

# Problems caused by biofilms

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Biofilms will form on critical locations in processing plants in the food and beverage industries, where nutrients are available and where cleaning and disinfection are inadequate. Formation of biofilms can lead to a number of problems such as product spoilage, food safety problems, and loss of production efficiency. Countermeasures against biofilms are therefore in focus in good manufacturing practice.

A general problem caused by biofilms is that some cellular by-products accelerate corrosion of stainless steel. Other problems are that biofilm formation in heat exchangers reduces the fluid flow and the heat transfer; and in membrane filtration units, biofilm will block membrane pores, which decreases the flux of permeate, degrades the membranes, and reduces the trans-membrane pressure.

The type of microorganisms found in biofilms in industrial environments depends highly on the available nutrients and on the temperature. Spoilage organisms present in biofilms in production lines will contaminate the product stream either by continuous release of individual cells or by breaking loose layers of biofilms, releasing large numbers of bacteria. This will reduce the shelf life of the manufactured products and if pathogenic microorganisms are part of the microbial community in the biofilm, this may compromise consumer safety.

## Biofilms in the dairy industry

Microorganisms forming biofilms in the dairy industry will often cause problems because dairy products are very sensitive towards contamination. Biofilms on surfaces in contact with raw milk stored at low temperature are dominated by psychrotrophic microorganisms typically *Pseudomonas* spp. such as *Pseudomonas fluorescens*, which is one of the most studied biofilm forming organisms. They can grow at low temperature, but higher temperature increases their growth rate and accelerates the formation of biofilms in farm cooling tanks and in silo milk tanks at dairy factories. Heat treatment will normally eliminate these bacteria, but their very heat stable, extracellular lipase and protease enzymes will in active form end up in the final products where they will reduce the shelf life by causing strong off-flavours such as bitterness, rancidity or aged taste.

Thermotolerant and thermophilic bacteria especially species of *Bacillus*, *Clostridium*, *Micrococcus*, *Streptococcus*, and *Lactobacillus* able to survive heat treatment will often colonise stainless steel surfaces downstream from the pasteurisation section and

will rapidly grow to large numbers. Bouman *et al.* (1982) found up to  $10^6$  bacteria per  $\text{cm}^2$  in regenerative sections of pasteurisers after 12 hours of operation. This will cause contamination for example with *Streptococcus thermophilus*, which is reported to reach a contamination level of about  $10^7$  cells per mL in milk after biofilms have developed for 8 hours in a pasteuriser (de Jong *et al.*, 2002). In other environments biofilms may take several days or weeks to develop.

Endospores produced by genera like *Bacillus* and *Clostridium* are able to survive high heat treatment. These spores can germinate in the manufactured products when conditions become favourable or they can colonise the process equipment and become a significant source of steady contamination. *Bacillus subtilis*, and *Bacillus cereus* will often cause sweet coagulation and bitter taste in milk and cream and the gas producing *Clostridium tyrobutyricum* may cause spoiled texture and late-blowing in semi-hard cheeses. In addition, spore forming thermoduric and thermophilic bacteria are commonly found in high numbers in milk powder after 16-20 hours production time due to biofilms formed on the large internal surface in evaporators and spray dryers.

Several pathogens like *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli* O157:H7, and species of *Salmonella* and *Staphylococcus* can also produce biofilms or be part of biofilm communities. These organisms are of concern, but also *Enterobacter sakazakii*, which grows well in biofilms, has been highlighted as an important safety risk in the milk powder industry, particularly in relation to production of infant formulas.

## **Biofilms in breweries**

Biofilms found in breweries are important reservoirs of spoiling organisms although beer is generally a hostile environment for most microorganisms due to the low pH value and the high content of ethanol and bitter hop components (Jespersen & Jakobsen, 1996). Bacteria adapted to brewery environments such as species of *Lactobacillus*, *Pediococcus*, *Pseudomonas*, and *Bacillus* and yeasts of the genera *Saccharomyces*, *Candida*, and *Debaryomyces* are found in biofilms in tanks, filters, pasteurisers and fillers where they can cause spoilage of beer for example sour taste and buttery off-flavour. The most common beer spoilage organisms appear to be *Lactobacillus brevis* and *Pediococcus damnosus* found in fermenting wort in the mashing process where they slow down yeast performance. Timke (2004) reported that about 25% of the biofilms found in a number of German breweries contained microorganisms able to multiply in beer. This clearly demonstrates that brewery biofilms will influence the quality of the products. On the other hand, pathogenic organisms are seldom found in standard beer products, where they are generally unable to grow (Storgårds *et al.*, 2009).

## **Biofilms in wineries**

Biofilms can also cause problems in wineries where microorganisms present on grapes form biofilms in fermentation and storage tanks, pumps, valves, and fillers. Although the high level of alcohol and acidity in wine inhibit development of microorganisms, it is wellknown that lactic and acetic acid bacteria and spoilage yeasts can survive in wine. Lactic acid bacteria cause malolactic fermentation, which is beneficial in some cases and undesirable in others if it forms unwanted flavour compounds resulting in mousy taint, bitterness, and overt buttery taste (Dharmadhikari, 1992). Flavour defects can also occur as a result of growth of *Leuconostoc oenos* and various species of *Lactobacillus* and *Pediococcus*. Traditionally, sulphur dioxide has been used to control contaminating microorganisms in winemaking but some bacteria species tolerate even high concentrations of sulphur dioxide and the overall trend to reduce the use of chemicals in the interest of public health has strengthen the focus on other means for prevention of biofilms in wineries.

## **Biofilms in soft drink and juice factories**

Many soft drinks and fruit juices are restrictive environments for microorganisms because of high sugar content, high acidity, or special ingredients with antimicrobial properties. However, biofilms can form in pasteuriser and filling equipment where processing runs of 24 hours are not unusual. Species of *Lactobacillus*, *Leuconostoc*, and other lactic acid bacteria are known to cause spoilage of fruit juices; and pathogens like *Escherichia coli* O157:H7 and *Salmonella* have been isolated from apple juice and apple cider probably contaminated via biofilms. Soft drinks rarely represent health risks, but some bacteria, and a variety of yeasts and molds are associated with spoilage of such products.

## **Biofilms in aquaculture**

Aquaculture, also referred to as aqua-farming, where marine organisms are cultivated in seawater, usually in sheltered coastal waters, is a very fast expanding industry worldwide. Biofilm formation in water recycling systems especially in the fry and juvenile fish-handling sector is problematic because biofilms will incorporate and multiply the microflora present in the water. Biofilms in breeding tanks and filtration units, pipelines, and pumps should be checked regularly in order to avoid outbreak of diseases, which may cause high financial losses. Diseases can be caused by pathogenic organisms such as species of *Listeria*, *Salmonella*, *Shigella*, *Vibrio*, *Bacillus*, and *Aeromonas*, all of which have been found in biofilms in aquaculture environments.

## **Prevention and removal of biofilms**

The many problems caused by biofilms in the food and beverage industries and several outbreaks of foodborne illness attributed to biofilms make it obvious that prevention and removal of biofilms are important. Many studies carried out in the past couple of decades have contributed to a more comprehensive understanding of the conditions that promote biofilm formation but more knowledge about the ecology and diversity of mixed microbial communities is still needed. In addition, it appears worthwhile to study the factors that affect microbial attachment on surfaces in order to be able to construct anti-adhesive materials with antifouling layers for example by making use of nano-technology. Another interesting research topic is the communication system (quorum sensing) between microbial cells in biofilms and the potential of retarding or impeding it, for example by enzymatic degradation of the signal molecules that control the microbial collaboration.

From an industrial perspective it is important to identify critical locations where biofilms can accumulate so such sites can be monitored regularly. The overall strategy for actions against biofilm in the food and beverage industries is to clean and disinfect before microorganisms have attached firmly to surfaces in mature biofilms. The various cleaning parameters may vary depending on the industry, the equipment, and the products manufactured, but if it turns out that the used procedure is inadequate prolonged exposure time, higher temperature, higher flux, and higher concentrations or other combinations of detergents, and disinfectants should be tried. In some cases use of oxidative agents such as, for example, hydrogen peroxide, sodium hypochlorite, peracetic acid, and ozone can be used for breaking-up biofilms. This procedure has gained a certain interest in some industries. Newer cleaning technologies based on use of enzymes for degradation of sediments are another interesting option.

Regardless of the used cleaning and disinfection strategy it is advisable to perform regular inspection of the hygienic standard of processing installations. Bactoforce has for more than 20 years conducted thousands of inspections of tanks, pipelines, pasteuriser, coolers, membrane filtration unit and fillers in various industries across Europa. This has clearly demonstrated that such inspections are a valuable supplement to an already established quality control programme because it helps prevent microbiological hazards and substantial financial losses.

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