

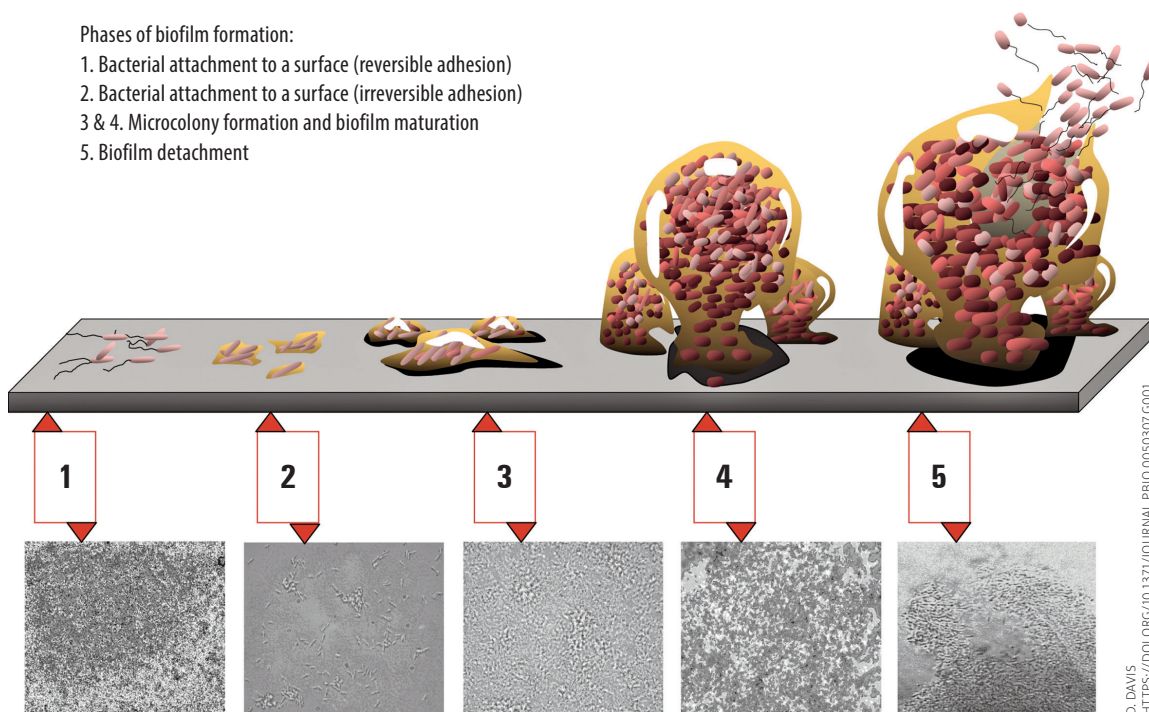


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Phases of biofilm formation:

1. Bacterial attachment to a surface (reversible adhesion)
2. Bacterial attachment to a surface (irreversible adhesion)
- 3 & 4. Microcolony formation and biofilm maturation
5. Biofilm detachment



D. DAVIS
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BACTERIAL “CITIES OF SLIME”

Foodborne diseases pose a major public health problem in both developed and developing countries. Bacteria that form biofilms are especially difficult to combat and require careful countermeasures.

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Bacteria have the ability to adapt to changing and diverse environmental conditions, so they can live practically throughout the entire biosphere. One defense mechanism that helps bacteria to survive involves forming structures known as biofilms. A biofilm can be defined as a consortium of microorganisms in which cells stick to each other and to a solid

surface. Microorganisms that form biofilms have the ability to adapt to their environment. They are often embedded within a slimy matrix called “glycocalyx” or simply “slime,” made up of self-produced extracellular polymeric substances (EPSs). EPSs cause the biofilm to become immobilized on the surface, while also allowing it to adapt to changing environmental conditions. In addition, the biofilm provides a physical and structural barrier against mechanical and physical stimuli and promotes genotype protection. Because the bacteria living in a complex three-dimensional biofilm are numerous and form a community of sorts, biofilms can be thought of as tiny bacterial “cities of slime.” Biofilms formed in food processing

environments, for example, are especially significant because they can act as a persistent source of microbial contamination, which can lead to food spoilage or disease transmission.

Biofilm

Most bacteria form biofilms in order to adapt to adverse environmental conditions. Biofilms are most commonly found on moist surfaces, such as food, food processing equipment, water and industrial pipelines, medical devices, air ducts, and human tissues and organs. Bacterial biofilm formation is determined by multiple factors, ranging from the characteristics of the microorganisms and the properties of the substrate on which the biofilm is formed, to the environmental conditions and availability of food for the bacterial cells.

Biofilm formation typically consists of four phases: adhesion (bacterial attachment to the surface), microcolony formation, bacterial biofilm maturation, and dispersal. The initial phase depends on the interactions between environmental changes and bacterial signal regulation. Adhesion is primarily influenced by surface properties, such as roughness and hydrophobic interactions. Bacterial cells attach to hydrophobic surfaces faster than to hydrophilic ones. Hydrophobic surfaces are ones that repel water molecules, and so water runs off them. Hydrophilic surfaces, on the other hand, tend to form bonds with water molecules, and so water tends to “stick.” Hydrophobic cell surfaces, the presence of fimbriae (hairlike appendages that allow bacteria to attach to other cells/surfaces) and flagella (organelles that grow from the surface of bacterial cells and provide motility), and the level of extracellular polymers produced by bacteria are the main factors that affect the rate and degree of bacterial adhesion to various substrates.

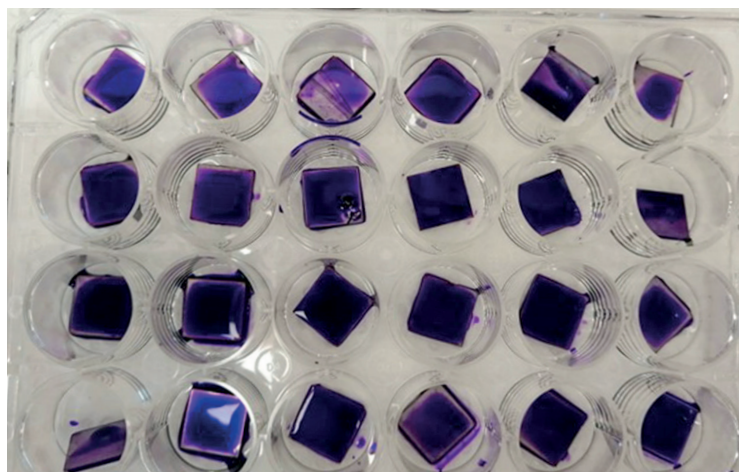
Bacterial adhesion to surfaces can be reversible or irreversible. In reversible adhesion, bacteria accidentally attach to the surface through certain extracellular organelles such as flagella, pili, and a small amount of EPS. At this stage, the biofilm can be relatively easily removed using chemical and physical techniques. When the bacterial cells are located within 1.5 nm of the adhesive surface, the strength of hydrogen bonds and hydrophobic interactions grows. Bacteria cover the surface they inhabit with a single layer, which is followed by increased EPS synthesis and secretion, which in turn causes the adhesion of more microbial cells. Adhesion then becomes irreversible, with strong attachment of microorganisms to the surface. This is followed by microcolony formation. Here, a mechanism called quorum sensing comes into play – as an internal system of communication for bacteria. It involves the secretion of signaling molecules that regulate the expression of the relevant gene for EPS secretion.

In the following phase of biofilm formation, bacterial cells multiply and differentiate, and pass acquired properties onto neighboring or daughter cells. Biofilm maturation and architecture are chiefly regulated by signals transmitted by various bacteria. The final phase in biofilm formation involves the detachment of cells from the biological membrane and their dispersal into the environment. Here, two categories of mechanisms come into play: active and passive. Active dispersal involves mechanisms initiated by bacteria that can actively detach from the biofilm to start a new cycle of colonization. Passive dispersal occurs when cells become detached because of external forces. Biofilm dispersal may result from increased shear stress, the absence of nutrients, or biochemical changes inside the bacterial cells.

Food

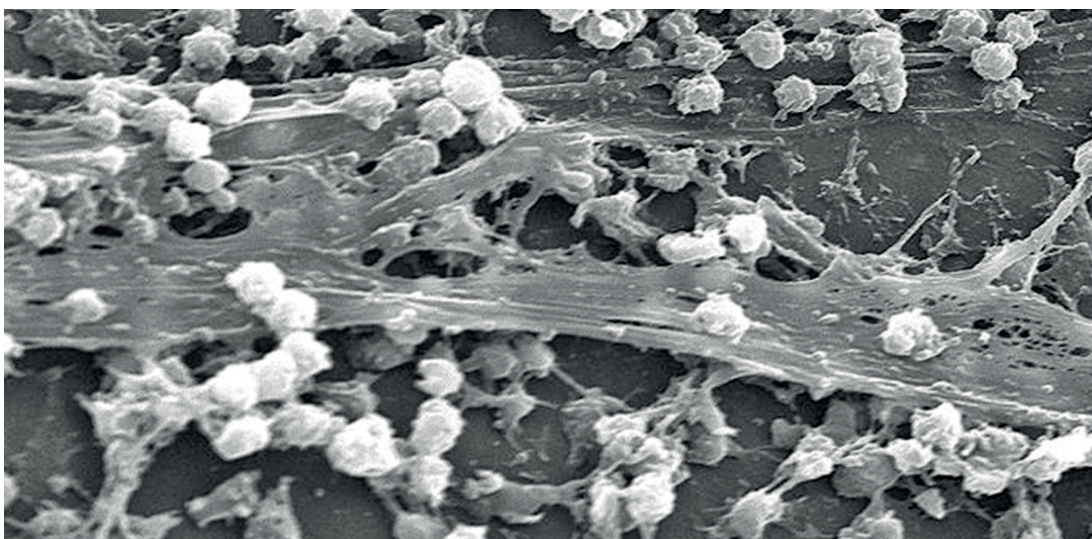
Once formed, a biofilm can impact negatively on sanitary conditions in the food industry and lead to cross-contamination of products. One example of perishable products prone to microbial contamination is milk. Bacteria of the genera *Enterobacter*, *Listeria*, *Micrococcus*, *Streptococcus*, *Bacillus*, *Pseudomonas*, and *Staphylococcus* are isolated in dairy processing plants. In the case of these pathogens, biofilm formation may lead to their spread and so to food poisoning. Other microbiologically sensitive products include fish and seafood. In fish processing, special attention should be paid to the proper hygiene of equipment and water, as its absence may contribute to cross-contamination of products with such bacteria as *Vibrio cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *Listeria monocytogenes*, *Salmonella* spp., *Bacillus* spp., *Aeromonas*, and *Pseudomonas* spp. Despite many treatment and disinfection procedures, seawater remains contaminated with the biofilm formed by *Vibrio* spp. in the water distribution system. *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp.

Analysis of biofilm formation by pathogenic strains on a stainless-steel surface



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Staphylococcus aureus biofilm on the surface of a medical catheter. The bacteria (round shapes) secrete a complex "slime" that helps them to attach to the surface and protects them from attack by antibiotics



CDC/RODNEY W. DONLAN, PH.D.; JANICE CARR (PHIL #7488), 2005

isolated from raw fish and surfaces in fish processing plants have the ability to form biofilm. In poultry processing plants, in turn, a major problem is posed by contamination with *Salmonella* spp. and *Campylobacter jejuni*. The most common sources of these pathogens are dust, feed, feces, and the transport of live poultry. A serious microbiological hazard may be posed by ready-to-eat food products. Strains of *Vibrio parahaemolyticus*, *V. vulnificus*, *V. fluvialis*, *V. alginolyticus*, *V. cholerae*, *V. mimicus*, and *V. harveyi* have been isolated from ready-to-eat shrimp, and more than 90% of these had the ability to produce biofilm. *Enterococcus faecalis*, *E. faecium*, *E. gallinarum*, *E. casseliflavus*, *E. hirae*, and *E. durans* bacteria capable of forming biofilm have also been isolated from seafood. In addition, ready-to-eat food products can be contaminated with a biofilm produced by *Listeria monocytogenes*.

Preventing contamination

Biofilms form relatively quickly, so in most cases it is hard to clean and disinfect surfaces and equipment often enough. It is necessary to accurately determine the appropriate frequency of disinfection to avoid biofilm maturation and the accumulation of absorbed organic material (product residues), which can impact on the hygienic status of the material and the availability of nutrients for bacteria. Recommended measures include monitoring the work time between the washing and disinfection of process lines, such as pasteurization lines in a dairy plant. Washing surfaces at short intervals is considered an effective way to prevent biofilm formation. Choosing the right equipment design and surface may facilitate washing and disinfection and lower the risk of biofilm formation. Various disinfectants used in the food industry include oxidizing agents (hydrogen peroxide, ozone, peracetic

acid), chlorine compounds, and surfactants. Chlorine compounds can effectively remove biofilm formed by *Staphylococcus aureus* and *Salmonella enterica*, but only from polypropylene and stainless-steel surfaces. For optimal disinfection, a combination of different agents should be used. For instance, sodium hypochlorite, copper sulfate, and hydrogen peroxide, when combined, can remove early-phase biofilms formed by *Escherichia coli* and *Klebsiella pneumoniae*. Using combinations of various substances may prove beneficial when a bacterial strain becomes resistant to one of the compounds.

To effectively prevent biofilm formation, it is essential to inhibit bacterial growth. In the food industry, food additives may exhibit bactericidal properties. For example, the orange pigment extracted from the *Monascus* mold inhibits the growth and development of *E. coli*. Fatty acid and sucrose esters, which are commonly used as surfactants, inhibit the growth and development of the following foodborne pathogens: *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli* O157:H7, and *S. typhimurium*. When combined with cinnamaldehyde (35 µg/mL) or citric acid (175 µg/mL), the antibacterial peptide nisin can combat the *L. monocytogenes* biofilm. Using food additives can significantly reduce the number of foodborne pathogens by limiting their capacity for biofilm formation.

Overall, biofilms that form in the food-production environment, on usable surfaces and machine components, are responsible for the spread of pathogenic microorganisms and may pose a significant threat, for example to the health of consumers. Effective prevention of biofilm formation in production facilities requires a well-prepared plan for maintaining surface and equipment hygiene as well as adherence to a sanitary regime. Implementing biofilm prevention measures not only helps ensure food quality but also crucially enhances food safety. ■

Further reading:

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