Wisconsin Center for Dairy Research

Biofilms: An Old Nemesis Achieving Well Deserved Notoriety

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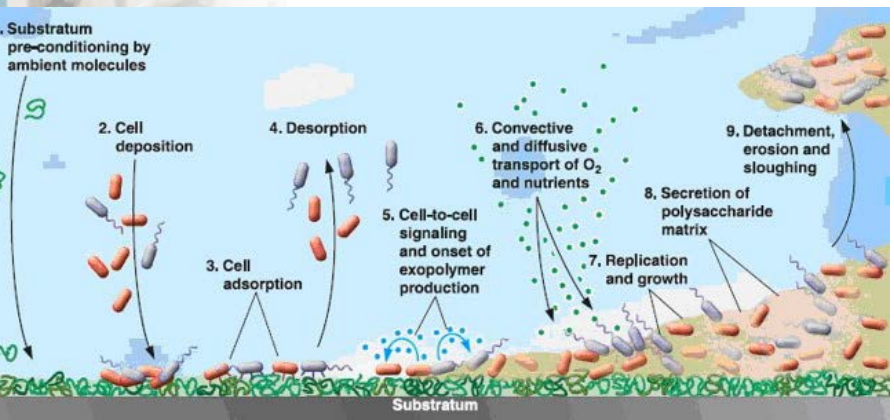
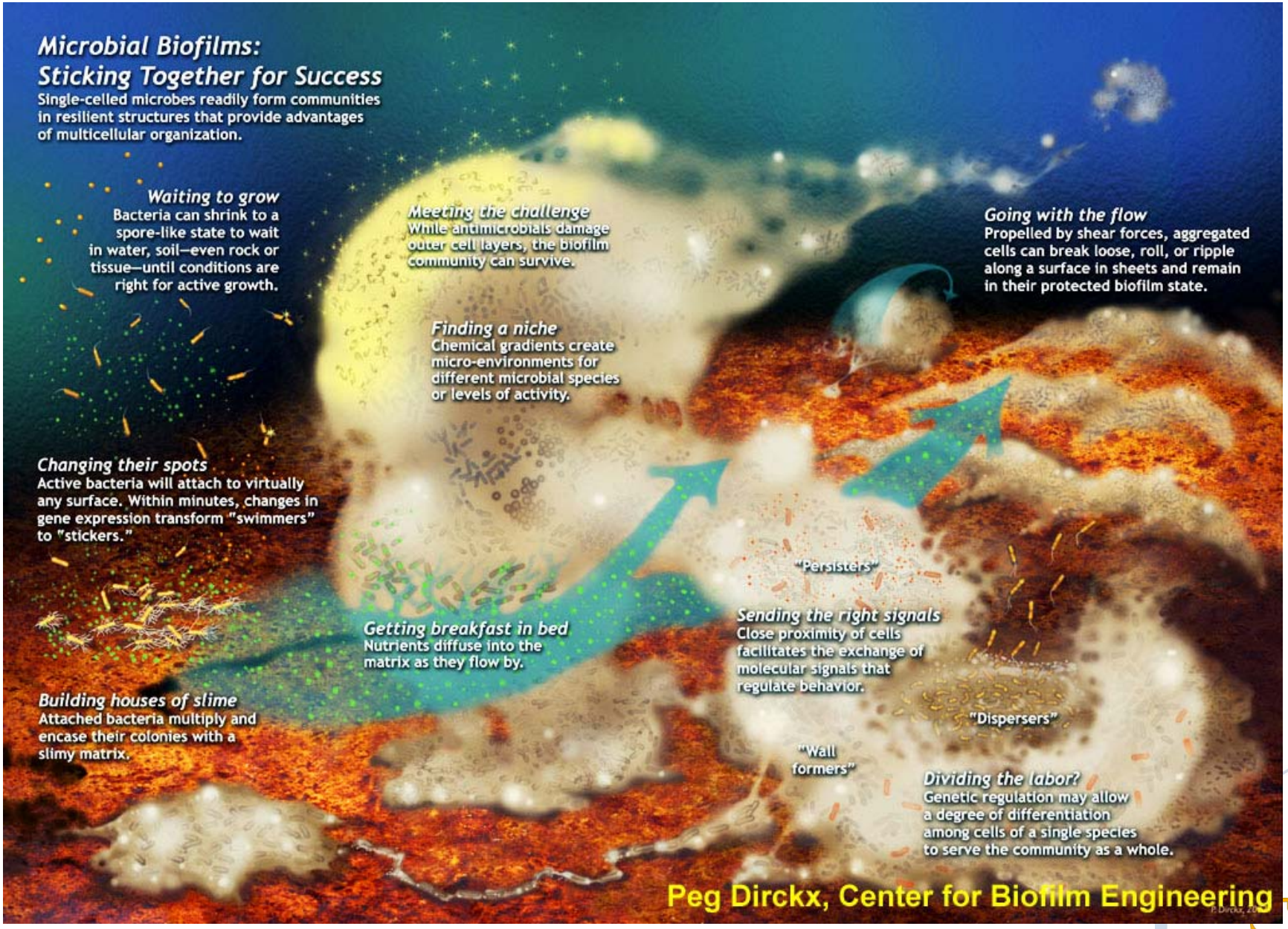
Senior Scientist



Why Talk About Biofilms?

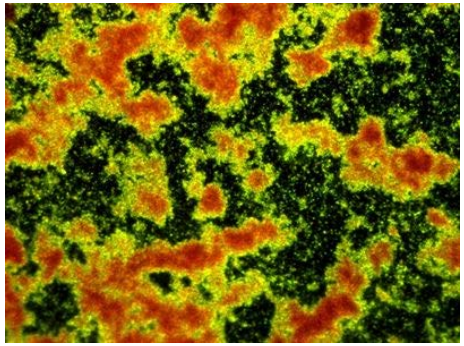
We are searching for the source of bacteria that cause issues during cheese making or in the finished product including whey or dried milk products.

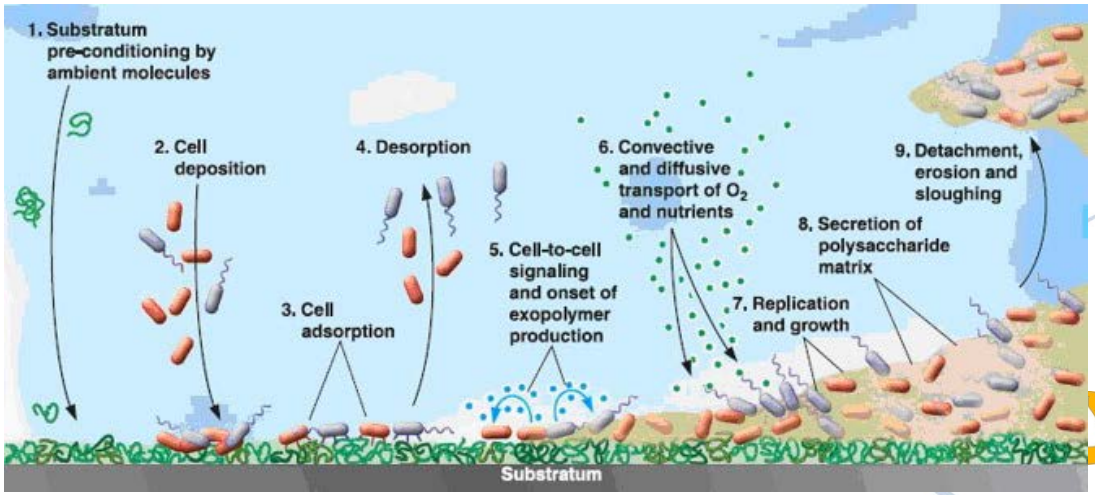
If we know the source at least we have a chance at controlling their numbers to a level they do not cause problems.


Microbial Biofilms:
Sticking Together for Success
Single-celled microbes readily form communities in resilient structures that provide advantages of multicellular organization.
Waiting to grow
Bacteria can shrink to a spore-like state to wait in water, soil—even rock or tissue—until conditions are right for active growth.
Meeting the challenge
While antimicrobials damage outer cell layers, the biofilm community can survive.
Going with the flow
Propelled by shear forces, aggregated cells can break loose, roll, or ripple along a surface in sheets and remain in their protected biofilm state.
Finding a niche
Chemical gradients create micro-environments for different microbial species or levels of activity.
Changing their spots
Active bacteria will attach to virtually any surface. Within minutes, changes in gene expression transform "swimmers" to "stickers."
"Persisters"
Sending the right signals
Close proximity of cells facilitates the exchange of molecular signals that regulate behavior.
Getting breakfast in bed
Nutrients diffuse into the matrix as they flow by.
Building houses of slime
Attached bacteria multiply and encase their colonies with a slimy matrix.
"Dispersers"
"Wall formers"
Dividing the labor?
Genetic regulation may allow a degree of differentiation among cells of a single species to serve the community as a whole.
Peg Dirckx, Center for Biofilm Engineering

Thinking about Biofilms

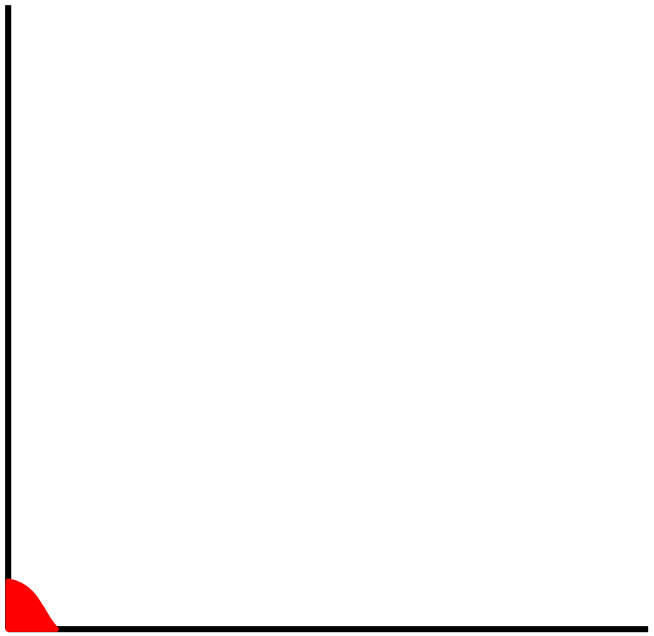
- Why do they happen?
  - Biofilms are the natural state of microorganisms in the environment
  - Biofilms are a means of protection and adaptation to a potentially changing environment that can be very competitive

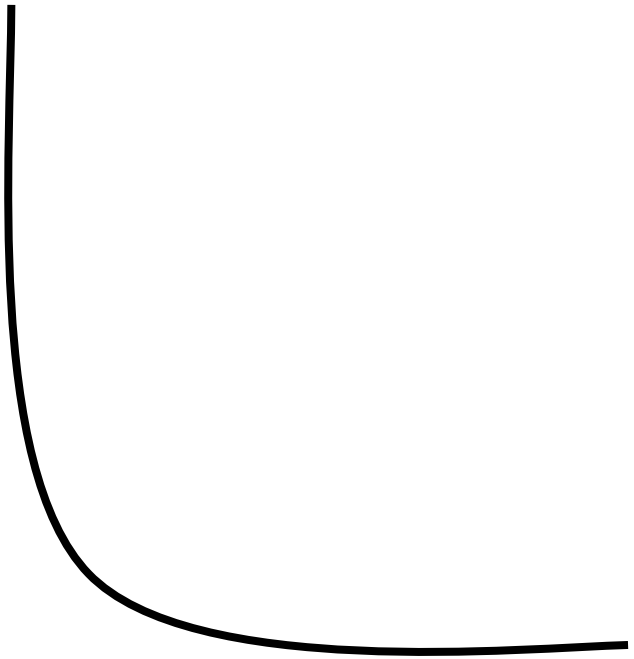




Microorganisms in Biofilms

- Attachment sites of biofilms on equipment are probably where they can "hide" : **Gaskets/ cracks**
  - **Where moisture/milk can accumulate or form a residue after drainage**
- Spread through cheese and biofilm as a creeping colony rather than through active motility of the bacteria
  - Harvard study indicated 5x increase in size of a biofilm in 24 hrs
  - Due to osmotic pressure (they swell-move)





Difficult to Reach Biofilms

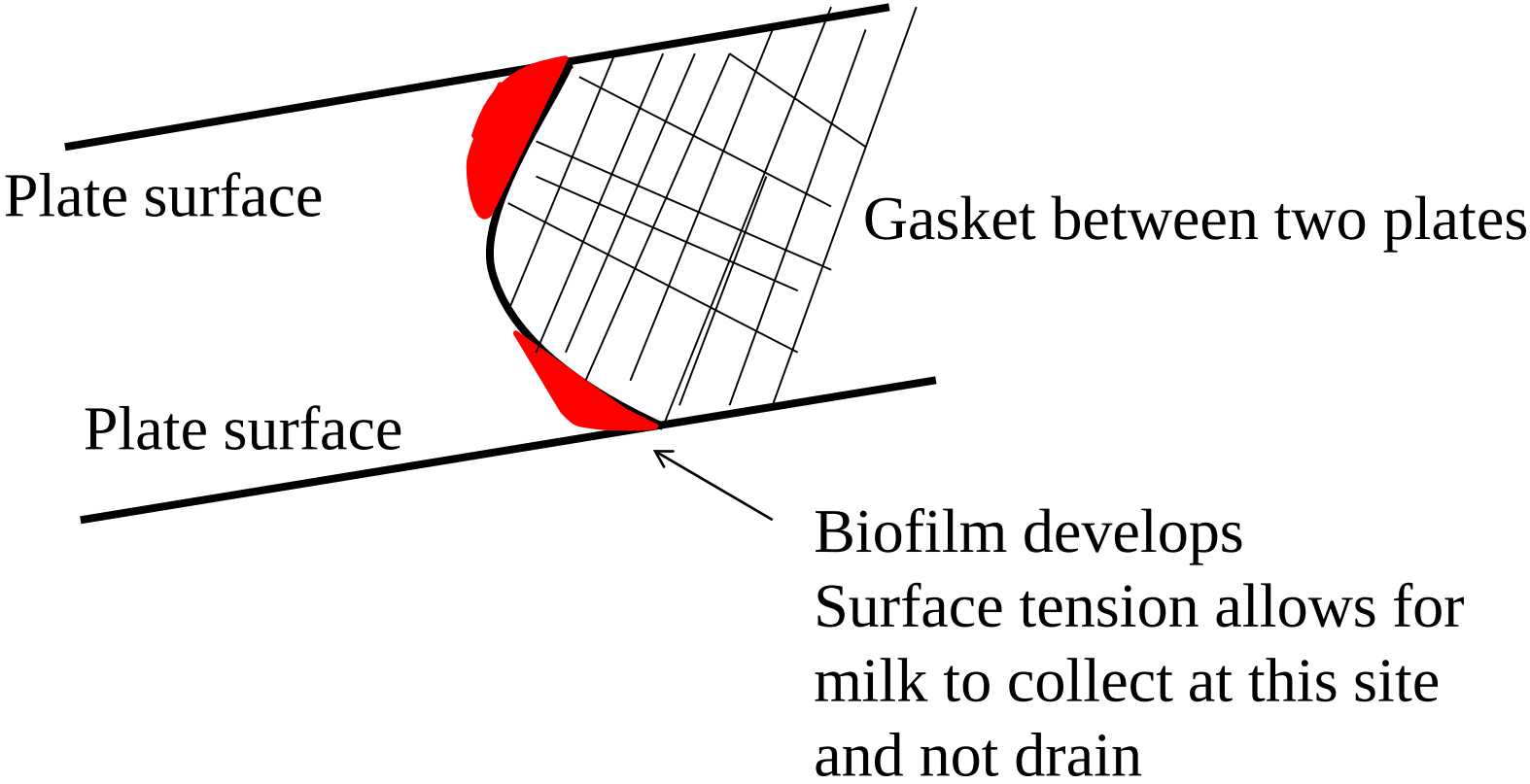
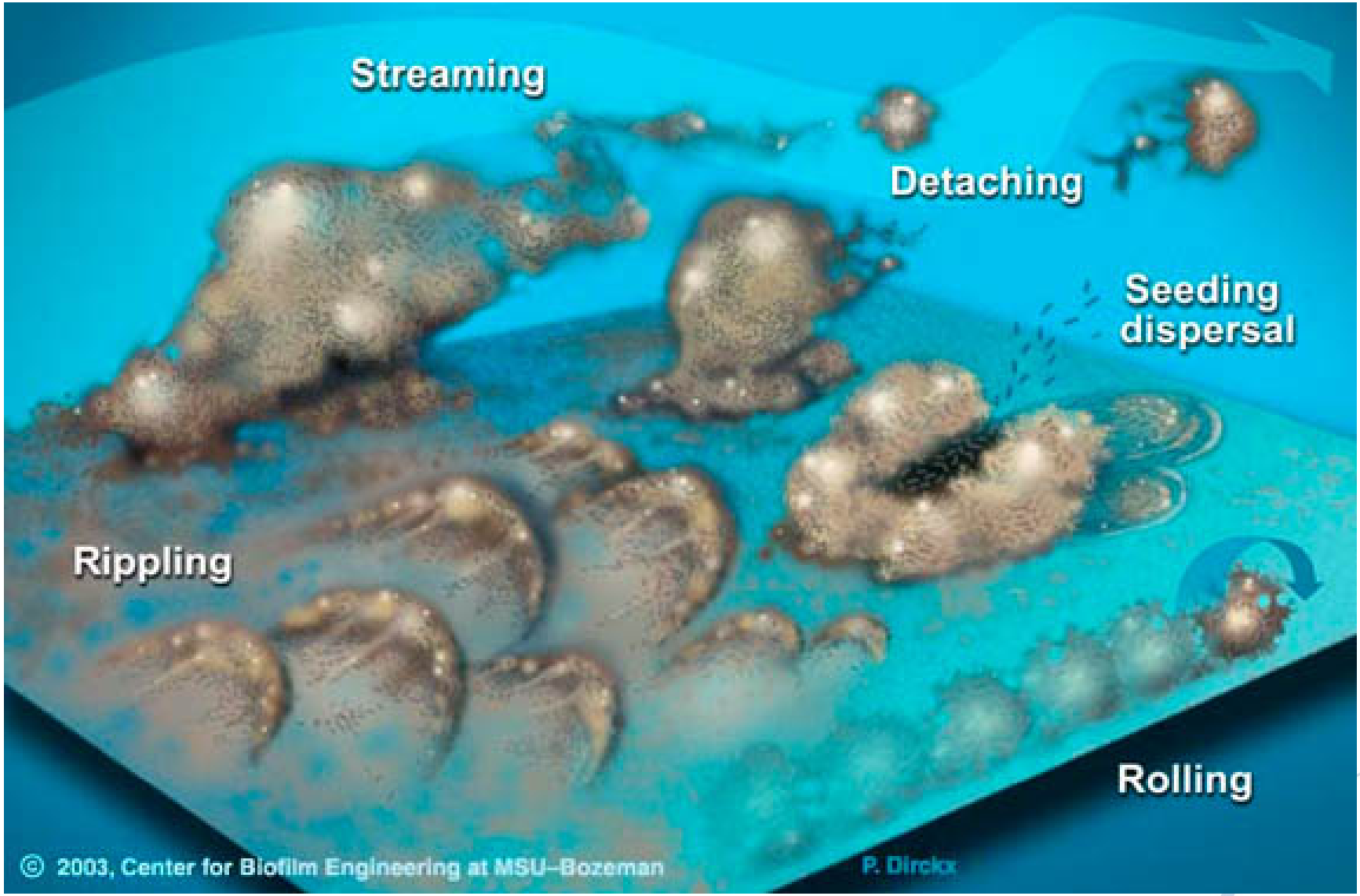
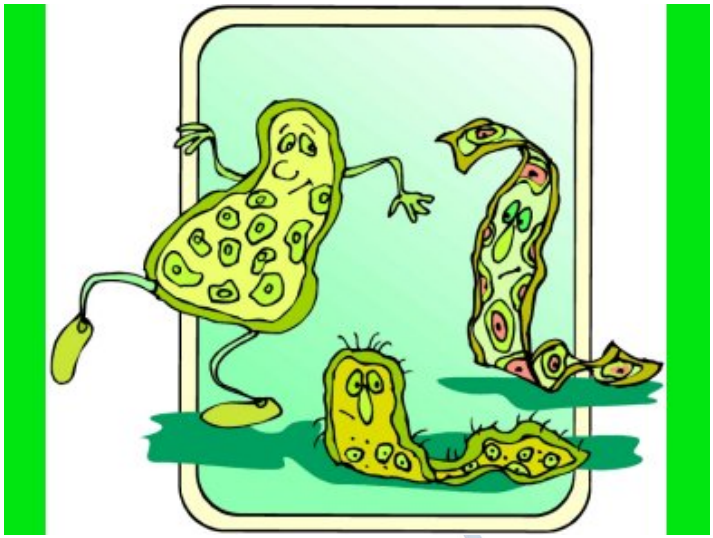


Plate surface
Gasket between two plates
Plate surface
Biofilm develops
Surface tension allows for
milk to collect at this site
and not drain

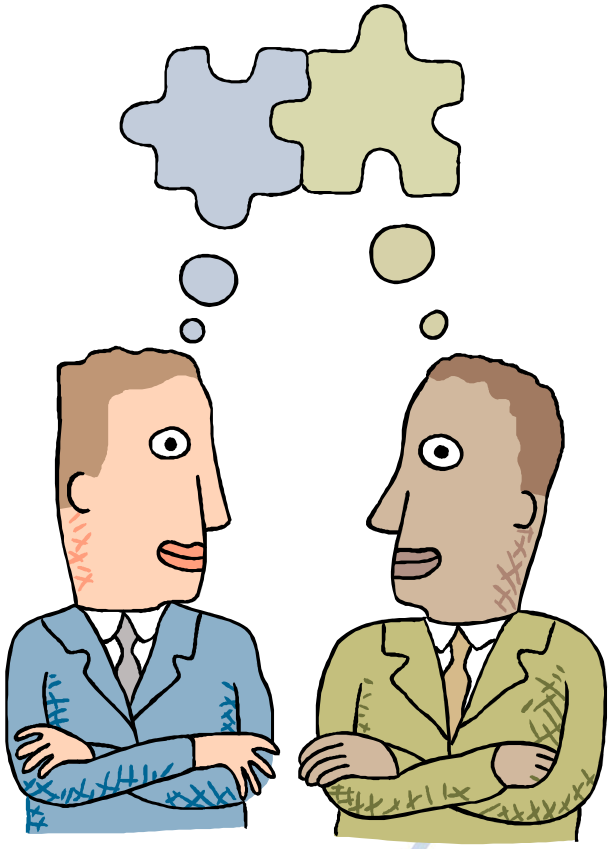

Streaming
Detaching
Seeding
dispersal
Rippling
Rolling
© 2003, Center for Biofilm Engineering at MSU–Bozeman
P. Dirckx

- Bacteria causing issues in cheese
  - Textural problems due to gas formation
    - *Lactobacillus sp.*
    - *Leuconostoc sp.*
    - *Weisella*
    - *Clostridia*
  - Increase rate of acidification during cheese making
    - *Streptococcus thermophilus*
    - *Streptococcus macedonicus*
- Bacterial spores in milk and dried whey and milks for human consumption (export markets)



The Process of Elimination:

- Is the original source of these bacteria an infection of the udder (mastitis)?
  - NO
- Is the problem the cleanliness of the animal?
  - YES
- Is the problem contaminated equipment?
  - YES
- Is the problem inadequate cooling?
  - YES



Original Source of Bacteria in Milk

- Number 1 original source of our problem bacteria are soils/vegetative matter
  - High numbers of bacteria in milk correlate to high amount of sediment in milk, which relates to teat cleanliness at milking.

Teats get contaminated with:

- **Bedding material (bio-solids**) and manure flushing (Doug Reinemann-UW Biological Systems Engineering)
- Improperly fermented or stored silage (generally through feces)
- Mud
- Feces
- Improper pre-milking teat treatment with inadequate cleaning of teats and legs being the most problematic

As a result of contact with contaminated teats, equipment and milk get contaminated

- Cracked rubber parts on milking equipment are also a source of buildup of residue **(biofilms)**
- Significant buildup of bacteria in milk residue **(biofilm)** may take several days to weeks to develop
- This indicates a **PERSISTENT CLEANING FAILURE**
- **Pam Ruegg** UW Dairy Science Extension Milk Quality Specialist: **“ wash failures**” improper water temperature (should be >158ºF), inadequate detergent strength, and low sanitizer strength.

Wash failures: Ramifications

- Warm milk (100ºF) is goes through a piping system until at some point the temperature is lowered to < 50ºF
- Biofilm is subjected to continuous warm temperature
- Thermophilic bacteria multiply rapidly in biofilms : (warning at >200 cfu/ml in raw milk)
  - *Streptococcus*
  - *Lactobacillus*

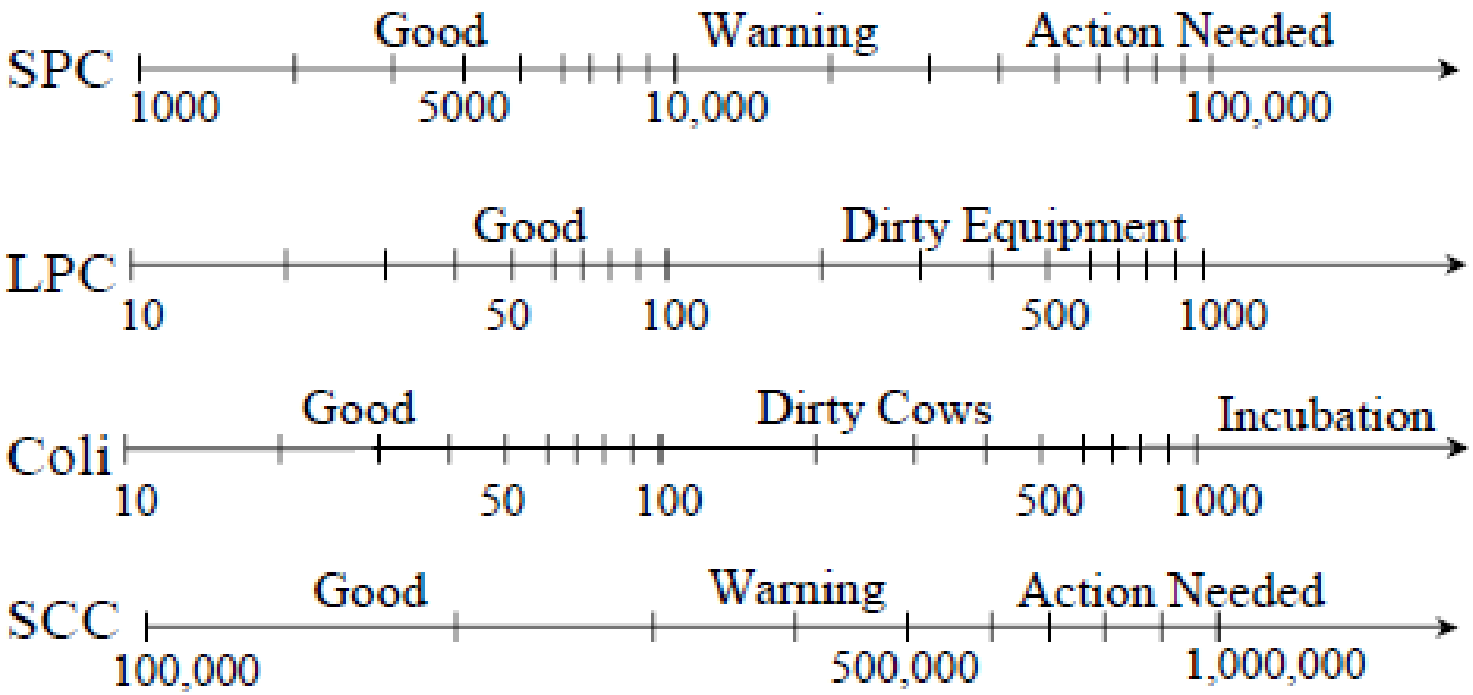
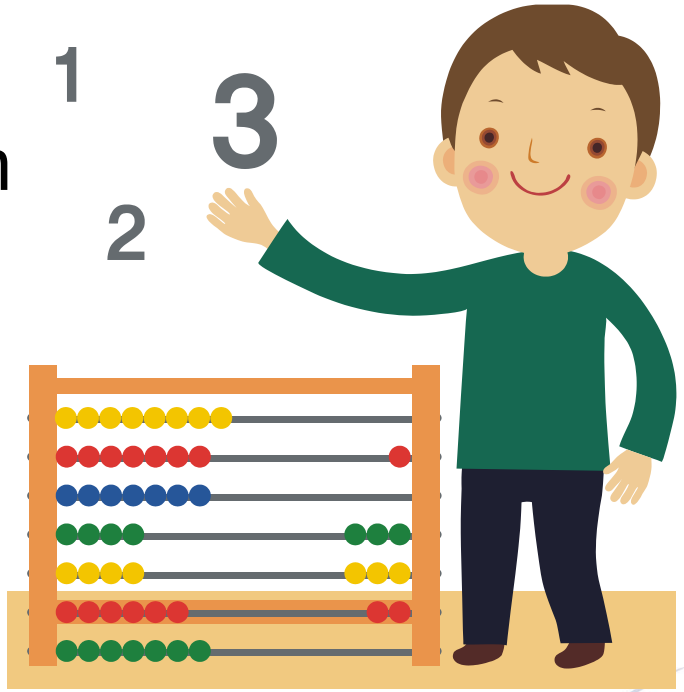


Figure 1. Diagnostic Chart for Bulk Tank Bacteria Counts.

Guterbok, W.M., and P.E. Blackmer, 1984. Veterinary Interpretation of Bulk Tank Milk.
Veterinary Clinics of North America: Large Animal Practice, Vol. 6, No. 2, July 1984. pp. 257-268

How bacteria are counted is important

- **Laboratory Pasteurization Count**
  - Simulates LTST pasteurization 145ºF/30 min
  - Standard Methods Agar at 90ºF for two days
  - Pick up thermoduric mesophiles including activated spores, some lactobacilli, micrococci and others.



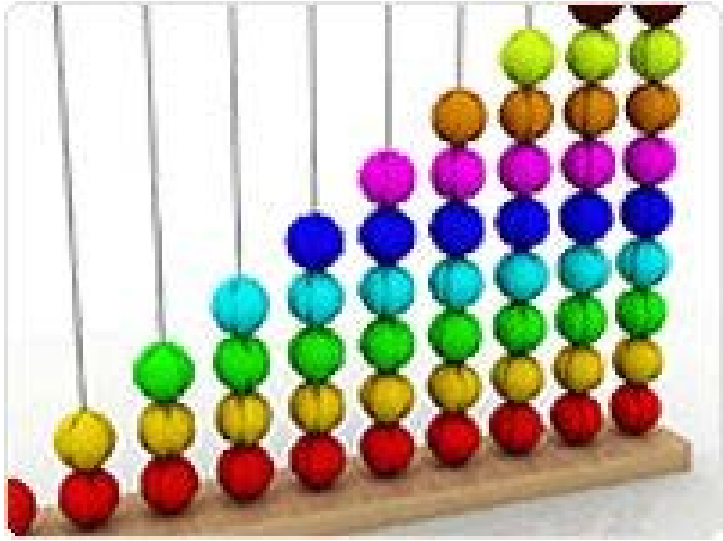
How bacteria are counted is important

- **Laboratory Pasteurization Count**
  - Probably will miss the thermoduric thermophiles: heterofermentative lactobacilli and *Streptococcus thermophilus*

  - Suggest using ST agar (at 108ºF/two days) for streps and Rogosa SL agar (at 90ºF and 99ºF for two days) for lactobacilli

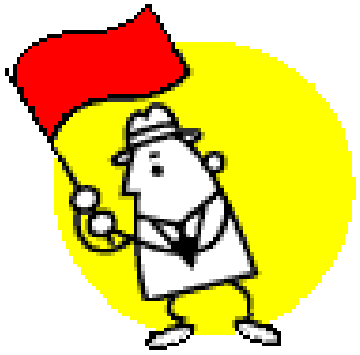
Lab Pasteurization Count (LPC)

- Compare LPC to actual number in plant pasteurization numbers
  - If numbers of selected bacteria are higher in the in-plant pasteurizer milk than in the LPC
  - Indicates biofilm exists in pasteurizer



Red Flags for Potential Biofilm

- High numbers of selected bacteria in pasteurized milk
  - If numbers in raw milk or LPC do not increase substantially during the day but …….
  - Selected bacteria increase in cheese from early vats to later vats
  - Counts of selected bacteria increase substantially over the day
  - Numbers of selected bacteria exceed certain values



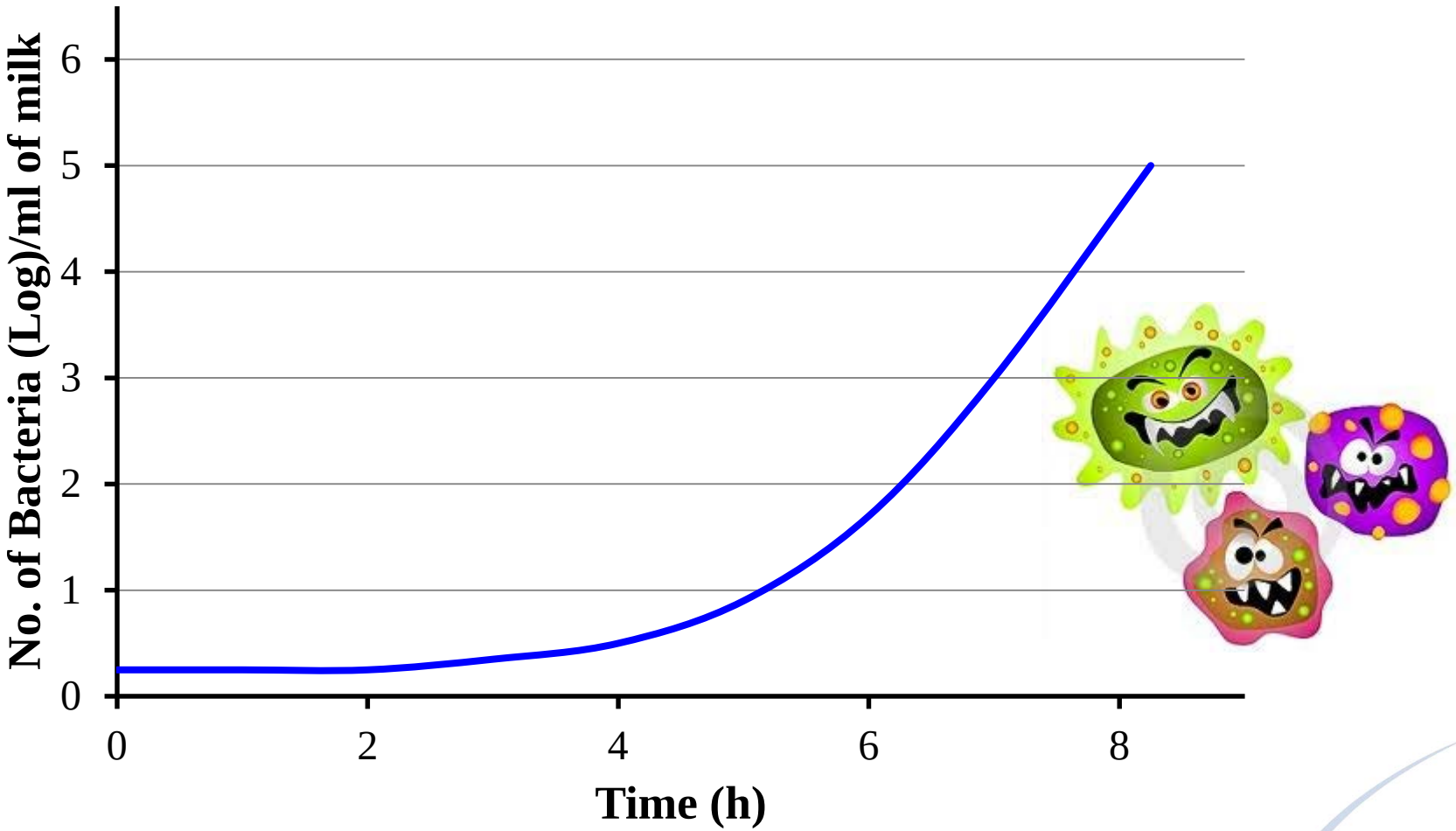
***Streptococcus* >100 cfu/ml milk**

Lactobacillus > **10 cfu/ml (generally is <1/ml)**

Biofilm Location in Cheese Plant

- Regenerative section of pasteurizer and piping from pasteurizer
  - Milk is warm : allows rapid growth of bacteria
  - Any location where flow is inconsistent
    - Hidden spots: gaskets, dead-ends, valves

Progression of a Biofilm: Samples taken from freshly pasteurized milk



Rapid increase indicates Biofilm already existed

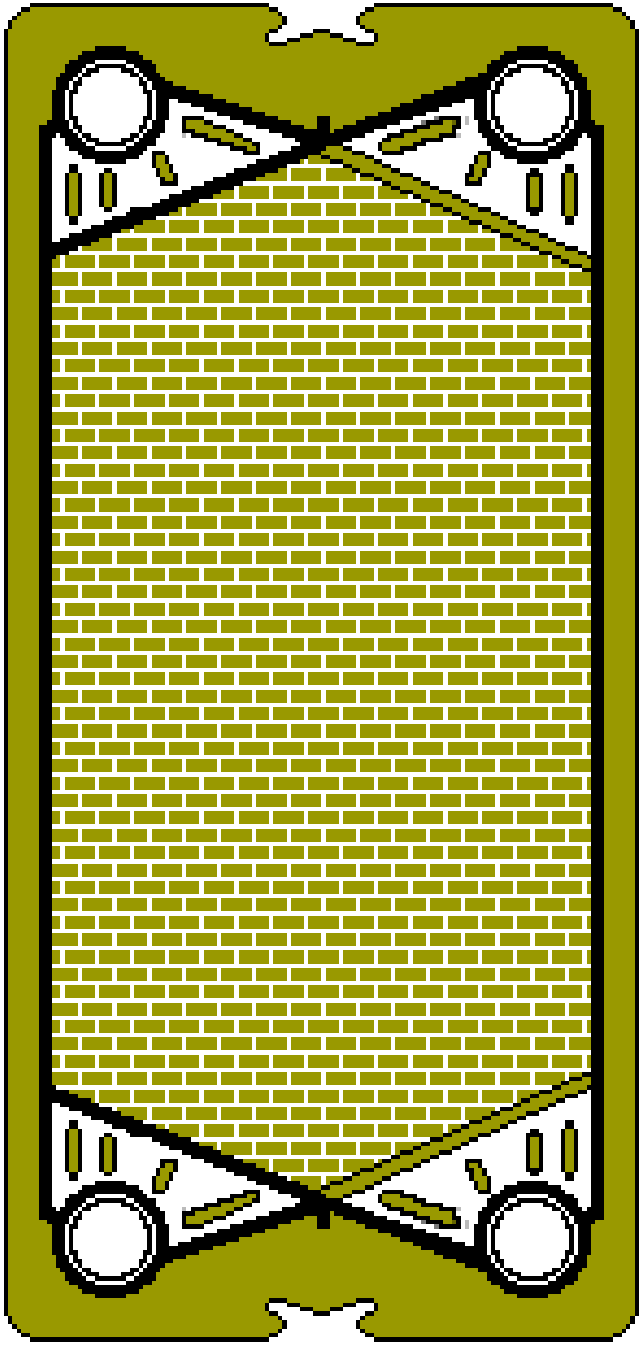


Plate from a HTST pasteurizer

In a large plate (~62 inches by 16 inches)
There are ~187 inches of gasket/plate

In a large unit (100,000 pounds per hr)
with ~240 plates there are

1250 yards of gaskets

How often are they changed?
Once every 3-5 years!

Case History: Biofilm

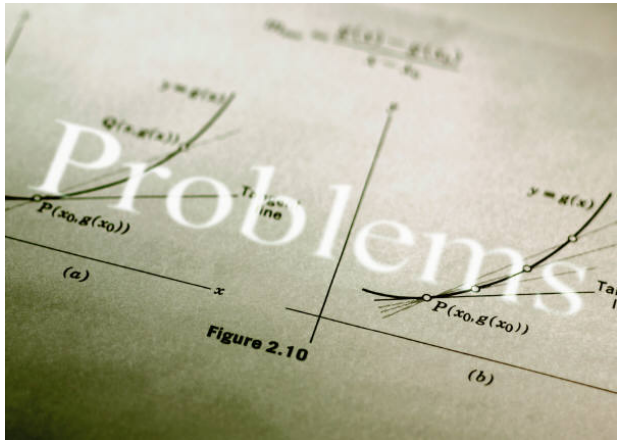
- Severe split cheese defect in Swiss, elevated histamine levels > 500 ppm
  - Isolated *Lactobacillus parabuchneri* from raw milk, and cheese
  - High counts of *Lb. parabuchneri* in incoming milk from just a few farms (~50-100 cfu/ml)
  - Checked on farm source and found biofilm on gaskets
  - Taught producers about proper cleaning and sanitation

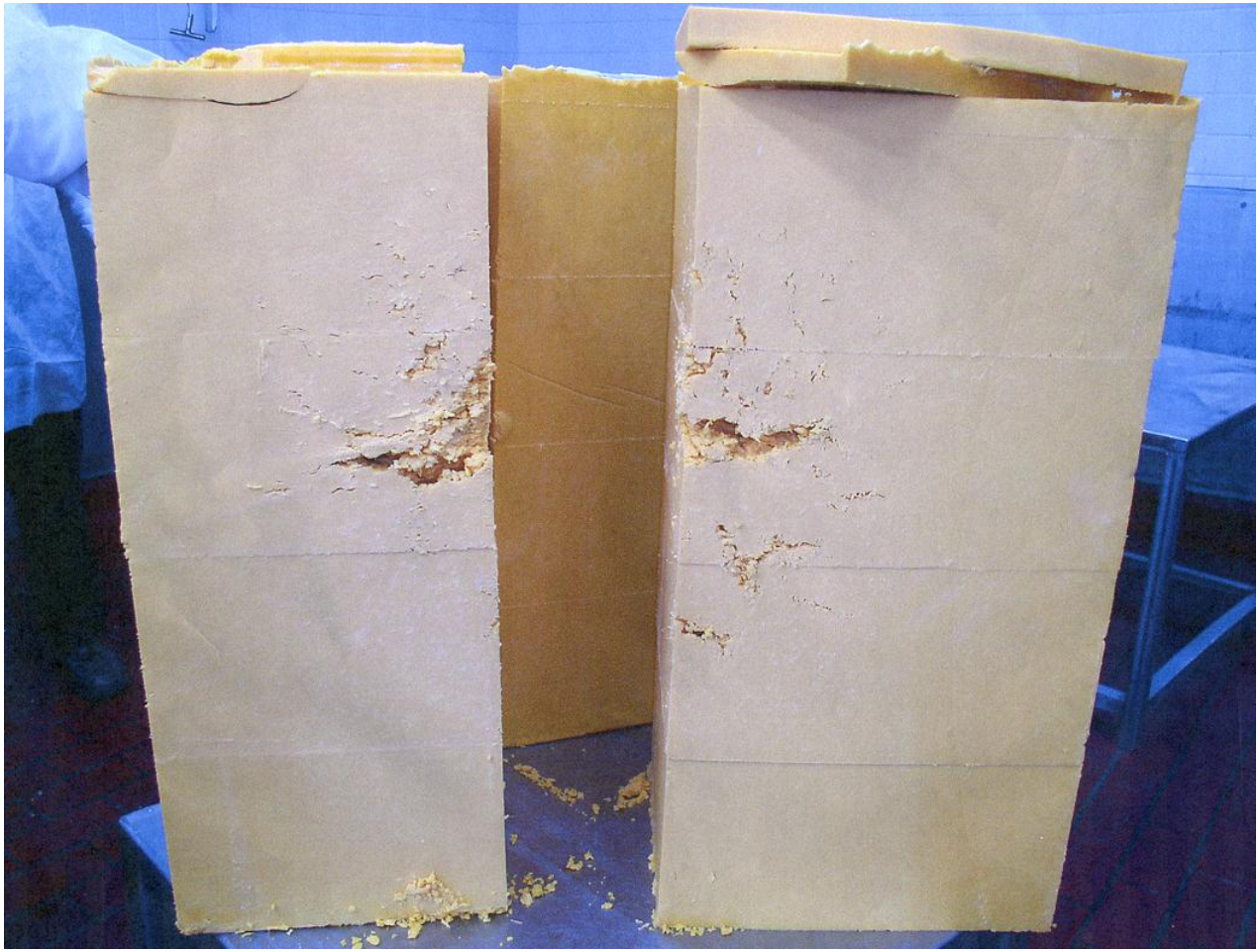
Case History: Biofilm

- Excessive acid development in pasteurized milk after ~8 hrs of continuous run
  - Isolated >100,000 cfu/ml milk of *Streptococcus thermophilus* from pasteurized milk by 8 hr
  - Desired titratable acidity at drain : **18**
    - Titratable acidity at drain by 10 hrs of running pasteurizer : **22**
    - Titratable acidity at drain by 12 hr of running pasteurizer : **31**
  - Reduced counts with mid-day wash at 8 hrs but biofilm persisted even after end of day wash
  - Biofilm found in regenerative section of pasteurizer

How much biofilm mass to be a problem?

- To get to 50,000 bacteria/ml of pasteurized milk would require at least 3 hrs of optimal growth in a static broth (starting with 100 bacteria per ml of milk).
- In a biofilm you would have to slough off about 1 g biofilm every 18 s. to get 50,000 bacteria per ml of pasteurized milk (in 100,000 pound/hr pasteurizer).
- About 0.002 g per inch of gasket





Case History: Biofilm

- Severe early (2 weeks) gassy cheese defect in Cheddar
  - Isolated *Lactobacillus fermentum* from raw milk, pasteurized milk and cheese
  - High counts of *Lb. fermentum* in incoming milk
  - Pasteurized milk had high lactobacillus numbers (Rogosa SL agar-99ºF) >1000 cfu/ml milk

Be Worried -Be Very Worried about an Established Biofilm

- It may take several days or weeks to establish a biofilm on clean equipment
- But once formed it will be very hard to completely remove and it will “seed” additional biofilm development
- Suggests vigilance in cleaning and sanitation before you see the numbers of bacteria in milk that would suggest a biofilm has formed

What to do?

- Prevention
  - Reduce bacterial numbers in raw milk
    - On-farm: proper cleaning and sanitation and cooling
    - In plant: Use bacterial removal systems
      - "centrifuge" : Bactofuge, Bactocatch systems
      - Microfiltration
  - Alternate equipment
    - 2 pasteurizers
    - 2 clarifiers
    - Change gaskets twice a year at least
- Aggressive wash targeted at biofilm
  - Cleaning /sanitation: including mid-day wash
- Suggests vigilance in cleaning and sanitation before you see the numbers of bacteria in milk that would suggest a biofilm has formed

Acknowledegements

- **WMMB**
- **DRI (Dairy Research Institute)**
- **CDR Industry Team**