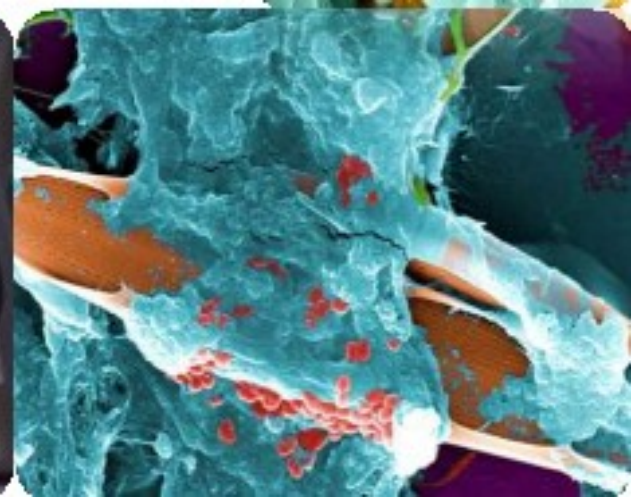
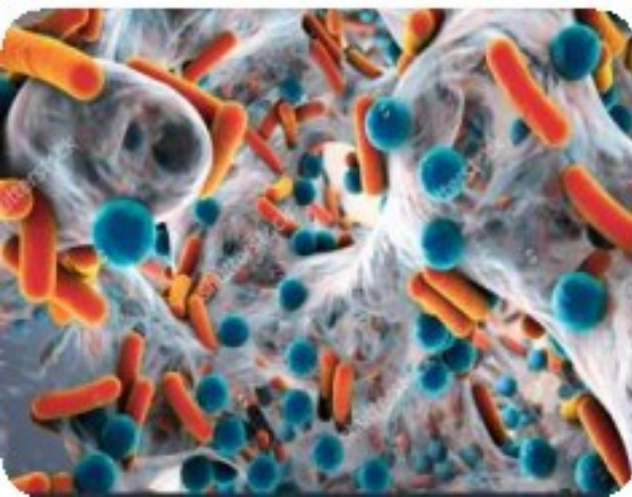


Biofilm formation during processing and their control measures



- Raw milk is an ideal culture medium for microorganisms.
- From farm to the consumer, milk comes into contact with the walls of the equipment in which it is being processed and transported.
- Since, European (and American) legislation has strict regulations concerning materials coming into contact with food and milk processing necessitates hygienic equipment material resistant to corrosion in alkaline and/or acidic conditions.
- **Requirements of materials in contact with food:** Material has to be chemical, bacteriological, and organoleptical neutral with regard to the food product, Material should be easy to clean so that the hygiene and appearance of the food product are guaranteed, and Durable

- **Stainless steel is mostly preferred in dairy industry due to its mechanical characteristics, thermal conductivity, and ease of use.**
- **Some range of plastics are food-approved (polypropylene (PP), polycarbonate (PC), high-density polyethylene (HDPE), polyvinyl chloride (PVC), and polytetrafluoroethylene) which is used with care.**
- **Surfaces of equipment used in food and milk processing and handling are commonly contaminated by microorganisms, even following cleaning and disinfection procedures. These contaminating microorganisms appear as adherent microorganisms or as more complex structures called **biofilms**.**

What is Biofilm??

- **Biofilm is an aggregation of microbial cells interconnected by extracellular polymeric substances which accelerate growth on different material surfaces adversely affect the dairy industry.**
- **Different biofilms exhibit different chemical and electrical properties.**
- **The cells coordinate among each other via intercellular communication using biochemical signalling molecules.**
- **Biofilms are generally resistant to nutritional and oxidative stress, desiccation, UV light exposure and sanitizing agents.**
- **Stainless steel, polyvinyl chloride, polyurethane are the prolific surfaces for biofilm attachment when they come in contact with food materials.**
- **The biofilm associated microbes are less susceptible to antimicrobial agents than those present in planktonic state. As a result, it becomes difficult to get rid of biofilms from the contact surfaces of food.**

Stages of bacterial biofilm development

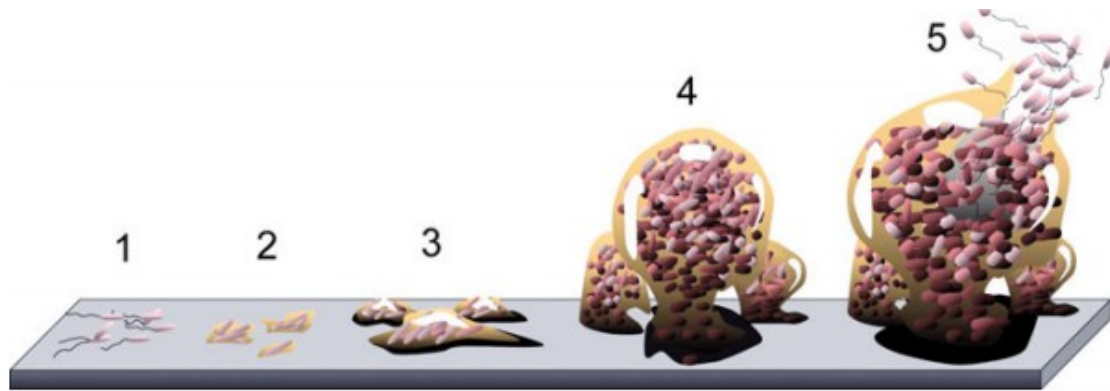
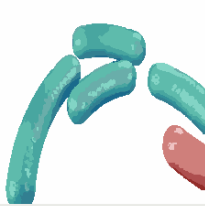

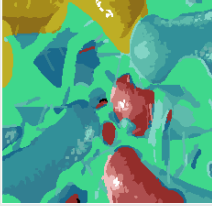
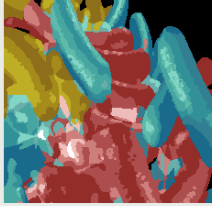



Figure 2. The five stages of biofilm development. Stage 1: Planktonic (free floating) bacteria adhere to the biomaterial surface. Stage 2: Cells aggregate, form micro colonies and excrete extracellular polymeric substances (EPS), i.e. slime. The attachment becomes irreversible. Stage 3: A biofilm is formed. It matures and cells form multi-layered clusters. Stage 4: Three-dimensional growth and further maturation of the biofilm, providing protection against host defense mechanisms and antibiotics. Stage 5: The biofilm reaches a critical mass and disperses planktonic bacteria, ready to colonize other surfaces. Figure adapted from Monroe²⁰ under the crea-

ATTACHMENT	GROWTH	MATURATION	DETACHMENT	RE-DEVELOPMENT
				
Bacteria attach to a variety of surfaces, from metal, to plastic, to skin tissue, using specialized tail-like structures.	The cells grow and divide, forming a dense matrixed structure, many layers thick. At this stage the biofilm is too thin to be seen.	When there are enough bacteria in the developing biofilm the bacteria secrete a slimy extracellular matrix of proteins and polysaccharides.	The slime protects the bacteria from the harsh environments, shielding them from many chemicals, antibiotics and immune systems.	As the colonies mature, the structures created weaken and cast off bacteria that look for new places to grow and prosper.

Biofilm Formation

Formation of a conditioning layer

- In dairy operations, the conditioning film mainly consists of organic milk components. This first stage occurs within the first 5–10 s after placement of an otherwise clean surface into a fluid environment. The conditioning also alters the physicochemical properties of the surface, such as surface free energy, changes in hydrophobicity, and electrostatic charges, which may affect the subsequent order of microbial events.

Bacterial adhesion

- During the second stage of biofilm formation, single bacterial cells are transported to surfaces and reversible bonds are formed between the cell wall and the substratum.
- Bacterial attachment is mediated by fimbriae, pili, flagella, and bacterial extracellular polymeric substances (EPSs) that act to form a bridge between bacteria and the conditioning film. The chemical structure of the EPS varies among different types of organisms.

- Biofilms accelerate corrosion and material deterioration.
- Dead ends, corners, cracks, crevices, gaskets, valves, and joints are all possible points for biofilm formation.
- Biofilms do not possess a uniform structure. The structures that are formed depend on a large variety of intrinsic and extrinsic factors such as species, temperature, flow conditions, pH, presence of salts, nutrients.

Biofilm formation and dispersal

- Once colonization has begun, the biofilm grows by a combination of cell division and recruitment. Polysaccharide matrices typically enclose bacterial biofilms. The final stage of biofilm formation is known as dispersion, and is the stage in which the biofilm is established and may only change in shape and size. Dispersal of cells from the biofilm colony is an essential stage of the biofilm life cycle. Dispersal enables biofilms to spread and colonize new surfaces.

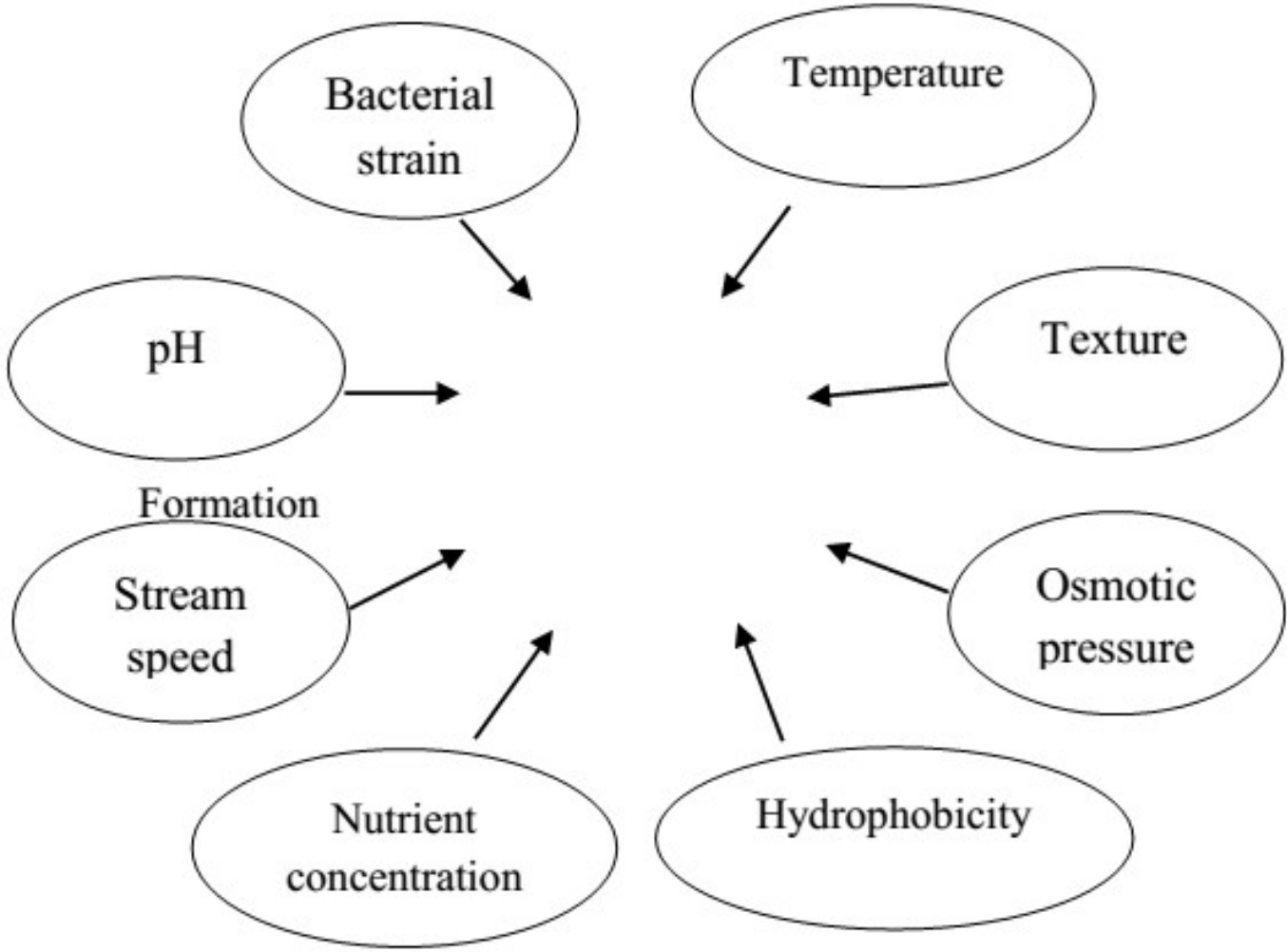
Biofilms in Dairy Industry...

Dairy industry is of utmost concern among food processing plants. In dairy plant, biofilms develop on floors, walls, drains, interior and exterior of dairy equipment and machines, transporting pipes and working surfaces. These can be seen in all product contact surfaces like milk cups at dairy farms to heat exchangers at the processing plants. This poses a great risk to safety, security and quality of dairy products and to the health of consumers who get exposed to such products.

Biofilm prone areas in Dairy Industry

Sampling point	Material	<i>Pseudomonas</i>	<i>Staphylococcus</i>
Balance tank	Steel	-	-
Aging tank	Steel	-	+
Conveyer belt of packaging machine	Rubber	+	-
Silo, welded joints	Steel	+	+
Air separators, inside	Steel	+	+
Tank truck, valve, gasket	Rubber	+	+
Milking tube	Rubber	+	+

Factors Affecting Biofilm Formation



Dairy Microbes Associated with Biofilm Formation

- Microorganisms occurring in the food industry could be a source of secondary contamination in food products. Their attachment to food contact surfaces in dairy plants and subsequent biofilm formation pose a risk of contamination in milk and milk products.
- Multispecies bacterial biofilms takes place by *Staphylococcus* and *Pseudomonas* which allow the adhesion of other pathogens like *Listeria monocytogenes*. In such biofilms, these microorganisms express an increased resistance to environmental stresses and can persist on food processing equipment.
- Important biofilm forming genera of dairy industry are *Bacillus*, *Pseudomonas*, *Listeria*, *Lactobacillus*, *Staphylococcus*, *Streptococcus*, *S. enterica* serovar *Typhimurium* and *Cronobacter sakazakii* etc.



Public Health Significance with Biofilm Formation

- **Biofilms contribute in the deterioration of food quality due to the action of various microbial enzymes like lipases, proteases.**
- **Because of the catalytic reactions the biofilms persuade corrosion of metallic food surfaces.**
- **Microbial quality of the products becomes inferior due to remarkable decrease in the heat transfer efficiency of the surfaces.**
- **The bacterial biofilms can persist in dairy plants and can potentially reduce the shelf-life of the pasteurized milk, cream, cheese and the other milk products.**
- **Food borne bacterial contamination leads to gastroenteritis creating a serious threat to public health and disbalance to the livelihood of mankind.**

Prevention and Control Measures of Biofilm....

- **Cleaning and Disinfection:** Most common measure of arresting the biofilm in the dairy industry is cleaning and disinfecting of all sites, equipment and instruments especially in the alarming zones. It prevent the development and spread of biofilms on the surfaces and in the food items. The efficiency and intensity of cleaning directly affect the final quality of the prepared products. Poor cleaning procedures are the causes of retention of biofilms as disinfectants are unable to enter the matrix of biofilms. Chlorine and ozone were effective for inactivating biofilm microflora.
- **Natural compounds:** Natural compounds like various plant extracts, honey, essential oil etc. are effective against microbial biofilm formation. Honey is a natural product having antimicrobial properties against about 60 spp. of bacteria and fungi. It was found that honey inhibited *Enterococcus* spp. biofilm production and reduced biofilm formation of EHEC O157:H7. Cumin oil and cinnamon oil are used in food industry for their aroma as well as potent antibiofilmers.

CIP wash steps for circuits with pasteurizers and other equipment with heated surfaces (UHT, and others)

- 1 Rinsing with warm water for about 10 min.
- 2 Circulation of an alkaline detergent solution (0.5%–1.5%) for about 30 min at 75 °C.
- 3 Rinsing out alkaline detergent with warm water for about 5 min.
- 4 Circulation of (nitric) acid solution (0.5%–1.0%) for about 20 min at 70 °C.
- 5 Postrinsing with cold water.
- 6 Gradual cooling with cold water for about 8 min.

CIP wash steps for circuits with pipe systems, tanks, and other process equipment with no heated surfaces

- 1 Rinsing with warm water for 3 min.
- 2 Circulation of a 0.5%–1.5% alkaline detergent at 75 °C for about 10 min.
- 3 Rinsing with warm water for about 3 min.
- 4 Disinfection with hot water 90–95 °C for 5 min.
- 5 Gradual cooling with cold tap water for about 10 min (normally no cooling for tanks).

- **Bio-cleaners (enzyme-based detergents):** Mixture of enzymes promote quicker degradation of biofilm.
- **Phages:** Phages having polysaccharide degrading enzymes can destroy biofilms rapidly and upto 80% of *Pseudomonas fluorescens* biofilms can be destroyed by phages and the bacteriophage T4 found to be effective against *E. coli* biofilms.
- **Biosurfactants:** Biosurfactants from *Lactococcus lactis* 53 inhibit biofilm formation on silicon rubber of the equipments. The Surfactin produced by *Bacillus subtilis* prevents *Salmonella enterica*, *E. coli* and *Proteus mirabilis* biofilms.
- **Other sources:** In dairy processing sectors biopreservatives like nisin, reuterin, pediocin protect from biofilm formation by various microbes including *L. monocytogenes*. Quorum sensing property of the microbes can be inhibited in order to prevent biofilm production at its very early stage. The use of ultrasound is one of the most recent advances to control and prevent microbial biofilm.