Formation and detection of biofilms

Børge K. Mortensen

Bactoforce International A/S

Biofilm, also referred to as biofouling, is an assemblage of microbial cells attached to a surface and encapsulated in a film or slime layer of extracellular polymers. Biofilms can cause major hygienic problems in the food and beverage industries, because the content of bacteria, including spoilage and pathogenic organisms can be very high, and these cells will gradually become detached and cause contamination during production. Microorganisms in biofilms are packed closely in a matrix that acts as a barrier to cleaning and disinfection, which makes it difficult to remove an established biofilm. Biofilms can form inside processing equipment and on open surfaces and will often lead to financial losses caused by food spoilage, food safety problems, and loss of production efficiency.

Problems related to microbial contamination in the food and beverage industries have been studied for more than a century. However, most of the knowledge acquired over the years relates to single-cells in a free-floating state, but today it is generally accepted that microorganisms grow and survive in organized communities where their physiology is very different. This has only been realized within the past few decades and is still not well understood.

Biofilm formation

Biofilms will form on almost any material where nutrients are available, but it happens more likely, if the attachment surface is rough, scratched, cracked, or corroded. Physical conditions, such as hydrophobicity, surface electrostatic charge, and fluid flow rate also affect the attachment. Several studies have shown that microorganisms attach more rapidly to hydrophobic, nonpolar surfaces such as Teflon and other plastics than to hydrophilic surfaces like stainless steel so some kind of hydrophobic interaction apparently occurs, which enables the cells to overcome the repulsive forces (Donlan, 2002).

A thin film of organic and inorganic components will quickly form a coating layer on a surface in contact with an aqueous fluid. This creation of a so-called conditioning film is an important step in the formation of biofilms, because it changes the properties of the surface so microbial adhesion becomes possible by electrostatic and van der Waals attraction forces.

A wide variety of microorganisms can form biofilms both gram-positive and gram-negative bacteria, yeast, and fungi, but some do it more easily than others, because

they produce extracellular attachment fimbriae, pili, or flagella. A single microbial species may form a biofilm, but it is more common that a mixed bacterial population is involved. Bacteria cells in biofilms communicate with each other via diffusible organic signal molecules that regulate gene expression, a phenomenon known as quorum sensing. This allows cooperation among cells in a way that benefits the entire biofilm population by regulating the supply of water and nutrients to the individual cells and the removal of waste products. This cooperation increases the level of protection, so that the cells resistance to hostile environments improves (Tarver, 2009).

Biofilms develop stepwise in a complicated process (Agle, 2007). First, individual cells attach to the conditioning film in an unstable and reversible manner, where it is still relatively easy to remove the developing biofilm by rinsing. In the next step, the cells secrete polymeric material that binds them more firmly in a heterogeneous, three-dimensional structure of extracellular polysaccharides (EPS), proteins, nucleic acids, fats, and water in which microorganisms are densely packed in clusters (Figure 1). This is an irreversible process, and now it is difficult to remove the formed biofilm by cleaning. Finally, the biofilm begins to disperse cells so they can move on to initiate formation of new biofilms elsewhere.

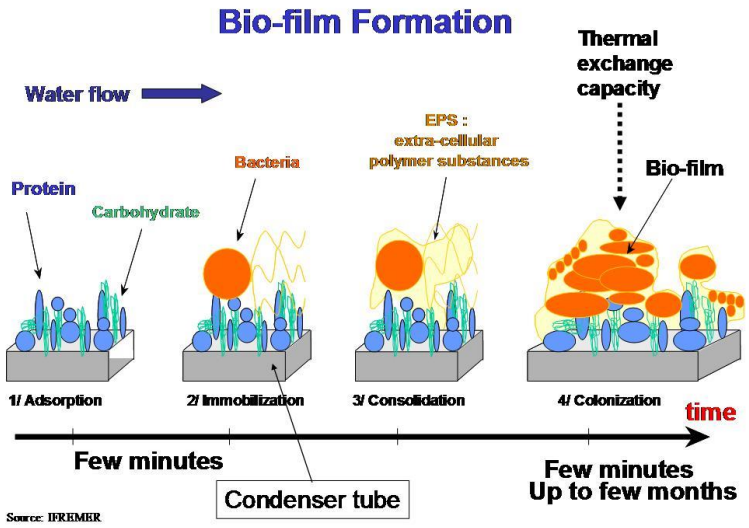

Fig.1. Biofilm formation in a condenser tube. (From Wikimedia Commons).

Critical locations in processing facilities

Insufficient cleaning caused by low cleaning temperature, cleaning flow, cleaning time, or concentration of detergents and disinfectants favours development of biofilms. Other causes of insufficient cleaning are damaged surfaces caused by corrosion or cracking, and inappropriate hygienic design, for example, around manholes, pipe connections, and agitators in storage and processing tanks. This will often make tanks and containers critical locations in dairies, breweries, wineries, and soft drink factories.

Creation of biofilms may also occur in pipelines, valves, and pumps, especially under gaskets and O-rings in joints and fittings. In addition, spots with problematic weldings are susceptible to biofilm accumulation. Other critical locations are floor drains, doormats, and areas that are difficult to reach in a cleaning process such as, for example, undersides of conveyor belts.

Plate heat exchangers, pre-heaters in evaporators, and coolers are among the most critical areas for biofilm accumulation because temperature in some sections of such equipment is favourable for bacteria growth. Biofilm formation in heat exchanger can occur due to sediments of burned particles of heat-denatured organic components primarily proteins or due to corrosion, cracks or holes in the plates. Such leakage results in influx of product to the waterside of the plates where microorganisms can form biofilms, which is not reached in a cleaning process. If the pressure in the equipment fluctuates, dispersed microorganisms may be sucked back through the leak into the product stream.

Filtration membranes are also important locations for accumulation of biofilms mainly because they are more complicated to clean than stainless steel surfaces, but also because they provide a large surface area for microbial colonisation. Fillers and bottling machines represent another critical area with many niches where biofilms can develop and contaminate fluid or semi-fluid products. Particular vulnerable spots are filler nozzles and conveyors.

Detection of biofilms

The problems caused by biofilms in the food and beverage industries make it clear that such sediments have to be removed by cleaning. This raises two vital questions: How can critical spots for biofilm accumulation be identified, and how can the effectiveness of the applied cleaning procedure be monitored?

An indirect and traditional procedure is to count the number of viable microorganisms by classical microbiological methods in samples taken at strategic positions in the production line and in the manufactured products. These methods are time-consuming and retrospective, as it can take days before the results are available, which is often too late because the manufactured products at that time may have left the premises.

There are several advanced methods available for detecting and studying biofilms in research laboratories, for example, microscopic, spectroscopic, and immunological techniques. However, these methods are complicated and require sophisticated instrumentation and highly specialized personnel, so such methods are not very suitable for practical use in industry.

In industrial environments, the simplest monitoring method is to use the human senses for visual inspection. Organic sediments appear as whitish or yellow coatings. They are not always detectable with the naked eye, but clearly visible under UV-light because the molecular configuration of organic material will cause sediments to fluoresce when illuminated by UV light. In addition, UV light inspections in connection with penetrant testing are useful for identifying damaged surfaces and surfaces not hit by the cleaning spray, which indicates spots favourable for biofilm formation (Mortensen, 2014). There are many advantages of using UV methods, they are inexpensive, readily available, do not require direct contact with the surface, and large areas are quickly inspected. Another advantage is that the outcome of an inspection is viewed directly on the tested surface, so that a visual presentation of the problem immediately appears. Biofilm formation in pipelines can effectively be monitored by endoscopic video inspections combined with UV illumination. This method can also be used for inspection of weldings in pipelines in order to reveal locations in which biofilms may assemble (Bactoforce, 2013).

Microbial contamination on accessible surfaces can be evaluated by swab methods such as the ATP swab test. ATP (adenosine triphosphate) is the principal energy carrier of all living organisms and measurement of ATP bioluminescence is a widely used rapid method for monitoring contamination. After swabbing of a surface and measuring the probe in a portable instrument, the result provides an estimate of the cleanliness. The method is easy to use, but the cost per analysis is relatively high and swab tests are not particularly useful when large areas need inspection. In addition, the detection limit of the ATP test is relatively high. It should also be realized that a biofilm may stick so tight to a surface, that swabbing only removes a small part of the film. This is especially critical in cases where swabbing is done after disinfection, where the outer layer of the film is inactivated, while an active flora still exists deeper in the three-dimensional structure of the biofilm.

In closed systems, application of a swap test is not possible; instead testing of rinse water can be used for validation of the cleaning efficiency. The rinse water is added upstream of the process and collected downstream. This has the advantage of sampling a large and inaccessible surface area without disassembling of the equipment. The rinse water can be tested by ATP bioluminescence or by measuring total organic carbon (TOC). TOC comprises both microbial organisms and organic sediment and the method is widely used in the pharmaceutical industry, where the hygiene standards are very high. Bactoforce International A/S has now developed an inspection method called CIP-Validation for use in the food and beverage industries. The method is based on measuring TOC in rinse water after cleaning and breaking up of organic sediments by adding an oxidative agent to the rinse water. This procedure makes it possible to validate the cleaning of closed systems, such as heat exchangers, membrane filtration units, pipelines, fillers, and bottling installations without

disassembling and with immediate presentation of the result (Mortensen, 2014). The determination of TOC is carried out according to ISO Standard 8245:1999.

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