Biofilms in Food Processing Plants

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Microorganisms are all around us—even on our own skin. We don't realize how many bacteria we are exposed to everyday because they can only be seen with a microscope and not with the naked eye. Figure 1 shows microorganisms on the surface of an apple under the scanning electron microscope (SEM, original magnification X 2,500).

All bacteria have a certain niche and remain in that niche, but when they have an opportunity to be in a new environment, they won't deny it. They become opportunistic, posing a hazardous threat to the new environment since they don't normally reside there. Thus, microbiological problems are found in every field, including the food industry.

Food processors should take extra precautions when handling not only food but also the equipment used to prepare it. Typically we think only in terms of pathogens and spoilage organisms contaminating fruits and vegetables, but we should also remember microorganisms can be found on stainless steel tables or packaging equipment used for processing. It is not only important to keep food free of contamination but also critical to maintain clean equipment.

Cleaning, Sanitizing Critical

One potentially damaging microbiological problem for today's food industry is formation of biofilms on food processing equipment. Because equipment is costly, it remains in service for long periods of time. If it is not properly cleaned and sanitized, microorganisms can aggregate on it and form biofilms. Sometimes the surface may appear to be clean after disinfecting, but the texture of the surface influences how thorough cleaning can be (1,8). If the surface has a rough texture, bacteria can hide themselves in crevices, where they are protected from bacteriocidal chemicals (1).

Bacteria attached to surfaces are more resistant to disinfectants than free-floating cells because they strongly adhere to the surface. This situation can be expensive for processors if equipment malfunctions or downtime is required to remove biofilms once they are discovered.

Figure 1: Microorganisms on the surface of an apple magnified 2,500 times.

Biofilms are simply layers of microorganisms, composed of the same or different species, bonded tightly to a surface. They can attach to high-tech, expensive machines or to stainless steel tables used for cutting meat. Biofilms may consist of anything including bacteria, yeast, molds, algae, etc. (7).

Microbes can attach themselves like glue to a surface by releasing their own biological material, exopolymeric substance (EPS). Made of carbohydrates and protein, this extracellular layer provides nutrients as well as protection against disinfectants and sanitizers. Bacteria attach to the surface and to each other, consuming available nutrients and growing into a lawn of microorganisms. This lawn of growth is visible as discoloration and corrosion. Microbes can also be the culprits in clogged water pipes.

Two Steps in Formation

For a biofilm to form, water (or any liquid) is needed because 85 percent to 90 percent of biofilms are water (4). Biofilm formation is a two-step. A reversible stage occurs when microbes are weakly bound to a surface by electrostatic and van der Waals forces (2). An irreversible stage occurs when the EPS helps microbes physically attach to a surface (13,14,).

If a surface is not properly cleaned and sanitized, residues of cut meat, for example, can remain, allowing bacteria to feast on rich nutrients and organic molecules. Such nutrient buildup is called a process-conditioning surface. It is also the first step to biofilm formation (16).

The buildup attracts bacteria through a concentration gradient to a surface and lays the groundwork for a lawn of growth. More organisms will grow on the bottom because more nutrients are on the surface. Subsequent layers of bacteria have fewer nutrients. They become adapted to nutrient-deprived conditions and can withstand harsh conditions better than surface bacteria (16).

The anatomical features of bacteria, including such things as flagella (a whip-like tail that enables movement) and cilia (hair-like projections on the cell that initiate movement and attachment), help in forming biofilms (1,2). Without flagella for motility, bacterial adherence decreases by 90 percent (12). The effectiveness of disinfectants may also depend on the peptidoglycan layer of the bacterium. In gram positives, the layer is thick (10-80 nm wide) whereas in gram negatives, it is thinner (2-3 nm wide). Therefore, being tightly attached to the surface can enhance resistance, but cell wall structure can also play a big role in disinfection. Gram positive bacteria have no outer membrane, while gram negative bacteria do. This membrane is composed of phospholipids, proteins, and lipopolysaccharides. These components are easily broken down with chemicals, making gram negatives more susceptible to cell lysis (destruction). If the organism also has an envelope, it is just another type of protection for the cell, especially if it's impermeable.

Terms of Attachment

In the food industry, microbes can attach to just about anything, including rubber, glass, polypropylene, and, stainless steel. The key extrinsic factors that play a role in cell attachment are temperature, time, and pH. Tests show more cells attach over longer periods of time, regardless of organism type. The rate of attachment due to these extrinsic factors, however, may vary according to the substratum to which cells are adhering (12). For example, biofilms form almost instantaneously (regardless of temperature and pH changes) on stainless steel rather than Buna-N rubber, but the detachment process is harder on rubber (12). Refrigeration temperature (4°C) and excessively warm temperatures inhibit biofilms, but room temperature (28°C) promotes growth (12). Research also shows changing pH from acidic to alkaline slows attachment (12). Lewis et. al. suggested that since the majority of surfaces in contact with aqueous solutions are negatively charged, electrostatic repulsion between bacteria and solid substrata would be greater at alkaline pH (12). Using proteolytic enzymes has also been shown to decrease attachment rates, thus proving proteins are involved in the initial attachment stage (12).

Researchers have used many applications to eliminate microbes, including agitation, environmental stresses, shock, and various chemical biocides. Since biofilms form on processing equipment, researchers have studied chemical disinfectants and sanitizers closely to determine which materials are most effective, yet safe for humans and the environment.

Chlorine vs. Ozone

Historically, the food industry has primarily used chlorine to rid processing facilities of microbes. It is the least expensive oxidizer and is considered very effective in killing microbes, but it can be dangerous in high concentrations (11). Chlorine is effective against biofilms because it can destroy the EPS and inhibit growth. Using chlorine at high concentrations for shorter periods is more effective against biofilms than low concentrations for longer periods. Chlorine dioxide is also an effective biocide against biofilms, but, much like ozone, it is unstable. Like chlorine, it is corrosive to metals and must be handled carefully (11).

Figure 2: Cell lysis or destruction occurs when a free oxygen radical attacks the cell walls of bacteria.

Ozone (03) is a colorless gas with a distinct odor to the human nose at concentrations low as 0.1 ppm (13). Over the years, researchers have compared ozone's effectiveness on bacteria to chlorine, the classic disinfectant. Studies have shown ozone is 51 percent stronger on bacterial cell walls than chlorine, while the kill rate is 3.125 times faster.

Most cleaning procedures require two steps: 1) cleaning surfaces and removing organic residues in which bacteria are embedded, and 2) sanitizing to eradicate bacteria adhering to a surface. According to an article from the Department of the Environment, no secondary biocide is necessary when using ozone. It can kill bacteria, fungi, viruses, spores (that withstand hostile environments), mold, mildew, etc. without the help of other products.

Ozone Dynamics

Ozone is unstable in water and must be produced on-site in required amounts precisely when needed (14). It has a half-life of about 10 minutes and is better stabilized at cooler temperatures than at warmer temperatures. Because of its instability, ozone wants more than anything to break apart into two molecules, diatomic oxygen and free oxygen radicals. Free oxygen atoms contact bacterial cell walls, attach to them and oxidize organic material in bacterial membranes, thus weakening the cell wall (14). Eventually, cell walls break down and lysis occurs (Figure 2, Copyright American Air Liquide, Inc., 2001, all rights reserved).

Although ozone is a good biocide that works on both types of cells, it has proved more effective against gram negatives than gram positives (9). Gram negatives have a thinner peptidoglycan layer; therefore, it's easier for ozone to penetrate the cell and destroy it. As a result, ozone may possibly work at lower concentrations for gram negatives (9).

Because they are not only a food safety concern but also an industrial concern, biofilms are a significant problem for a variety of industries. Only one small bacterium is needed to initiate the growth of billions of cells in a relatively short time. That's why it's important to stop biofilms before they form. ■

Editor's note: References are available from Dr. Yuan at Air Liquide America, Countryside, IL. Call him at (708) 579-7907. References are also available at Fresh Cut magazine (800) 900-2452.

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