Dairy Thermodurics

Module 4: Persistence of Thermodurics in Dairy Processing Plants

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Content

- Are thermodurics in raw milk a real cause of worry for the milk processors?
- What makes thermodurics so persistent?
- The concept of bacterial biofilms

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Why thermodurics are so difficult to eliminate from the processing environment?

- Resistant to thermal processing such as industrial pasteurization
- Many form spores having greater resistance to heat and chemicals
- Wide temperature growth range
- Thermoduric thermophiles exhibit a faster growth rate
  - generation time 15-20 min
- Readily form biofilms

(Flint et al, 1999 & 2001; Parker et al, 2001, Scott et al, 2007; Burgess et al, 2010; Hassan et al., 2010)

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Persistence; The biofilm angle

- Biofilms are congregation of sessile bacteria attached to a surface
- Bound together with extracellular polymeric substance (EPS)
  - Proteins, lipids, and polysaccharides
- Occur in areas of initial mineral deposits or organic matter buildup
  - Soiling within milking or processing equipment
- Biofilms formed by thermoduric bacteria
  - Both spores and vegetative cells can attach to stainless steel and fouled surfaces
  - Foulant or biofilms may protect spores and vegetative cells against CIP chemicals

(Costerton et al.,1994; Hassan et al., 2010, Anand et al., 2012)

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Areas prone to thermoduric biofilms

- Following are the major areas prone to biofilms in the processing plants
  - Pasteurizer heat exchange plates; regeneration section
  - Cream separators
  - Regeneration section of evaporators
  - Preheater of evaporation unit in spray drying
  - Membrane applications in milk and whey processing
  - Cream heater and recycle loops in butter plants
- Remain ~45 - 60°C/ 113 - 140°F
  - Support growth of thermodurics, especially thermophiles
- Other problem areas
  - Rubber seals, corners, hard to clean areas

(Scott, 2007; Burgess, et al., 2009)

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Factors influencing biofilm formation

- The ratio of planktonic (free-floating) cells to biofilm embedded cells is a function of several interrelated factors
  - Types of bacteria
  - Surface energetics
  - Materials of construction
  - Microtopography of surfaces
  - Hydraulic factors
  - Biofilm chemistry

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Stages of a typical biofilm formation

- Stage I: (within 5-10 sec) surfaces are coated with milk proteinaceous components and calcium phosphate
- Stage II: (within 6-8 hours) primary colonization of bacterial cells with the substratum
  - Extrapolysaccharides (EPS) appears to support this process
- Stage III: (within 10-12 hours) irreversible biofilms
  - Formation of mature biofilms with embedded bacteria in the organic film matrix

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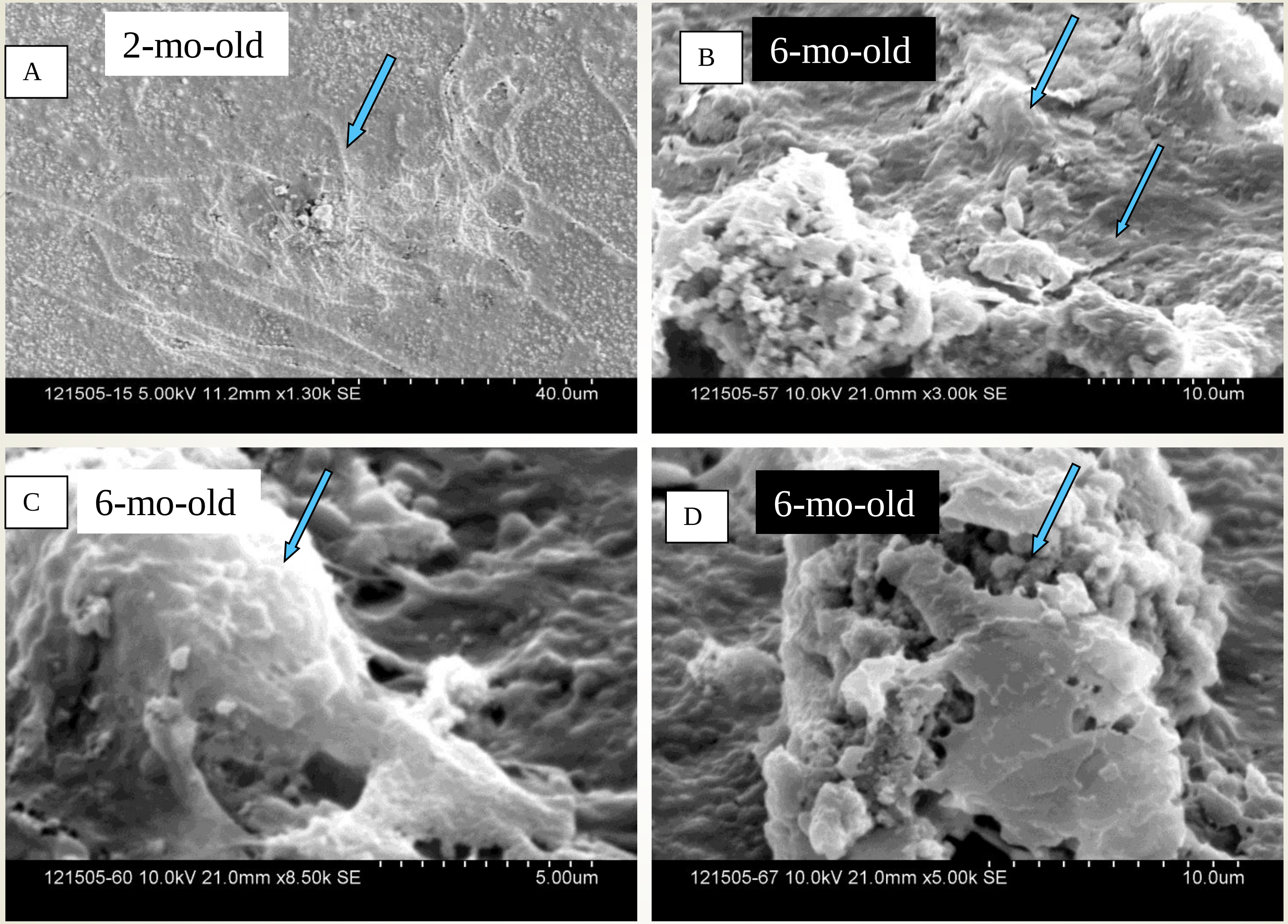
SEM showing biofilm structures on used RO membranes



2mo-old membrane showing long chains of cocci (A), 6mo-old membrane showing hill and valley structure (B), Mushroom like structure (C), and Ruptured mushroom (D). *(Hassan et al., 2010)*

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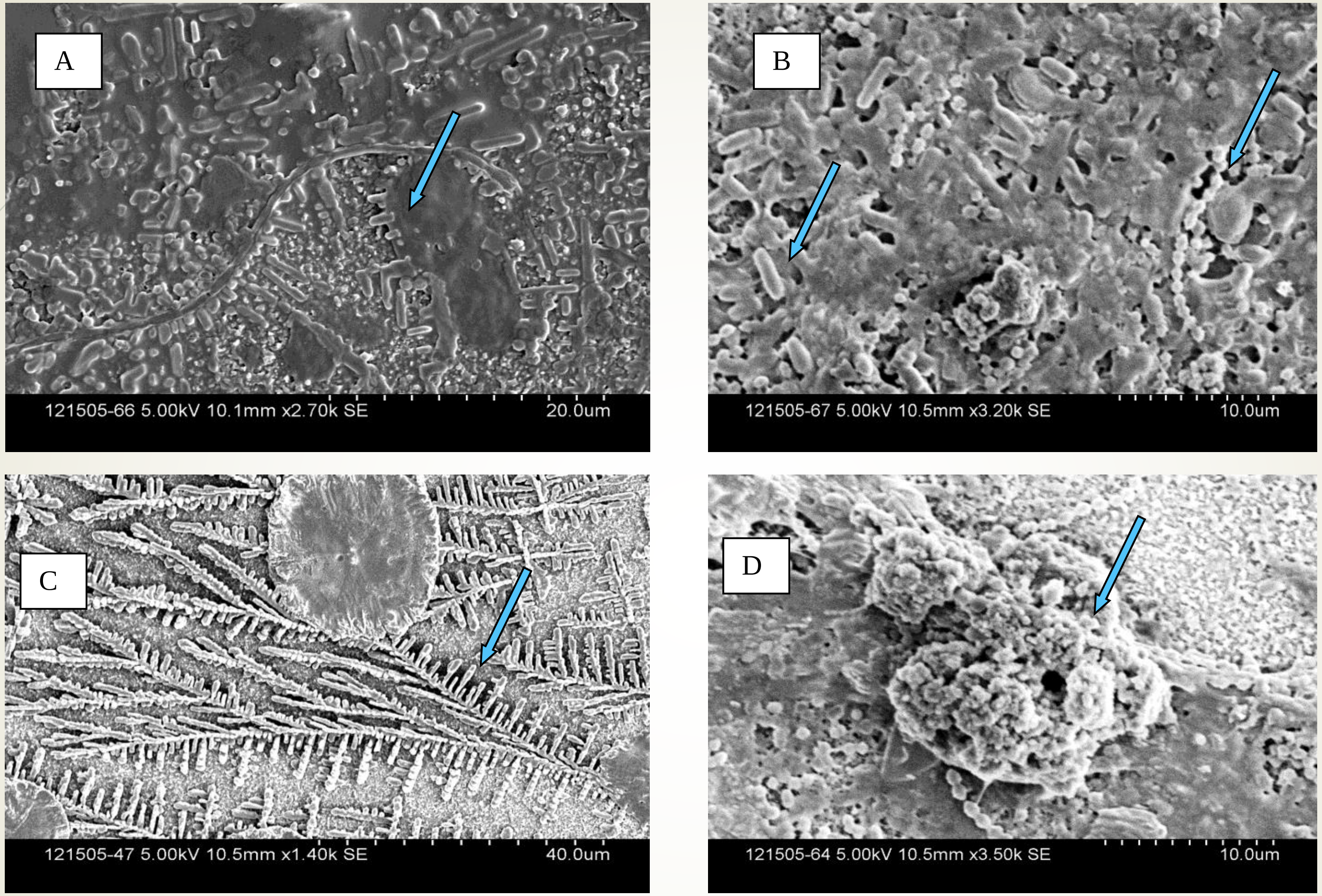
SEM showing different biofilm structures on aged membranes



12mo-old membrane showing monolayer of rods and cocci connected by EPS (A), 14mo-old membrane showing rods and cocci embedded in biofilm matrix (B), Distinct biofilm structures (C&D) *(Hassan et al., 2010)*

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Evidence of cross contamination of whey retentate

- In a study the presence of the same *Bacillus* sp. was reported in both membrane biofilm and the corresponding whey retentate
- The absence of this species in the feed whey confirmed the cross contamination of the retentate from the biofilm embedded bacteria

(Anand et al., 2012)

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Resistance of biofilm embedded bacteria to regular CIP

- Biofilms embedded bacteria are more resistant to cleaning as compared to their planktonic counterparts
- The existing CIP protocol from a whey concentration plant was tested against the biofilm consortia isolates obtained from used whey RO membranes (2 to 14 months old)
- The six steps CIP process was based on; alkali, surfactant, acid, enzyme, a second surfactant, and a weekly sanitizer application
- The individual isolates showed a great variability in their sensitivity towards different chemical treatments
  - In general, the spore forming *Bacillus* spp. showed greater resistance as compared to the other genera such as *Enterococcus, Escherichia, Streptococcus, Staphylococcus,* and *Klebsiella*

(Anand and Singh, 2013)

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Making CIP more effective using certain enzymes as biocleaners

- Different enzymes including a protease, lipase, lactase, a-glucosidase, and β-galactosidase were selected to evaluate their ability to degrade the biofilm matrix
  - A 24 hour old membrane biofilm formed by a resistant *Bacillus* isolate was used for this evaluation
  - β-galactosidase showed highest reduction in biofilm counts

(Anand and Singh, 2013)

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Credits

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