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Biofilms

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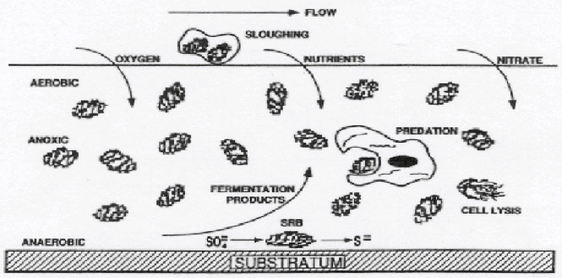
Chlorine, iodophors and most quaternary ammonium compounds are ineffective against removing them. Water at a temperature of 180°, the temperature permitted by the USDA in lieu of a chemical sanitizer, actually aids in their formation. Once they have established residence, they can cause pathogen contamination or decrease the shelf-life of food products. Biofilms are a mixture of microorganisms, components of food products, nutrients and exopolysaccharides (EPS), which are substances produced and excreted by the organisms that have attached to solid surfaces. As seen in the schematic below, biofilms can be thought of as a community of substances that once formed, take on a life of their own. Typically, anywhere that there is a flow of water, organisms and a solid surface, a biofilm can be formed. (Figure for Natural Biofilm).

Figure 4. Mature Biofilm



Shown are sloughing of the biofilm at the biofilm-water interface; the input of oxygen, nutrients, and nitrate from the aqueous phase; the aerobic, anoxic, and anaerobic zones resulting from limitations to oxygen diffusion; predation by an ameba; the production of fermentation by-products; cell lysis; and the activity of SRB.

An excellent example of a biofilm is the gastrointestinal tract, in which a large number and diverse array of bacteria colonize the lining of the intestinal mucosa. Biofilms in the gastrointestinal tract act in a protective fashion because they help to inhibit pathogen adhesion. However, in food applications, biofilm formation is destructive because biofilms can contain food-borne pathogens and spoilage microorganisms. As such, they can lead to product contamination or decreased shelf-life if the organisms contained within the biofilm are released into product.

In the dairy industry, improperly cleaned and sanitized equipment and air-borne organisms are considered the major sources of contamination of milk and milk products (2).

The solid surfaces that can harbor biofilms in food plants include stainless steel, aluminium, glass, nylon materials, Buna-N and Teflon seals. Surfaces that are pitted, scratched or cracked provide an excellent opportunity to trap food particles and bacteria, which begins the formation of a biofilm. Corrosion patches and dead ends are also areas where biofilms can grow. These settings are hard to reach giving optimal conditions for the formation and development of biofilms because they provide protection from sanitizers while being exposed to a water and nutrient flow.

Biofilm formation: Step one-the conditioning layer

Biofilm formation takes place in a step-by-step manner with the first step involving inorganic or organic molecules adsorbed to the surface creating what is termed a conditioning layer. Many times, proteins from meat or milk products are important elements of this conditioning layer because they actually aid in the adhesion of bacteria. Whey proteins, for example, have been shown to not only cause an increase in bacterial adhesion, but selectively increase adhesion of several milk-associated organisms (2). Studies have shown that in environments where nutrients are plentiful, the nutrients act as a bacterial primer that increases the ability of the bacteria to attach to a surface (5).

Biofilm formation: Step two-bacterial adhesion

Once a conditioning layer is formed, the next step in biofilm formation is the adhesion of organisms to this layer. There are many factors that effect bacterial attachment including, the pH and temperature of the contact surface, flow rate of the fluid passing over the surface, nutrient availability, length of time the bacteria is in contact with the surface, bacterial growth stage and surface hydrophobicity. Bacterial attachment is mediated by the use of fimbriae, pili, flagella and EPS that act to form a bridge between the bacteria and the conditioning film (1,2). The EPS bridge is actually a combination of electrostatic, covalent and hydrogen bonding, dipole interactions and hydrophobic interactions. Initially the bonds between the bacteria and EPS may not be strong and can easily by removed by flowing water. However, with time, these bonds are strengthened making attachment irreversible. At this stage, the removal of cells requires much stronger action such as scrubbing or scraping.

Biofilm formation: Step three-bacterial growth and biofilm expansion

There are many reasons why forming a biofilm for an organism is advantageous.
Once firmly attached to the surface, injured or small nutrient deprived cells can repair, metabolize fatty acids and proteins contained within the conditioning layer, grow and reproduce (3). As the bacteria grow and reproduce, they excrete greater volumes of EPS that provides a protective barrier around the cells (2). Also, as debris floats over the biofilm, it too can become trapped adding to the biofilm size and providing nutrient sources. Biofilms can form quickly and within 24 hours be considered "mature", but continue to grow to millimeter proportions in a matter of days.

At this stage, extensive scrubbing or scraping, in conjunction with sanitizers, is required for biofilm removal. Simply passing sanitizers alone over the top of the biofilms is not adequate for removal. This will remove only the top layer of the biofilm exposing the subsequent layers to nutrients flowing over them and actually hastening biofilm growth and development. In this manner, bacteria below the top layer actually benefit from the sanitizers because they are now directly exposed to the flow of nutrients. Repeated sanitizer applications tend to favor growth of bacteria producing large amounts of EPS that protect the cells from the sanitizers (3).

It is well established that bacteria contained within biofilms exhibit increased resistance to antimicrobial treatments compared to individual cells grown in suspension (2). As the biofilm matures, resistance against various disinfectants is greater than with younger (less than 24 hours) biofilms (2). The need for motility is also greatly reduced, reserving energy, as nutrients are directly within reach. Biofilms afford a protection that allows for extended bacterial longevity. For example, a study performed by the National Food Processors Association demonstrated an 85% recovery rate of bacteria sustained within a biofilm on a stainless steel surface that had been placed in a dry sterile environment (4).

Detachment and sloughing off of bacteria within biofilms

Periodically, large particles of the biofilm will slough off due to flow rate dynamics, the shearing effects of the fluid, chemicals within the fluid, or changing properties of the biofilm bacteria. The released bacteria can then be transported to a new location and restart the biofilm formation process (2).

Control and removal of biofilms

Needless to say, the best way of controlling biofilms is to prevent their development. Effective cleaning and sanitation, which combines physical and chemical methods within the program, will often prevent the accumulation of food product residues and bacterial cells on equipment surfaces. Cleaning by brushing, scrubbing and scraping surfaces is often necessary because once a bacterial cell is released from the protection of a biofilm, it is much less resistant to subsequent sanitizers. Breaking the EPS conditioning layer bonds is a goal of cleaning. Acid cleaners can be used to remove inorganic soil or material such as rust and using soft water for cleaning aids in the effectiveness of cleaning chemicals. Using water at 180°F will coagulate proteins on equipment, making them more difficult to remove and actually aids in the formation of a biofilm. Chlorine, iodophors and quaternary ammonium products have been shown ineffective at removing biofilms. However, peroxide and peroxide containing sanitizers have been found to be highly effective in removal of biofilms. Their reaction rate is very rapid (1-2 minutes) and they are relative non-corrosive.

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