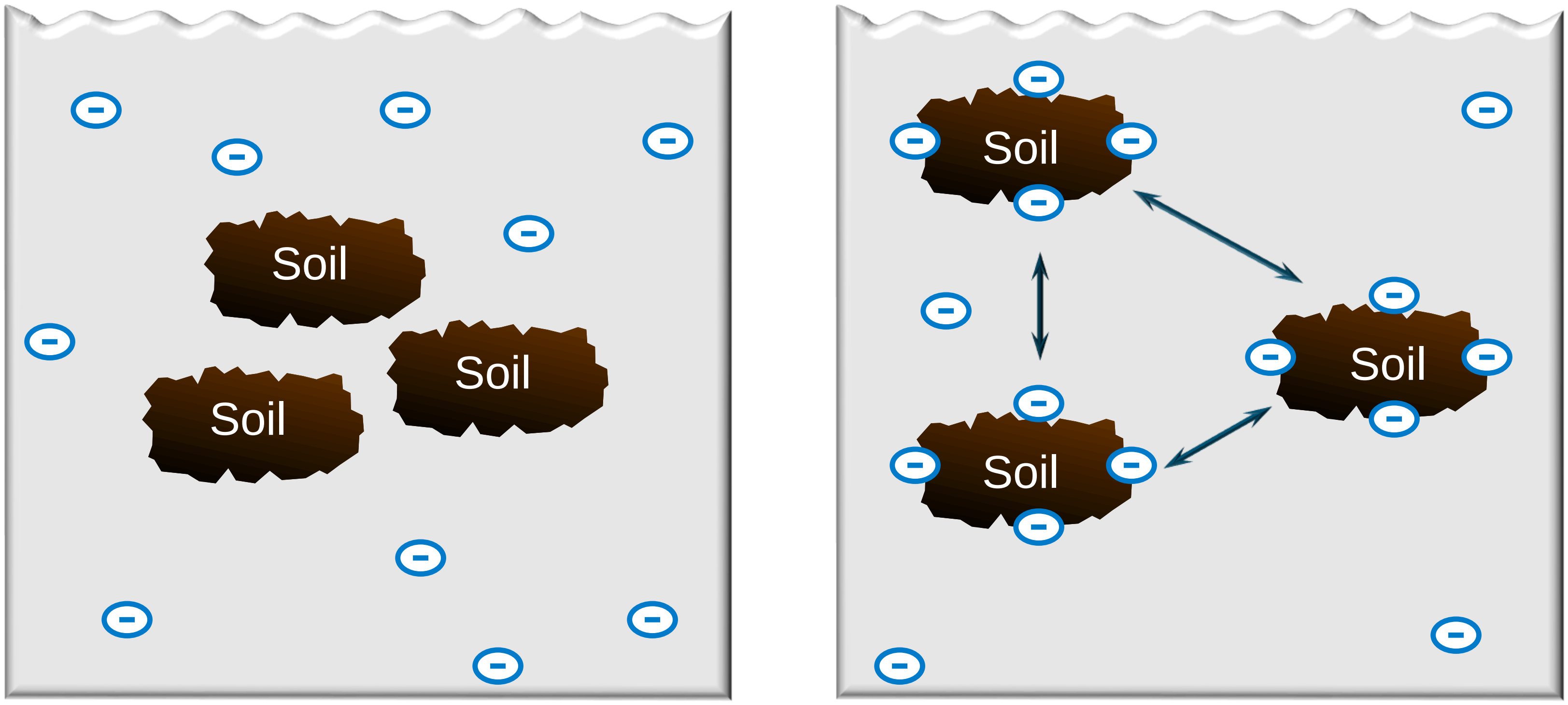
CHEMISTRY OF CLEANING

Alkaline

Alkaline ingredients disperse or dissolve organic soil particles by charge repulsion



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CHEMISTRY

Alkaline | Acid | Oxidizer | Enzyme | Solvent | Surfactant

- 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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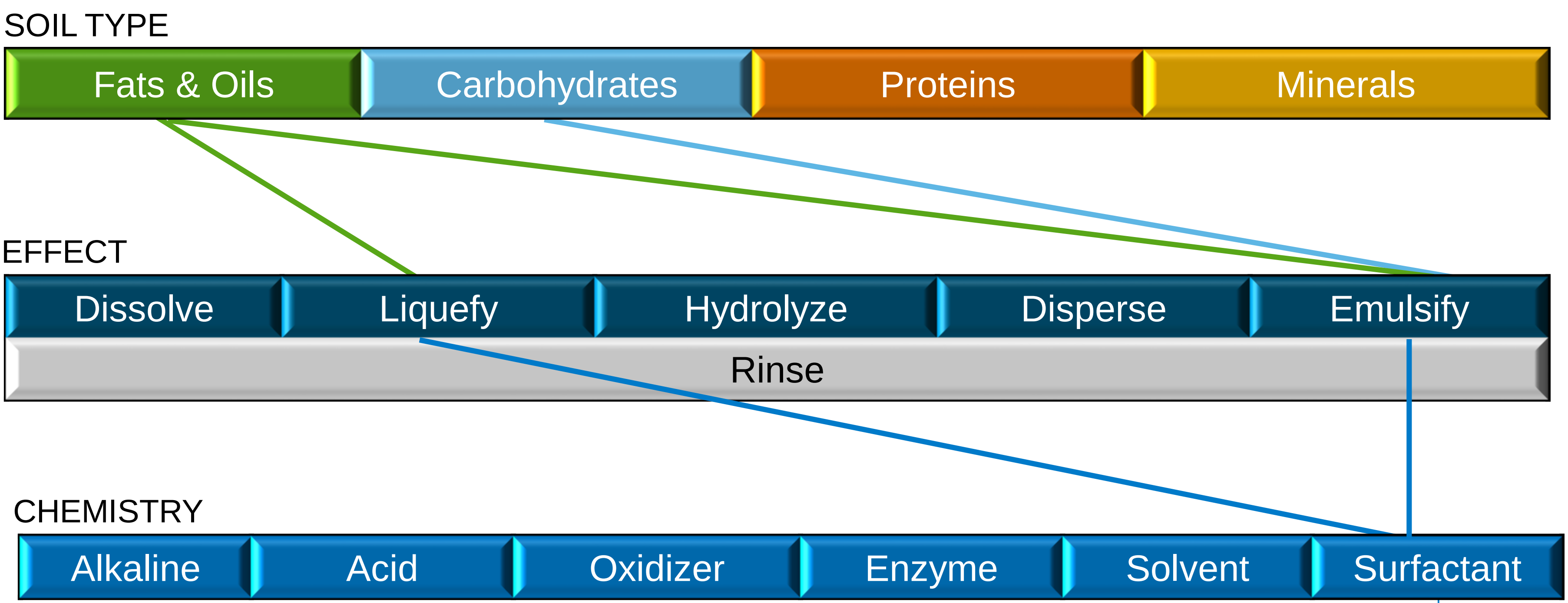
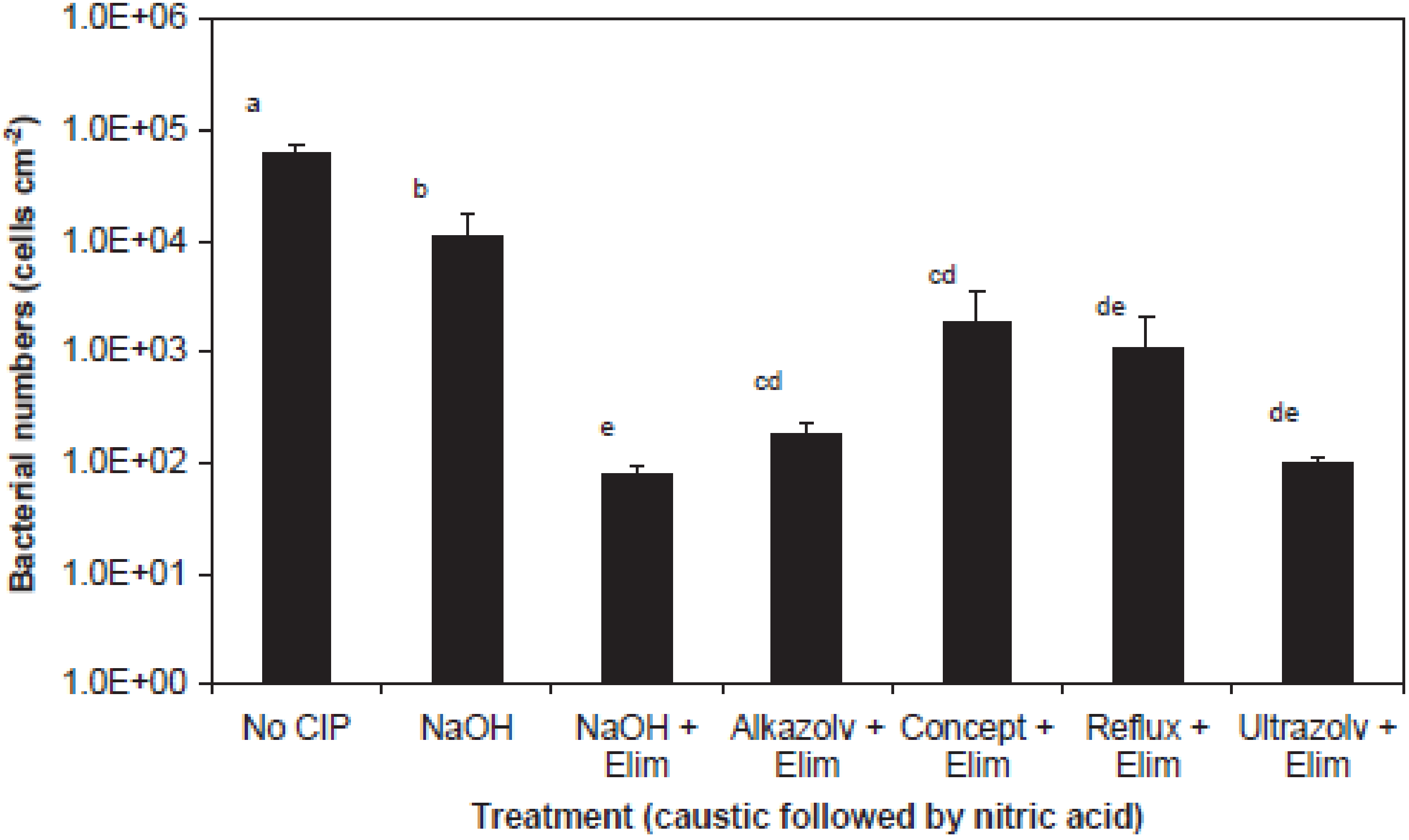
CHEMISTRY OF CLEANING



- 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

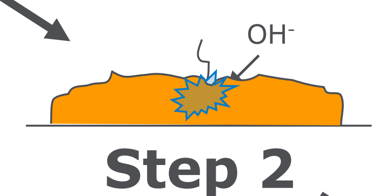
Improved cleaning with formulated caustic



Bremer et al. (2006) Intl J Food Microbiol

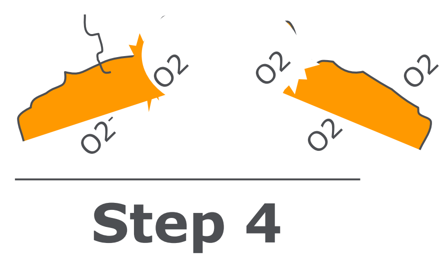
Adding mechanical force to chemistry

- **Pre-treatment** circulates through system prior to alkaline wash. Active ingredients penetrate biofilm.



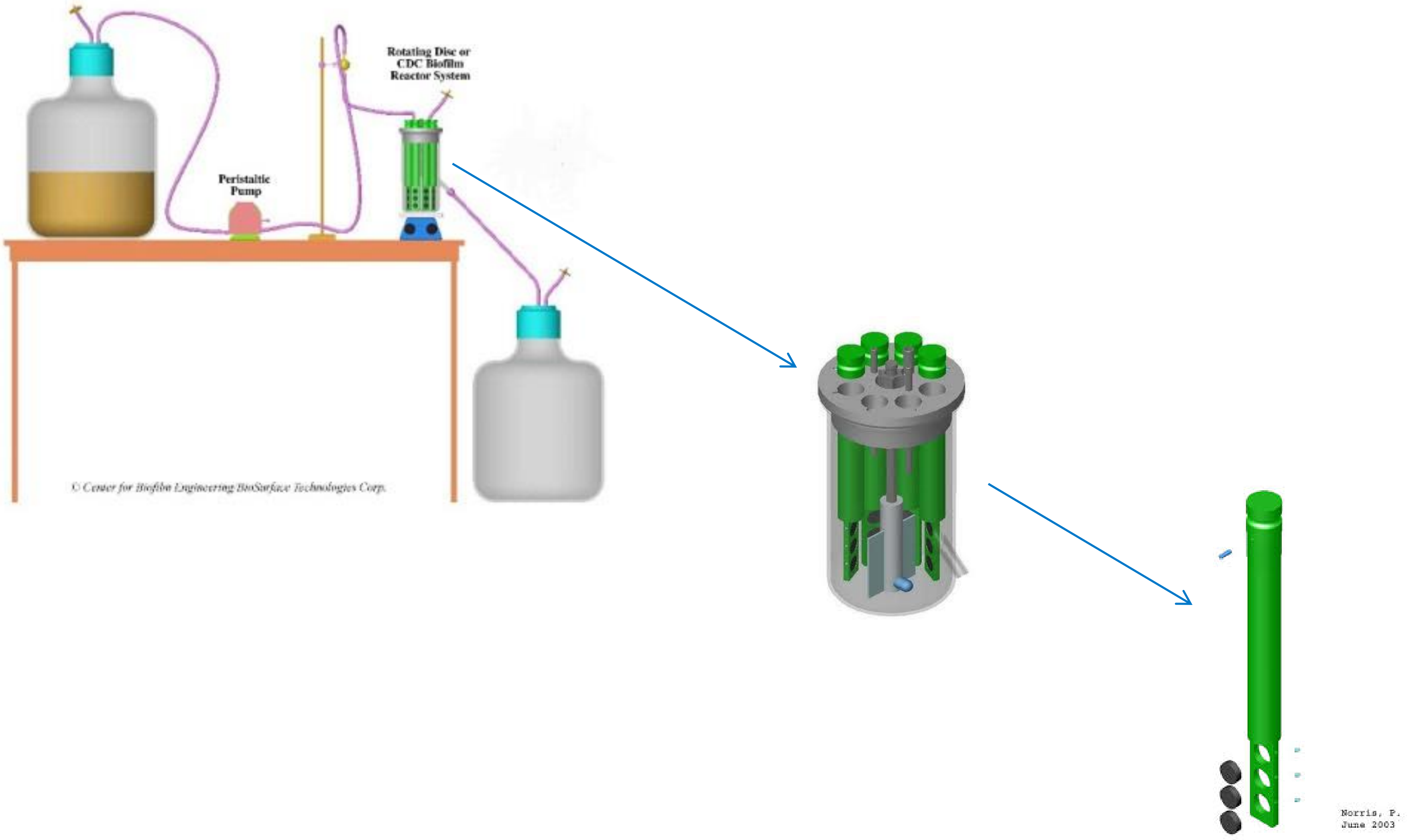
- **Alkaline detergent addition:** Immediately following Step 1, an alkaline detergent is circulated through the system; the rise in pH and temperature triggers Step 3.

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



- **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

Conventional	Enhanced
Pre-rinse Caustic Wash Water Rinse Acid Wash Hot Water	Pre-rinse Pre-treatment Built Caustic Wash Water Rinse Acid Wash Sanitizer

Laboratory Investigation: Biofilm Removal

Conventional

Pre-rinse
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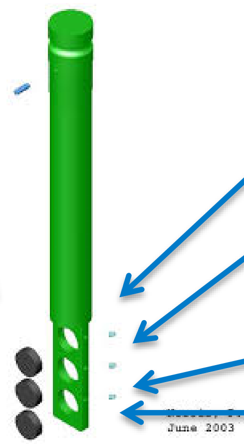
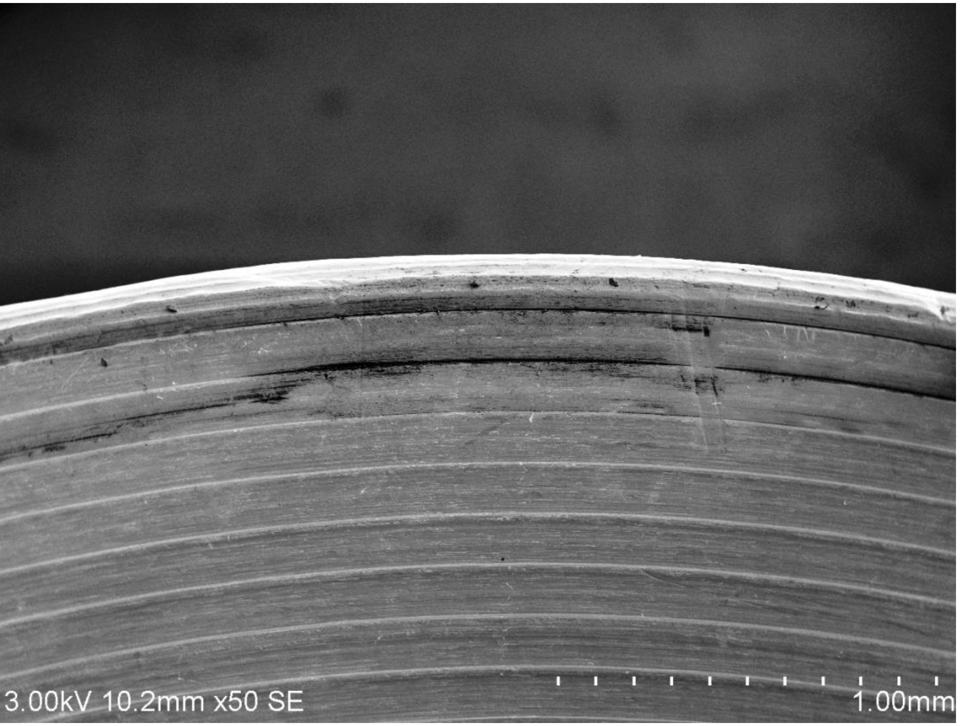


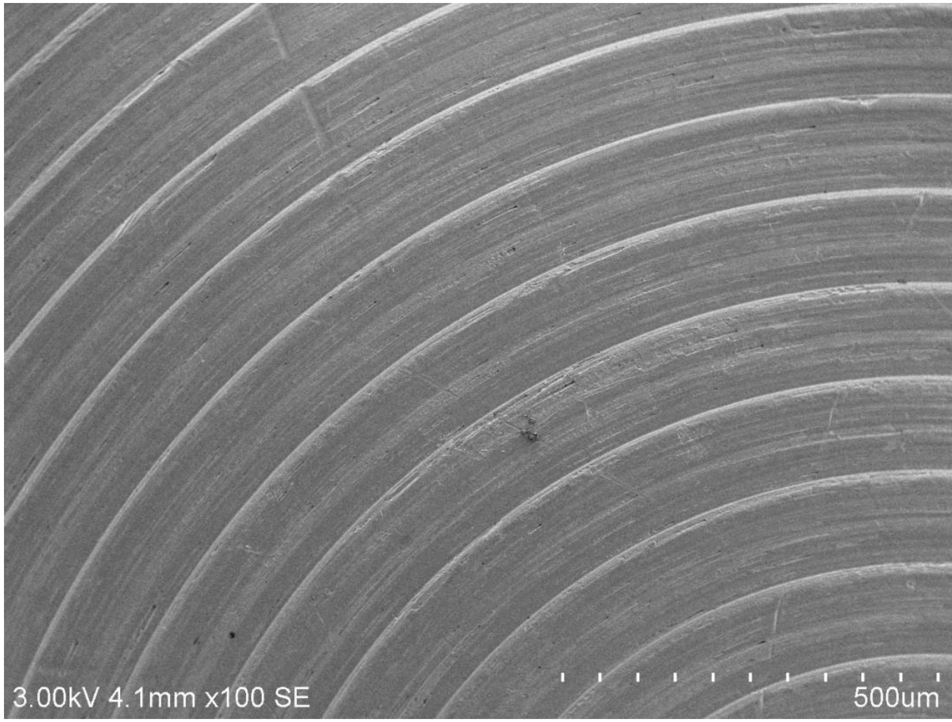
Enhanced

Pre-rinse
Pre-treatment
Built Caustic Wash
Water Rinse
Acid Wash
Sanitizer

SEM of SS Coupon

Control, SS Coupon, No Biofilm

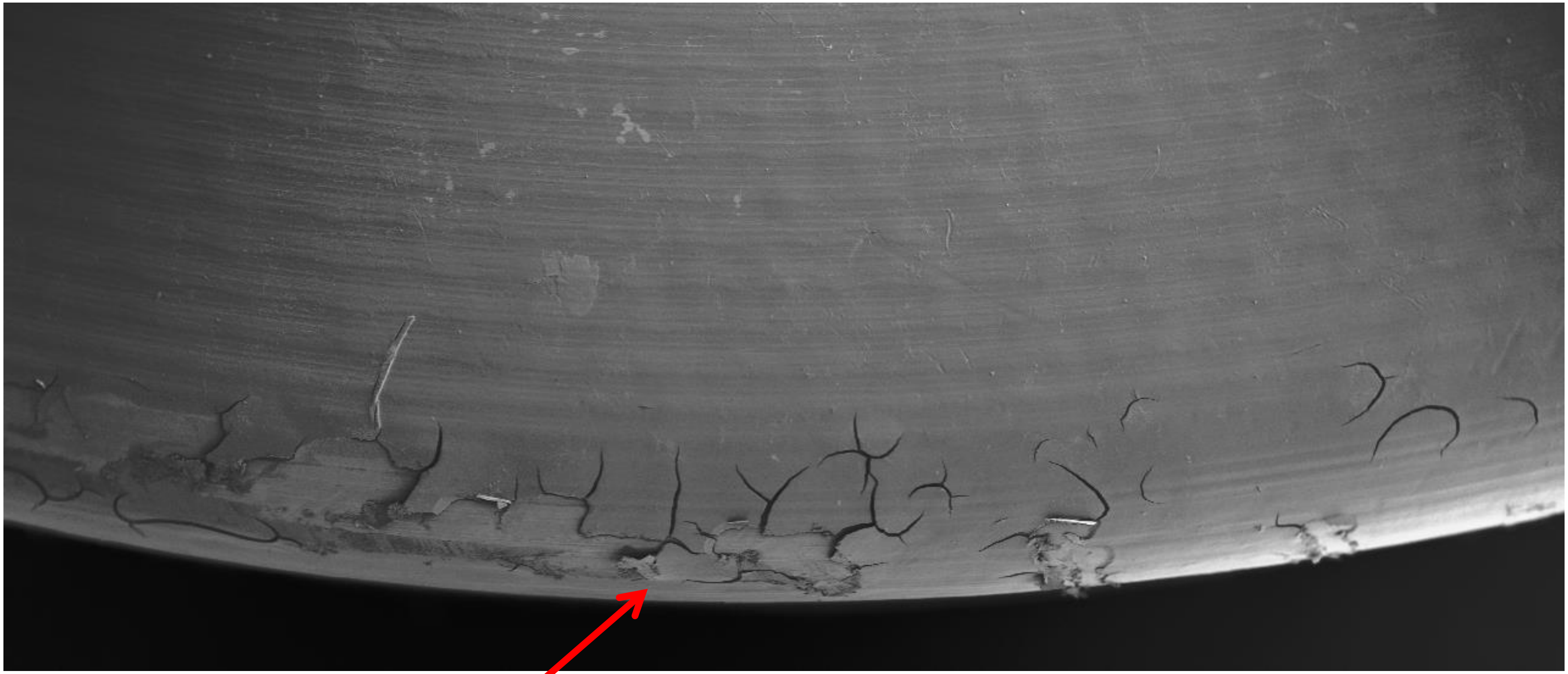




SEM of Biofilm

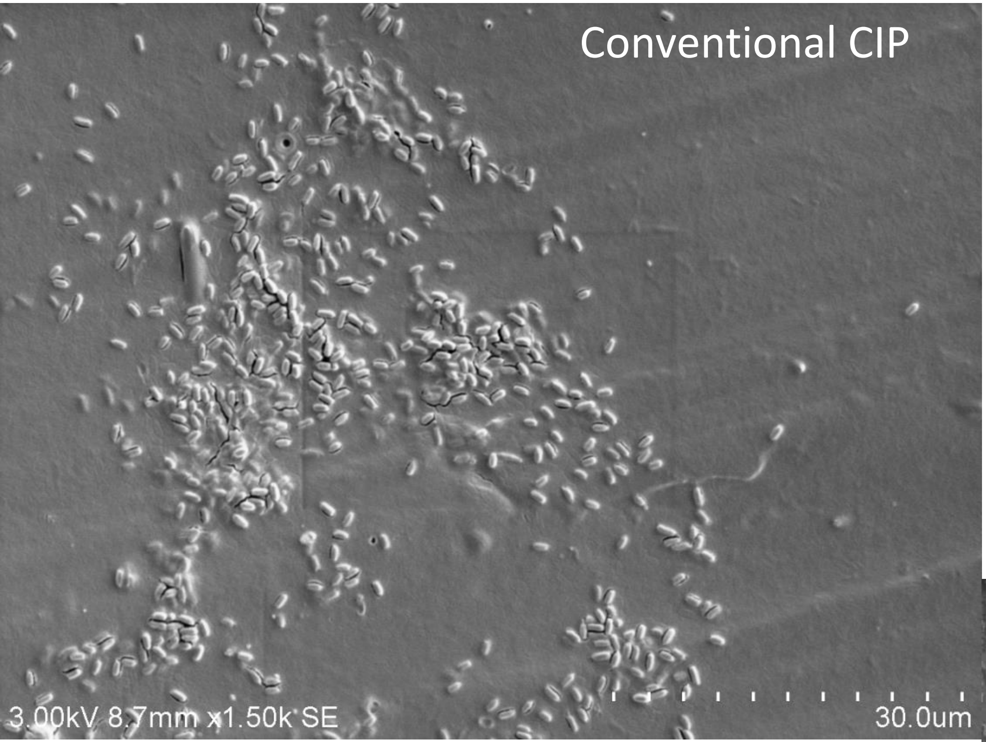
Demonstration of biofilm layers on stainless steel control coupons

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



Note biofilm layering, visible near edge of coupon

SEM – Pre-treatment step

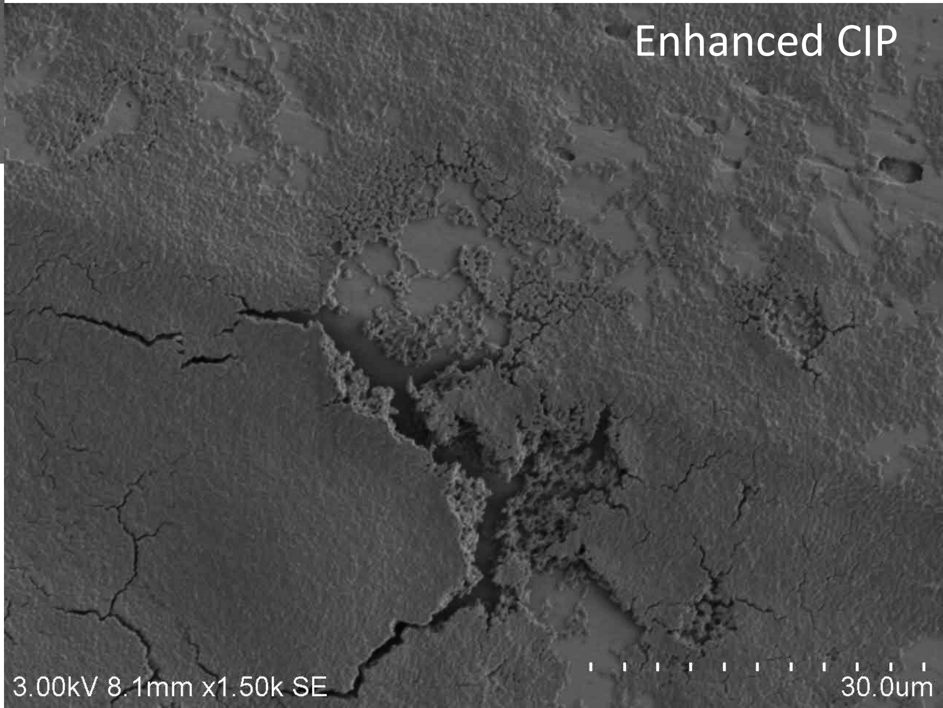


Control – condition before caustic step

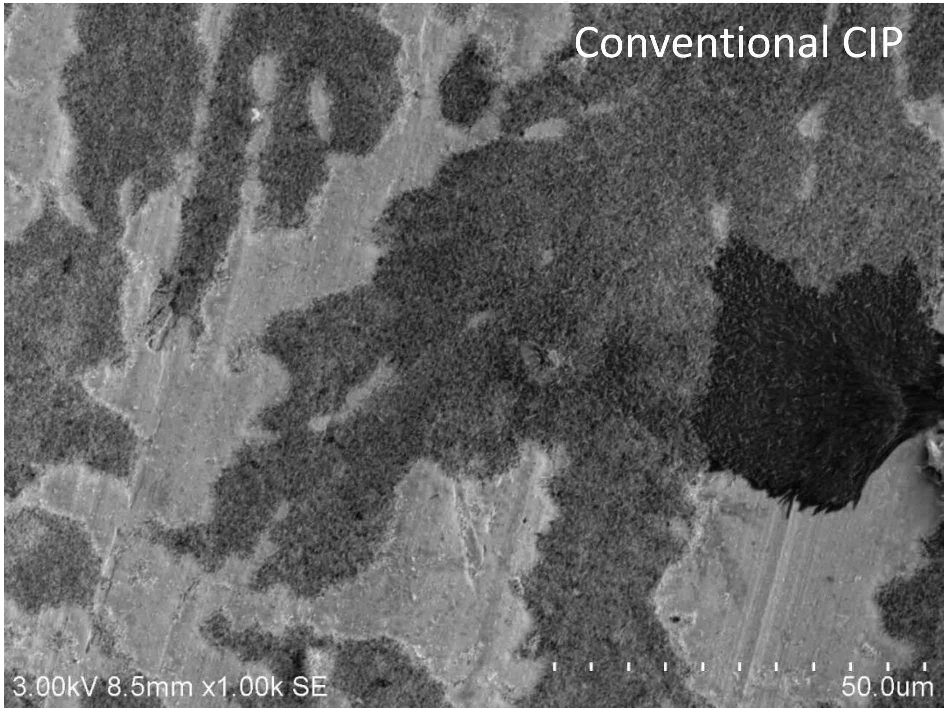
Note smooth EPS coating with potential Bacillus spores present

Condition after pretreatment, before caustic step

Note exposed biofilm layers for more efficient further attack



SEM – Caustic step

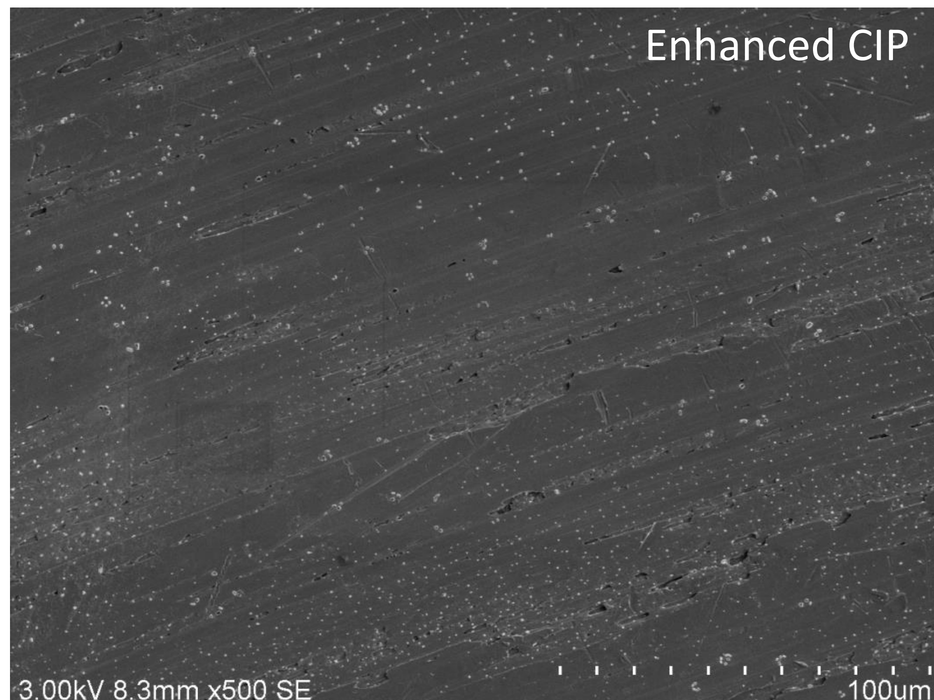


After exposure to NaOH

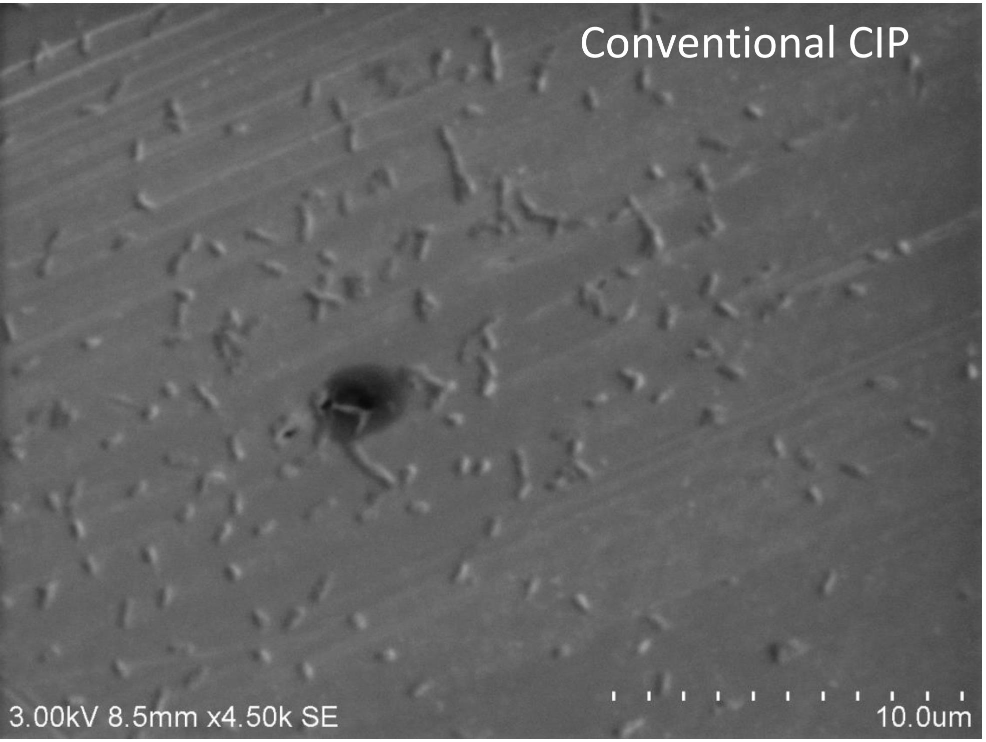
Note uneven attack of biofilm structure

After pre-treatment and
built caustic

Note even removal of biofilm structure down
To base metal surface



SEM – Acid step

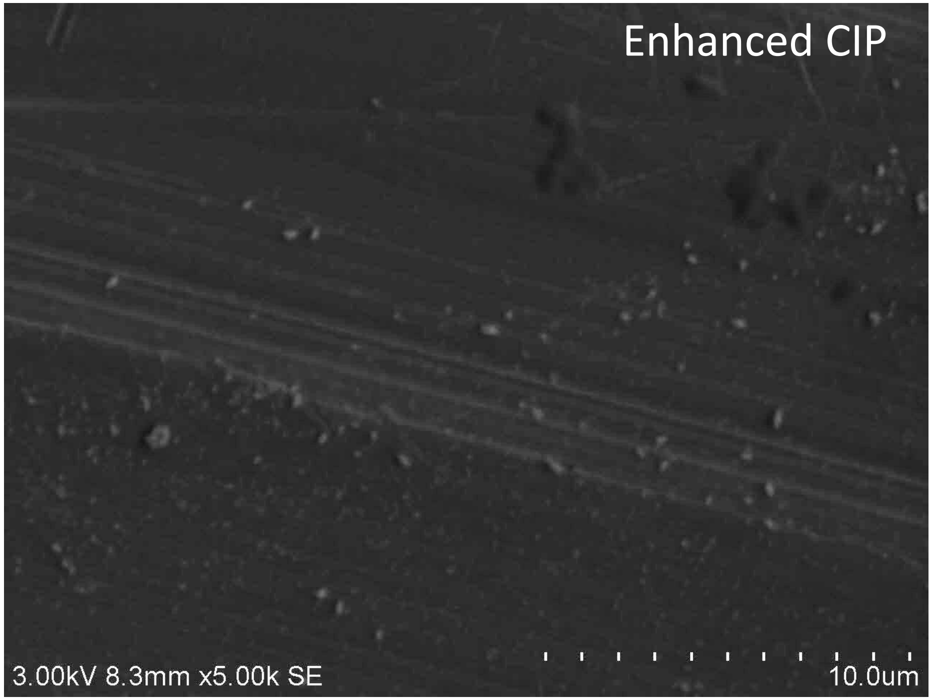


After exposure to NaOH + HNO3

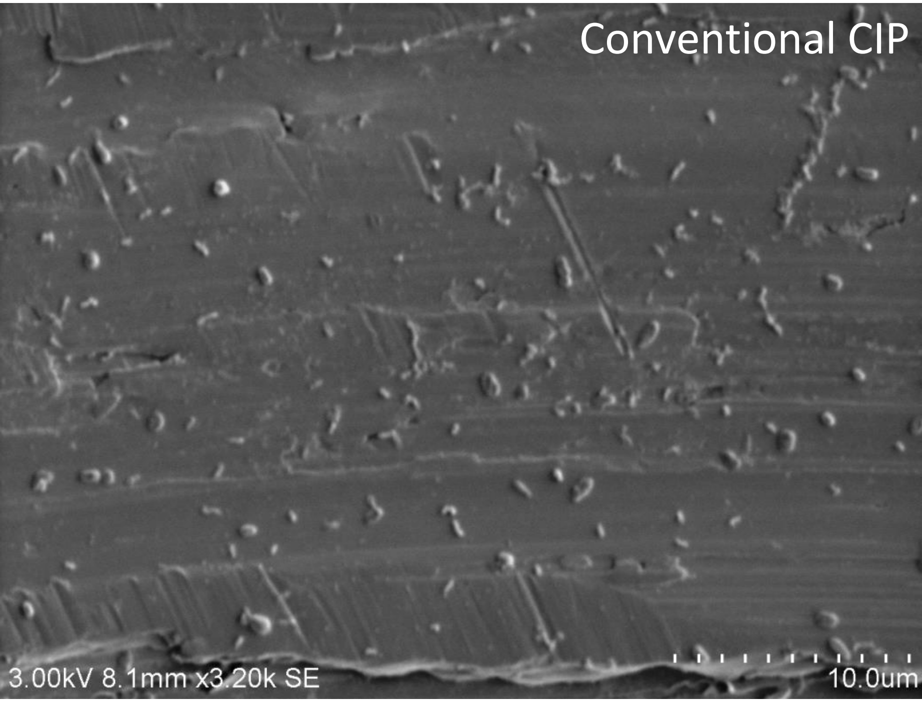
Note populations of microorganisms remaining on surface

After pre-treatment, built caustic, & built acid

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



SEM – Hot Water or Sanitizer step



Final Result from NaOH + HNO_3 + Hot Water

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

Final result from Enhanced CIP

Note nearly complete removal of organic material observed after sanitizer step

FIELD OBSERVATIONS

Cleaning observations

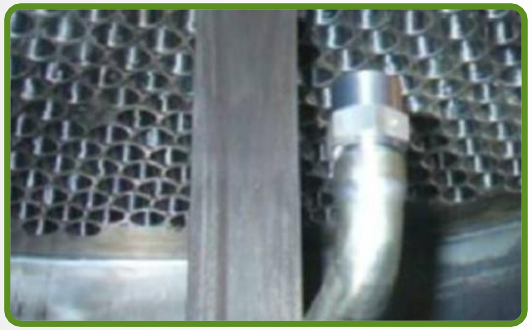
Evaporator

CONVENTIONAL CIP

ENHANCED CIP

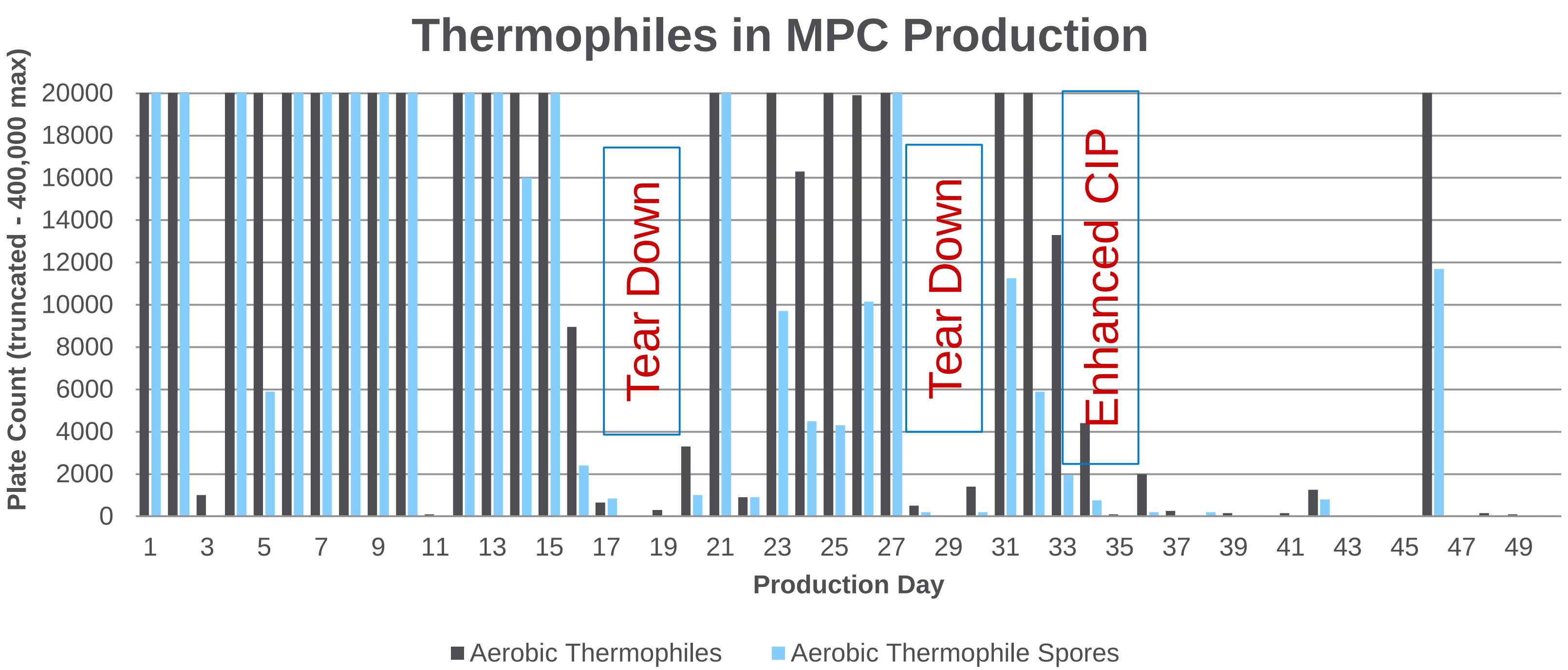
Evaporator

CONVENTIONAL CIP



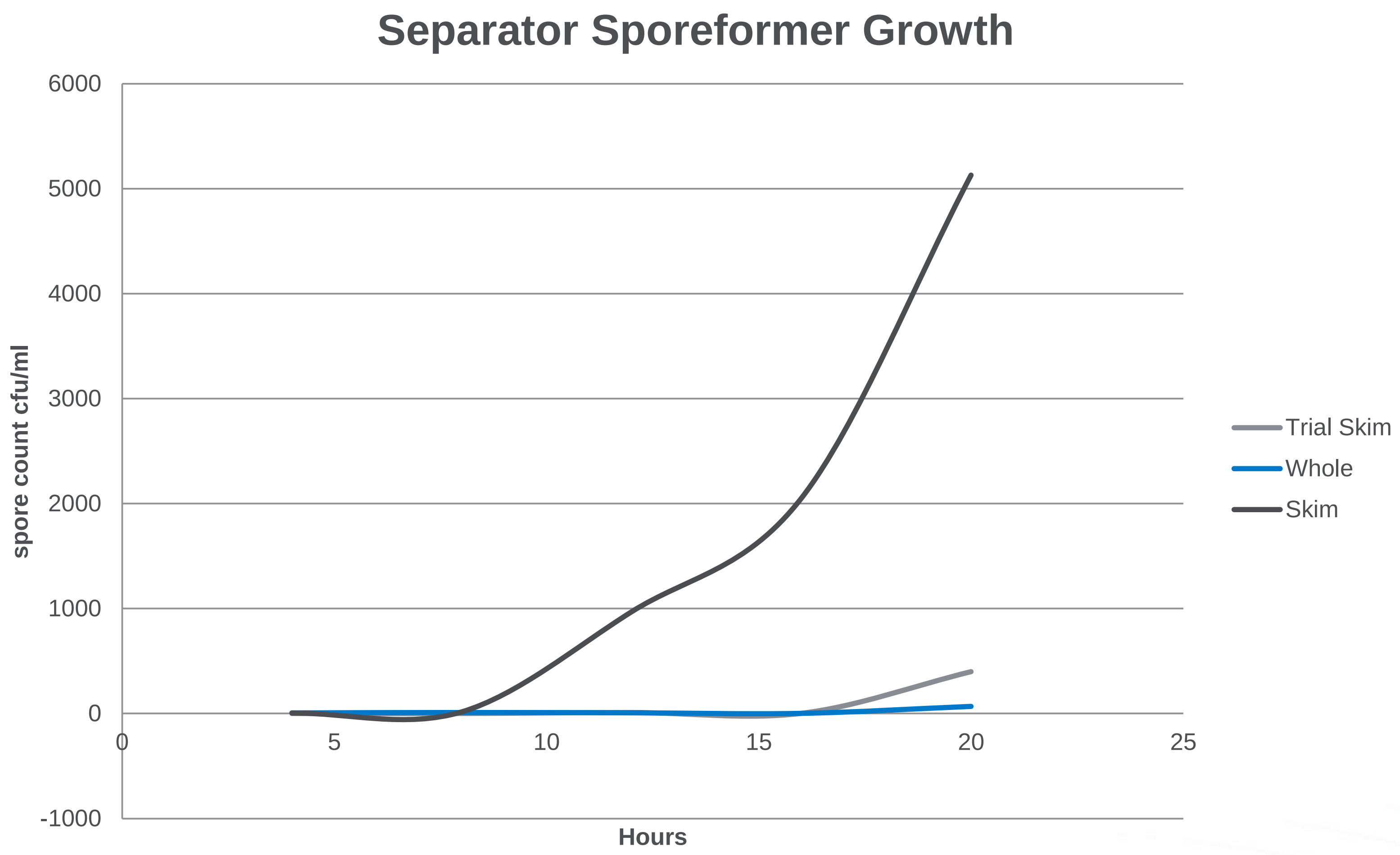
ENHANCED CIP

Spore control through Enhanced CIP



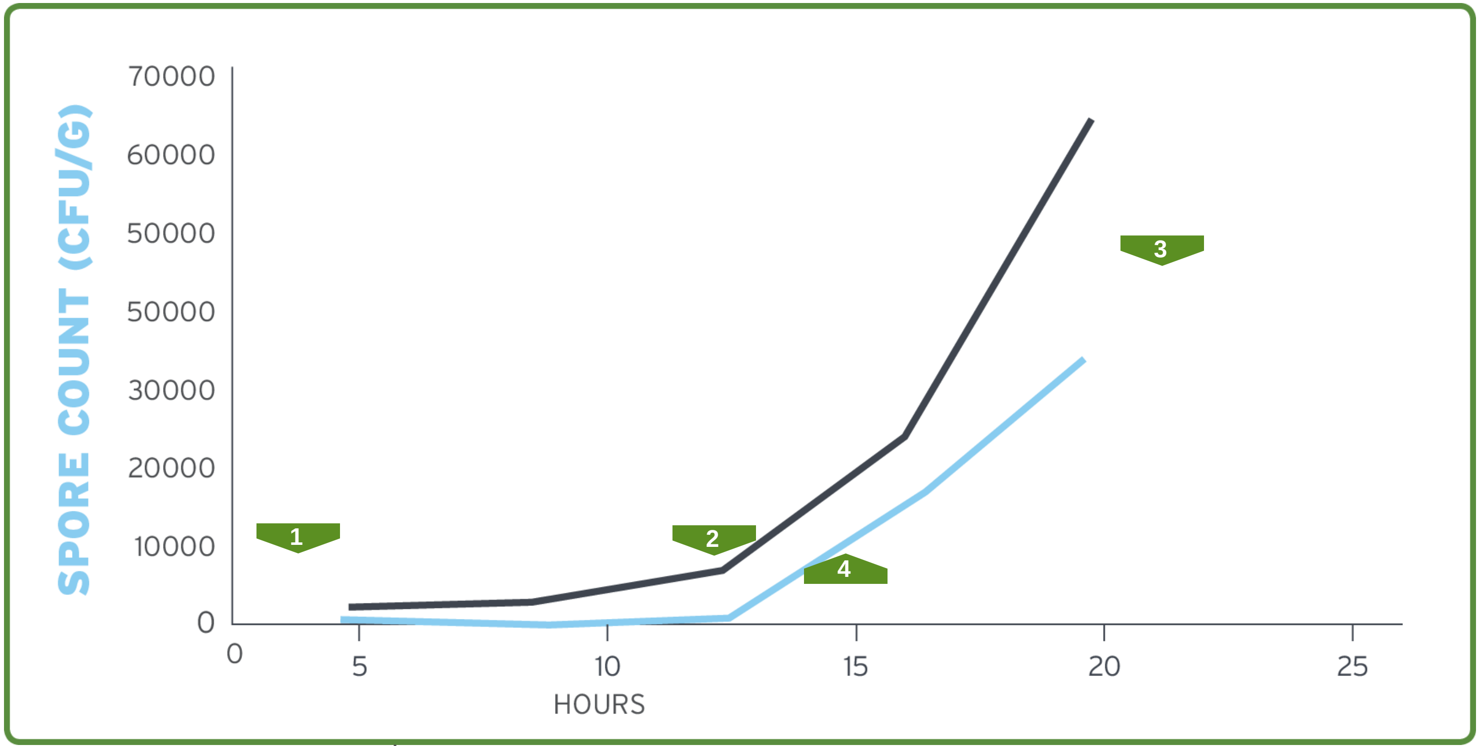
- Spore control program cleared up persistent infection
- Returned to low counts after 'events'

Spore control in separators



Spore control for extended production

LARGE NONFAT DRY MILK PRODUCER



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

2 Powder out of target range within first few hours

3 Baseline showed high variability in day to day quality

4 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

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Thank You

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<u>And THANKS to:</u>
Jeff Hutchison
Josh Luedtke
Justin Valenstein
Linda Grieme
Tony Erickson
Paul Schacht
Caleb Powers