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Biofilm Formation and Control in Food Processing Facilities

R.A.N. Chmielewski and J.F. Frank

ABSTRACT: Microorganisms on wet surfaces have the ability to aggregate, grow into microcolonies, and produce biofilm. Growth of biofilms in food processing environments leads to increased opportunity for microbial contamination of the processed product. These biofilms may contain spoilage and pathogenic microorganisms. Microorganisms within biofilms are protected from sanitizers increasing the likelihood of survival and subsequent contamination of food. This increases the risk of reduced shelf life and disease transmission. Extracellular polymeric substances associated with biofilm that are not removed by cleaning provide attachment sites for microorganisms newly arrived to the cleaned system. Biofilm formation can also cause the impairment of heat transfer and corrosion to metal surfaces. Some of the methods used to control biofilm formation include mechanical and manual cleaning, chemical cleaning and sanitation, and application of hot water.

Introduction

Biofilms as they occur in nature consist primarily of viable and nonviable microorganisms embedded in polyanionic extracellular polymeric substances anchored to a surface (Carpentier and others 1993, Wimpenny and others 1993). Extracellular polymeric substances (EPS) may contain polysaccharides, proteins, phospholipids, teichoic and nucleic acids, and other polymeric substances hydrated to 85 to 95% water (Costerton and others 1981, Sutherland 1983). EPS provide protection to the biofilm inhabitants by concentrating nutrients, preventing access of biocides, sequestering metals and toxins, and preventing desiccation (Carpentier and Cerf 1993). Food industry biofilms may also have a high food residue and mineral content that originate with product and process water. These constituents also provide protection to microorganisms held within the biofilm.

Biofilms in nature can have a high level of organization, as they may exist in single or multiple species communities, form a single layer or 3 dimensional structure, or take the form of aggregates such as flocs or granules (Allison and others 1987, Bagge and others 2001, Bryers 1987). A natural biofilm community may function through collective behavior and coordinated activity, which assists survival of constituent cells in stressful environments. The majority of microorganisms in natural habitats are attached to surfaces (Davey and others 2000), indicating the extent of the selective advantage for biofilm growth. Environmental stresses such as low nutrient availability trigger phenotypic changes of planktonic (free living) cells to the sessile (attached) form (Carpentier and Cerf 1993, Costerton and others 1987). Other factors that influence biofilm formation are substratum composition, surface chemistry and topography, and fluid flow (Mittelman 1998). Biofilm formation can cause mechanical blockage in fluid handling systems, the impedance of heat transfer (Sandu and others 1991), and corrosion to metal surfaces (Bryers 1987), though these problems are not common in the food industry.

Poor sanitation of food contact surfaces, equipment, and processing environments has been a contributing factor in food borne disease outbreaks, especially those involving *Listeria monocytogenes* and *Salmonella*. Improperly cleaned surfaces promote soil buildup, and, in the presence of water, contribute to the development of bacterial biofilms which may contain pathogenic microorganisms (Boulange-Peterman and others 1993). Cross contamination occurs when food passes over contaminated surfaces or via exposure to aerosols or condensate that originate from contaminated surfaces (Barnes and others 1999, Boulange-Peterman 1996, Bower and others 1996). Frank and Chmielewski (1997) and Holah and others (1990) demonstrated that the type of food contact surface and topography play a significant role in the inability to decontaminate a surface. Abraded surfaces accumulate soil and are more difficult to clean than smooth surfaces. Surface defects provide protection against the removal of soil and bacteria (Boulange-Peterman 1996, and others 1997; Bower and others 1996; Mafu and others 1990), with the result that surviving bacteria can regrow and produce a biofilm. Bacteria within a biofilm are more resistant to disinfectants, which may assist the survival of *Listeria* spp. and other food borne pathogens in the food processing environment (Bower and others 1996). Direct evidence that pathogen-containing biofilms play a role in the spread of foodborne illness is lacking, as identification and characterization of biofilms has not been included in foodborne illness investigations.

Biofilm Formation

Biofilm formation consists of initial attachment, microcolony and EPS (extracellular polymeric substances) production, fol-

lowed by maturation (Davey and O'Toole 2000). This process is diagramed in Figure 1. Bacterial transition from planktonic to the sessile state is triggered by environmental signals. Natural ecosystems are generally low in available nutrients and biofilm formation is an important adaptation for survival under these conditions (Mittelman 1998). Therefore, biofilm formation principles derived from natural ecosystem observations may not apply to nutrient-rich food industry environments.

Attachment

Adhesion to a substratum can be active or passive depending on cell motility. Passive attachment is driven by gravity, diffusion and fluid dynamics. In active adhesion, the bacterial cell surface facilitates initial attachment. Cell surface properties such as flagella, pili, adhesin protein, capsules, and surface charge influence attachment (Kumar and others 1998). Flagella allow bacteria to move to a specific attachment site, while changes in cellular physiology that affect surface membrane chemistry, surface proteins such as pili and adhesins, synthesis of polysaccharides, and cell aggregation all influence adhesion (Davey and O'Toole 2000). Attachment often occurs within 5 to 30 s and occurs in 2 stages: reversible followed by irreversible adhesion (Mittelman 1998).

Reversible attachment is an initial weak interaction of bacteria with a substratum. It involves van der Waals and electrostatic forces and hydrophobic interactions. During reversible attachment, bacteria still exhibit Brownian motion and are easily removed by application of mild shear force.

Irreversible attachment results from the anchoring of appendages and/or the production of extracellular polymers (Sutherland 1983). Repulsive forces usually prevent direct bacterial contact with the substratum (often both the substratum and the bacterial cell are negatively charged). Bonding between bacterial appendages, (that is pili, flagella, adhesin protein) (Pratt and others 1998, Vatanyoopaisarn and others 2000) and the substratum involves short range forces such as dipole-dipole interaction, hydrogen bonds, hydrophobic, and ionic covalent bonding (Boulange-Peterman 1996, and others 1993; Bower and others 1996; Briandet and others 1999; Gilbert and others 1991; Sorongon and others 1991; Stanley 1983). This bonding usually occurs within a few hours of contact (Hood and others 1997). Several studies indicate that irreversible attachment takes from 20 min to a maximum of 4 h at 4 to 20 °C (Gilbert and others 1991, Lunden and others 2000, Mafu and others 1990, Smoot and Pearson 1998, Sorongon and others 1991, Vatanyoopaisarn and others 2000). Removal of irreversibly attached cells is difficult and requires application of strong shear force (scrubbing or scraping) or chemical breaking of the attachment forces through the application of enzymes, detergents, surfactants, sanitizers, and/or heat (Bower and others 1996, Gelinas and others 1994, Oh and Marshall 1995, Richards 1999, Sinde and Carballo 2000).

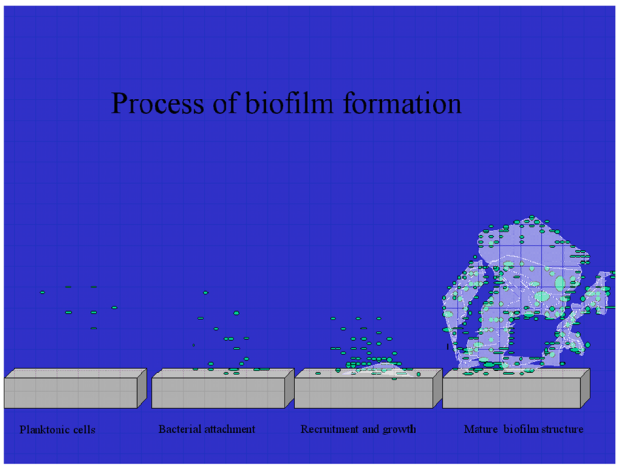

Figure 1—The process of biofilm formation. Planktonic cells attach to a surface, biofilm genes are turned on, and other cells are recruited through quorum sensing. Cells grow on the surface, forming microcolonies. Microcolonies continue to grow producing a mature biofilm structure with channels (blue). The green represents bacterial cells and the white and light blue opaque areas represent EPS.

Factors that affect microbial attachment to abiotic surfaces

Adhesion is affected by the chemical and physical properties of the cell and substratum surfaces and the composition of the surrounding medium. Abiotic substrata are modified by conditioning films that originate with the surrounding medium. Adherent properties of the cell are influenced by the cell envelope, whose chemistry changes in response to environmental stimuli and quorum sensing. Irreversible attachment is a physiological process under genetic regulation. Studies using *S. aureus*, *E. coli*, and *S. epidermis* demonstrate that genes responsible for surface protein expression, attachment and EPS production are activated in response to external stimuli such as population density, stress or nutrient limitation (Adams and Mclean 1999, Cramton and others 1999, Dalton and March 1998, Gilbert and others 1991, Kim and Frank 1994, Mclean and others 1997, Pratt and Kolter 1998).

Properties of food contact surfaces

Bryers (1987) and Boulange-Peterman and others (1993) observed that a critical surface tension value promotes bacterial adhesion. Maximum attachment of bacterial cells depends upon high free surface energy or wettability of a surface. Surfaces with high free surface energy, such as stainless steel and glass, are more hydrophilic. These surfaces generally allow greater bacterial attachment and biofilm formation than hydrophobic surfaces such as Teflon, nylon, buna-N rubber, and fluorinated polymers (Blackman and Frank 1996, Hyde and others 1997, Mafu and others 1990, Snide and Carballo 2000). Smoot and Pierson (1998) observed that initial attachment of *L. monocytogenes* to stainless steel was more rapid than to rubber, even though attachment to buna-N rubber was stronger. Additional evidence for the importance of free surface energy in attachment was reported by Bos and others (2000), who found that bacterial adhesion occurred mostly at the hydrophilic region of the hydrophilic–hydrophobic interface of a stainless steel surface. In addition, a study by Boulange-Peterman and others (1993) noted that the spreading pressure (βE) of bacteria as well as the balance of free energies (polar and van der Waals force) influenced adhesion; for instance, polar interaction of stainless steel and *Streptococcus thermophilus* resulted in decreased adherence. Boulange-Peterman and others (1993) and Sinde and Carballo (2000) also demonstrated that cleaning stainless steel conditions the surface temporarily changing its properties. Cleaning with alkali or strong acid (4 *M* nitric acid) caused the surface to be hydrophilic, while cleaning with weak acid produced a hydrophobic effect. Once stainless steel is exposed to air or water, it is passivated by forming a chromium oxide layer. Organic soil adheres to the oxide layer, producing a conditioned substratum to which bacteria adhere (Verran and others 2000). In most cases, bacteria attach more to hydrophilic than hydrophobic surfaces, but the differences in attachment are not necessarily of practical significance (Black man and Frank 1996, Cunliffe and others 2000, Hyde and others 1997, Sinde and Car-

ballo 2000), since high growth rates on the surface can make attachment differences a minor factor in the development of microbial load.

Substrate type also influences the attachment pattern. Bacteria tend to attach to glass (a hydrophilic surface) uniformly in a monolayer, while on hydrophobic surfaces such as nylon and tin, they tend to adhere in clumps (McEldowney and others 1987).

Topography of food contact surfaces

Stainless steel type 304, with either 2B (cold rolled), nr 4 (mechanically polished), or electropolished finish are usually used for fabricating equipment and utensils. Rubber, Teflon, and nylon are used for gaskets, various utensils, and equipment parts. These surfaces are abraded with repeated uses, increasing their ability to entrap bacteria and soil (Holah and Thorne 1990). This condition creates a harborage for bacterial growth and protection from cleaning and sanitation. Frank and Chmielewski (2001) and Boulange-Peterman, and others (1997) observed that the average surface roughness (Ra) of stainless steel does not correlate to cell adherence, but other measurements of surface roughness that indicate surface defects (Rdin and Rmax) correlate more closely with soil retention and removal (Frank and Chmielewski 2001). Studies by Jones and others (1999) and Holah and Thorne (1990) also demonstrated that surface defects were associated with significant increases in bacterial attachment.

Contact time

Contact time between the cell and the substratum is required for irreversible attachment. Lunden and others (2000) demonstrated that the most prevalent strain of *L. monocytogenes* (strain 1/2c) found in food processing plants had good adhesion ability and required only a short contact time for attachment.

Adhesive properties of the bacterial cell surface

Adhesion of bacterial cells is influenced by the physicochemical properties of the cells' surface, which in turn are influenced by factors such as microbial growth rate, growth medium, and culture conditions (time and temperature). Bacteria have a net negative surface charge and usually behave as hydrophobic particles, but the degree of hydrophobicity can change with growth phase. Hydrophobicity generally decreases as growth rate increases (Boulange-Peterman 1996, and others 1997). Herald and Zottola (1988), Hood and Zottola (1997), and Smoot and Pierson (1998) demonstrated that *Listeria* and *Yersinia* exhibited increased attachment when the microorganisms were at their highest metabolic activity. For *Listeria*, the optimum metabolic activity and attachment conditions were at 30 °C and pH 7, and for *Yersinia* the optimum was at 21 °C and pH 8 to 9. Sorongon (1991) observed that starvation of *Cytophaga* increases surface hydrophobicity. Other studies have correlated adhesion with surface charge and hydrophobicity. The adhesiveness of *Staphylococcus epidermidis* correlates directly with surface electronegativity and hydrophobicity, while the adhesion of *Escherichia coli* is inversely proportional to the degree of negative surface charge but is not influenced by hydrophobicity (Carpentier and Cerf 1993, Gilbert and others 1991). Spores adhere better to food contact surfaces than vegetative cells due to the high hydrophobicity of their hairy surfaces (Bower and others 1996). Growth media pH and nutrients influence the surface charge of bacteria. Glucose and lactic acid in the growth medium decreased the electronegativity of *L. monocytogenes'* cell wall through the neutralization of the surface charge and the production of acid stress proteins (Brocklehurst and others 1987). *L. monocytogenes* grown in tryptone exhibited less attachment ability than those grown with amino acids (Kim and Frank 1994). Growth temperature also affects the surface properties of *Listeria*. At high temperatures (37 °C), *Listeria* lose their flagella and their cell surface becomes less electronegative. When *Listeria* are grown at 15 to 20 °C, the cell surface has a negative charge, suggesting that a negative charge results from the presence of flagella and glycolipids (Briandet and others 1999). Smoot and Pierson (1998) also found that *Listeria* grown at 30 °C was more hydrophilic than when grown at 10 or 40 °C. High growth temperature is also associated with increased attachment ability (Smoot and Pierson 1998), possibly due to the production of heat stress proteins associated with the cell surface. Some studies (Briandet and others 1999, Piette and Idziak 1991, Smoot and Pierson 1998) suggest that attachment ability is controlled by surface proteins other than flagella. The importance of flagella being primarily to bring the cells to attachment sites.

Structures that protrude from the cell membrane such as lipopolysaccharide (LPS), adhesins and other proteins, and lipoteichoic acids can play an important role in microbial attachment. *E. coli* and *L. monocytogenes* utilize flagella, pili, and membrane proteins to initiate attachment (Davey and O'Toole 2000, Vatanyoopaisarn and others 2000). The loss of these cell appendages changes surface properties, which may lead to decreased attachment ability on some abiotic surfaces (Gilbert and others 1991, Heilmann 1996). LPS plays a role in initial attachment. *Pseudomonas* mutants deficient in the B-band of LPS exhibit reduced surface hydrophobicity and reduced ability to attach to hydrophilic surfaces, while in *E. coli*, the loss of LPS resulted in decreased ability of cells to attach to surfaces (Davey and O'Toole 2000). Pili act like velcro to anchor bacteria to some surfaces (Butler and others 1979, Netting 2001, Pratt and Kolter 1998) and also act as chemoreceptors, directing cells to move to specific attachment sites. Briandet and others (1999) and Smoot and Pierson (1998) demonstrated that the greatest adhesion of bacteria on stainless steel occurred in a high ionic strength solution, while the lowest attachment occurred under alkali conditions. This indicates electrostatic repulsion between cell and the attachment surface and demonstrates the importance of the suspending solution in providing conditioning layer at the attachment surface to overcome this repulsion.

Substratum preconditioning

Clean surfaces submerged in solution are rapidly changed by the adsorption of organic molecules and charged ions. This process is called preconditioning. Adsorption of an organic layer onto a substratum can occur within seconds of exposure to an aqueous environment. Numerous studies demonstrate that bacterial attachment occurs best on preconditioned surfaces in the presence of ions (Barnes and others 1999, Briandet and others 1999, Stanley 1983). Initiation of bacterial attachment is dependent on the surface properties of the preconditioned substrate (Bryers 1987, Sanford and others 1995). Bryers (1987) and McEldowney and Fletcher (1987) also pointed out that the presence of a surface layer of organic molecules can promote bacterial cell adhesion and that the maximum adsorption of organic molecules occurs on surfaces with high free energy. The importance of an organic preconditioned surface in bacterial adherence to stainless steel has also been demonstrated (Verran and Jones 2000). Verran and Jones (2000) concluded in their review that hydrophobic protein macromolecules adhered more to high free energy surfaces, and that fatty acids adhered better to hydrophobic polymeric surfaces and metals cleaned with solvents. McEldowney and Fletcher (1987) and Criado (1994) suggested that the adhesion of bacteria to an inert surface is greatly influenced by the compatibility of the preconditioning macromolecules with that of the surface properties of the bacteria. McEldowney and Fletcher (1987) observed that hydrated layers of polymers and proteins that form on inert surfaces can either facilitate or reduce bacterial adhesion.

Milk and milk components will adsorb to surfaces within 5 to

10 s, forming a conditioning film that may encourage or inhibit bacterial attachment (Mittelman 1998). Hood and Zottola (1997) demonstrated the effect of dairy soil on attachment by using stainless steel exposed to whole, chocolate, and diluted milk . Attachment of *L. monocytogenes* and *S. typhimurium* was inhibited by preconditioning with whole and chocolate milk, and was enhanced when using diluted milk. Data of Wong (1998) and Barnes and others (1999) supported this finding by reporting that preconditioning with milk inhibits attachment of *Listeria* to stainless steel and buna-N rubber. Fletcher and others (1976), Bower and others (1996), and Wong (1998) found that various proteins such as bovine serum albumin (BSA), gelatin, fibrinogen, and pepsin inhibited bacterial attachment to various surfaces. Although Fletcher (1976) showed the inhibitory effects of BSA, this effect may not be entirely due to the properties of the conditioning layer, as serum albumin may also have modified the bacterial surface.

Sequence of attachment of multiple species influences the species composition of the resulting biofilm. The initial population that attaches can change the surface so that the following species can attach via cell-to-cell association. In some cases, attachment of a 2nd species can increase stability of the biofilm population (McEldowney and Fletcher 1987). Hood and Zottola (1997) demonstrated that *L. monocytogenes* was more likely to adhere to stainless steel in the presence of *Pseudomonas fragi*.

Microcolony formation

Microcolony formation will proceed after irreversible attachment given appropriate growth conditions. Microcolony formation results from simultaneous aggregation and growth of microorganisms and is accompanied by the production of EPS. Images of microcolonies produced by water system bacteria on a polyvinyl chloride surface are presented in Figure 2. Studies of bacterial species in natural systems showed that aggregation may involve recruitment of planktonic cells from the surrounding medium as a result of cell-to-cell communication (quorum sensing) (McLean and other 1997, Pesci and others 1999). In *P. aeruginosa,* the *algC* gene is transcribed upon attachment, which results in down-regulation of flagellum synthesis and up-regulation of *alg T* for the synthesis of alginate, the major component of EPS for this species (Davey and O'Toole 2000). The production of acylhomoserine lactones (AHL) and other quorum sensing molecules (Lon protease) regulate the formation of typical biofilm structure of *P. aeruginosa* as well as various virulence factors (Davey and O'Toole 2000, Pesci and others 1999). In other microorganisms, adhesion and biofilm formation are under distinctly different genetic regulation (Crampton and others 1999; Heilmann 1996, and others 1996).

P. aeroginosa, *E. coli*, and *Vibrio cholerae* lose their flagella and increase their EPS production upon attachment to a surface (Davey and O'Toole 2000). EPS is also produced in response to attachment and environmental stimuli such as osmotic pressure, pH, temperature, and starvation. Hood and Zottola (1997) found that *P. fragi* only adhered to stainless steel under starvation conditions and produced EPS to anchor itself to the surface. The *crc* gene in *P. aeroginosa* codes for biofilm development as well as catabolite repression. This *crc* gene also regulates the *pilA & B* genes, which encode for the main protein of type IV pili (Davey and O'Toole 2000). The genetic control mechanism that links carbon metabolism *(crc* gene), and pilus assembly (*pil* gene) is unknown but data suggests a link between nutrient availability and biofilm formation (Kjelleberg and others 1983). The composition of biofilm EPS is not known, but is likely a mixture of polymers. It cannot be assumed that EPS material produced in broth culture is similar to that produced when attached to a surface. The EPS of pseudomonad biofilm attached to stainless steel contain galactose, glucose, rhamnose, and uronic acid (Lindberg and others 2001). Allison and Sutherland (1987) provide evidence that EPS production does not always occur immediately after attachment. They demonstrated that polysaccharide production in Gram negative bacteria was initiated 5 to 6 h after attachment. However, attachment EPS can also be produced by planktonic cells resulting in enhanced attachment (Bryers 1987).

Maturation of the biofilm

If conditions are suitable for sufficient growth and agglomeration, biofilm in nature may develop an organized structure. This process is called maturation. The mature biofilm may consists of a single layer of cells in porous extracellular polymer or multilayered loosely packed microcolonies held together with EPS and interspersed with water channels. Examples of mature biofilms are presented in Figure 2 and 3. Lawrence and others (1991) observed the spatial redistribution of cells after microcolony formation to produce the mature biofilm structure by using confocal laser microscopy.

Biofilm structural models

Various models have been proposed to explain the development and properties of biofilms. These models are based on observations of biofilm structure and are therefore limited by the available visualization technology.

The monolayer biofilm theory

The first biofilm structure theory, the continuum model, described biofilms as smooth, planar, and homogeneous. This model was used in water engineering to predict the rate of biofilm chemical activity based on diffusion, the physical effects of flow and pressure, and cell detachment rate (Bishop 1997, Wimpenny and others 2000).

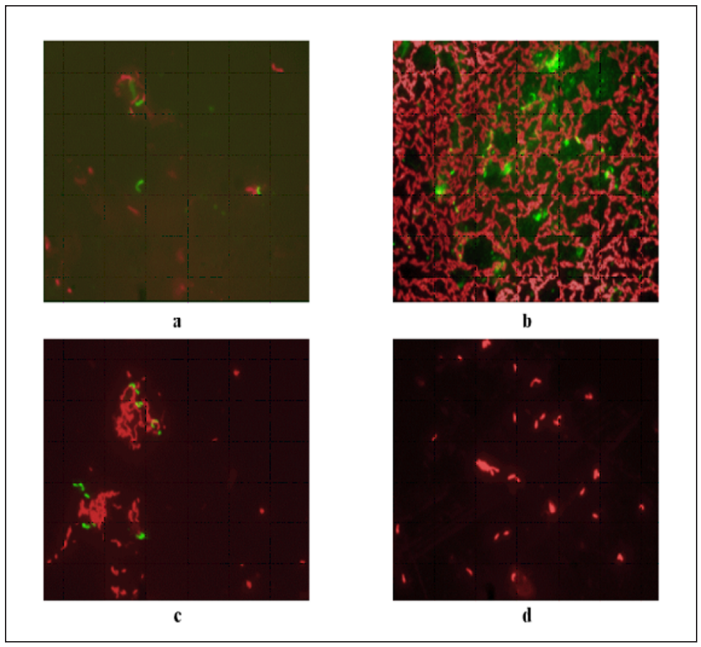

Figure 2—Epifluorescent micrographs of 4 unidentified water biofilm isolates grown on polyvinyl chloride surface with *Campylobacter jejuni*. All biofilms were produced in 7d at 21 °C. Red represents the water biofilm isolates stained with Syto™ red and green represents *Campylobacter* labeled with fluorescent antibody. Images illustrates (a) biofilm at the beginning stage of production; (b) a typical mature biofilm structure; (c) and (d) different degrees of microcolony formation. Images courtesy of Nathanon Trachoo.

The multilayer- 3D structure theory

The continuum model was adequate for engineering predictions, but observations using differential interference contrast (DIC) microscopy indicated a heterogeneous structure. The *Kreft* or swarm model described biofilms as a heterogenous mosaic with stacked microorganisms held together by EPS (Wimpenny and others 2000). This model was based on simple microbial physiology concepts. Cells metabolize substrates, excrete, maintain an energy level, divide, or die, depending on nutrient availability. If a cell divides, it pushes other cells apart. If it dies, the cell components are recycled as nutrients. The model assumed that biofilm cells had a specific affinity for each other, causing them to stick together. This model was able to explain the incorporation of diverse microbial communities with their own distinct characteristics into a biofilm.

The current theory

The discrete model is based primarily on evidence provided by confocal laser scanning microscopy. This instrumentation has allowed the visualization of a mushroom/ tulip structure of the biofilm with towers, pedestals, and water channels. The upper portion is mushroom shaped with a narrow stalk penetrated by channels (Wimpenny and others 2000). The structural characteristics of this model are diagramed in Figure 1. This model proposes that cell growth in the periphery is rapid and that growth in the interior is slow. The towers or mushroom portion may have streamers, which may break off and repopulate other sites. Zhang and Bishop (1994) demonstrated that the porosity of mature biofilms range from 89% in the top layer to 5% in the bottom layer; evidence that confirms this model. In the discrete model, the effect of an individual cell or each microcolony is evaluated in relation to the entire group. For example, in a low concentration of substrate, independent stacks of microorganisms form, but as solute concentration increases, microcolonies appear denser, forming mushroom-type structures with water channels interspersed within the structure. Davey and O'Toole (2000) and Wimpenny and others (2000) described biofilm structures ranging from monolayers of single-scattered cells to patches of cells which are interspersed throughout thick mucoid 3 dimensional layers (Figure 2 and 3). Organisms within the biofilm may compete, operate independently, cooperate, or be predatory.

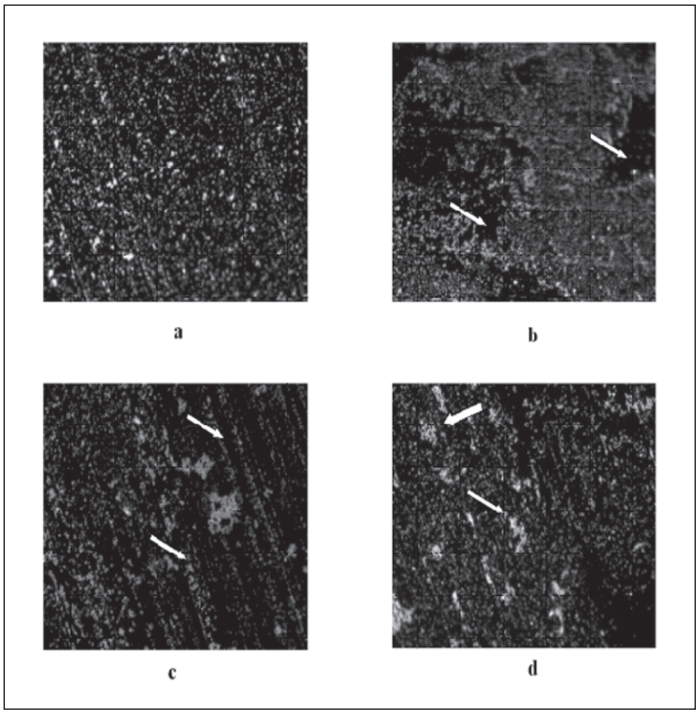

Figure 3—Epifluorescent micrographs of biofilm produced on stainless steel by 4 strains of *Listeria monocytogenes*. Images illustrate (a) confluent growth of biofilm; (b) channels within the biofilm; (c) growth along the striations of the stainless steel; (d) multilayered microcolonies. Images courtesy of James Folsom.

Factors influencing biofilm development

Some of the factors affecting biofilm development include surface and interface properties, nutrient availability, composition of microbial community, hydrodynamics, interspecies interaction, and cellular transport. A study (Jones and others 1999) of biofilm in mineral water bottles showed that the interface properties of a surface can create a microenvironment that selects for different microfloral communities. Although the smooth surfaces of the polyethylene terephthalate (PET) bottles were sparsely populated, they were colonized by rod-shaped bacteria, while the rougher and more hydrophobic surfaces of the high density polyethylene (HDPE) caps were populated with clumped coccoid bacteria.

Nutrient availability has a major influence on biofilm structure and the composition of the microbial community. Studies showing the effect of nutrients on pure culture *Listeria* biofilm development (Kim and others 1995) suggest that low levels of phosphate initially stimulate biofilm development, but after several days the effect was reduced. The type of sugar provided also influenced the development of biofilm, with trehalose and mannose allowing for only poor biofilm formation.

As the biofilm matures, it adapts to nutrient, oxygen, and population changes and forms discrete microcolonies separated by water channels. The structural density of the matrix increases at the core while the top layers remain porous (Bishop 1997). Most metabolically active bacteria remain at the top layers of the biofilm matrix, near water channels (Zhang and Bishop 1994). Water channels allow the dispersion and exchange of dissolved organics, metals cations, and metabolites. Nutrients become trapped and concentrated in the biofilm matrix and move throughout the matrix by diffusion (Bryers 1987, Davey and O'Toole 2000), which results in a stratified habitat that selects for different microbial species (Stewart and others 1997). These species can then become involved in nutrient exchange with their neighbors. According to Wimpenny and others (2000) and Kumar and Anand (1998), heterogeneous biofilms can possess different nutritional requirements. For example, when citrate was provided, both *Pseudomonas* and *Burkholderia* grew as separate microcolonies, but when an intermediate substrate was provided, both organisms cooperated to create a metabolic symbiosis and stayed together as a mixed culture biofilm (Wimpenny and others 2000).

Bishop (1997) demonstrated that mature biofilms generate a dynamic redox potential gradient throughout the structure. At the biofilm core there was a 25% diffusivity rate for oxygen, while biofilm/liquid interface exhibited 90% diffusivity. Such a structure creates a habitat for a heterogeneous and dynamic microbial population (Bishop 1997). At the liquid-biofilm interface there may be a high population of multiplying aerobic cells. Towards the attachment surface where there are less available nutrients and oxygen, a niche is created for a population with various metabolic rates and processes that can recycle cell components and survive in low redox potential. The diverse communities or guilds form a food web creating symbiotic relationships. Other research indicates that the coexistence of microbial species in a biofilm depends on the ability of the microbial species to attach to a substratum and out-compete the competitors (stewart and others 1997). Biofilms of mixed cultures are thicker and more stable to environmental stress than monospecies biofilms. The stabilization of

mixed culture biofilms may be due to the production of a variety of EPS materials that result from the activity of different microorganisms (Kumar and Anan 1998).

Most of these investigations focused on biofilms in water and waste water systems. The implication of microbial diversity in food industry biofilms has not been determined. Some processing plant biofilms form in environments likely to have high microbial diversity (such as a floor drain), whereas other biofilms form in environments likely to be dominated by only one or a few microbial species (such as a plate heat exchanger). Jeong and Frank (1994) grew multispecies biofilms containing *L. monocytogenes* and biofilm microflora from dairy and meat processing plants. This study demonstrated that *L. monocytogenes* is able to survive and grow at 10 and 21 °C in the mixed microflora.

Hydrodynamics influence biofilm structure. After bacterial attachment, flow rate or shear force of the liquid environment affect biofilm structure and content. In the intracellular transport, molecular diffusion is dominant within laminar flow (Bryers 1987, Davey and O'Toole 2000). Laminar flow causes patchy and rounded cells aggregates that are separated by cell free spaces, whereas turbulent flow produces patchy and elongated structures with streamers (Davey and O'Toole 2000). High turbulence is also associated with increased EPS production (Lazarova and others 1994) even though adhesion generally decreases with increased shear.

Detection methods

Biofilm development and structure has been analyzed using various methods. Biofilms have been grown in capillary tubes and flow through devices. Light, fluorescence (Blackman and Frank 1996, Jones and others 1997, Kim and Frank 1995), differential interference contrast (DIC), transmission electron (TEM) (Sanford and others 1995), scanning electron (SEM) (Carr and others 1996), atomic force (AFM), and confocal laser scanning microscopy (CLSM) (Bishop 1997, Stewart and others 1997, Wimpenny and others 2000, Zhang and Bishop 1994) are used to analyze biofilm structure. Microelectrodes can detect the presence of O_2 and observe molecular diffusion within the biofilm. Molecular biological methods, including 16-23S rRNA hybridization and fluorescent *in-situ* hybridization (FISH) with CLSM (Davey and O'Toole 2000), have been used to observe microstructure and metabolism of biofilms (Wimpenny and others 2000). The FISH method was used to confirm the decrease in viability of cells as the biofilm ages. Researchers were able to detect viable cells in the biofilm and determine that young biofilm had about 80% viable cells and about 50% in old biofilm (Wimpenny and others 2000).

The structure of biofilms found in the food industry has not been sufficiently studied to determine if the discrete model applies. This model was developed through observations on water and wastewater biofilms that are probably unlike many biofilms found in food processing facilities. The presence of high levels of nutrients, macroscopic and microscopic deposits of food residues, and frequent stress from cleaning, sanitizing, or processing treatments will all influence biofilm structure. The influence of these factors is not accounted for in current biofilm theory.

Factors influencing detachment

Layers of biofilm can detach through sloughing and shear. As biofilm matures, it thickens, creating an anaerobic environment on the interior. Bryers (1987) suggests that the anaerobic condition results in an increase in acid and insoluble gas accumulation that weakens the biofilm structure, causing sloughing of polymer layers from the supporting surface. Biofilm sloughing may also occur when there is an imbalance or fluctuation of nutrients. Low carbon availability can cause increased EPS production (Kim and Frank 1995), which leads to detachment. High levels of available carbon can also trigger sloughing off. Shear force aids in sloughing off, especially for biofilm with the mushroom type structure and peripheral streamers ((Wimpenny and others 2000). Bryers (1987) states that after biofilm reaches a certain thickness, the rate of biofilm removal increases under constant laminar flow. Chae and Schraft (2001) evaluated the growth and biofilm production of different strains of *L. monocytogenes* attached to glass slides in static conditions at 37 °C over a 10-d period. They noted that overall, there was a cycle of cell population and biofilm production. There was an initial increase in population within the first 3 d, then the cell numbers and EPS production decreased. After 6 d, there was a resumption in population and biofilm production.

Foodborne Pathogens and Spoilage Organisms in Biofilms

Listeria monocytogenes

L. monocytogenes is a hardy pathogen with ability to proliferate in cold wet environments that are ideal for biofilm formation. Listeria forms biofilms in pure culture (Figure 3), and can survive and grow in multispecies biofilms (Blackman and Frank 1996, Chae and Schraft 2001, Charlton and others 1990, Cox and others 1989, Fatemi and Frank 1999, Mafu and others 1990). *L. monocytogenes* forms biofilms on stainless steel, plastic, and polycarbonate surfaces and many other food contact surface materials (Dhir and Dodd 1995, Frank and Koffi 1990, Helke and others 1993, Jeong and Frank 1994, Kim and Frank 1995, Mafu and others 1990, Nelson 1990). Therefore, *Listeria* species are well suited for growth and survival in various microniches found in food processing facilities. *L. monocytogenes* was isolated from the wooden shelves in the cheese ripening room (Noterman 1994) implicated in a listeriosis outbreak. *Listeria* has been isolated from environmental surfaces such as conveyor belts, floor drains, condensate, storage tanks, and hand trucks (Charlton and others 1990, Cox and others 1989, Nelson 1990). These are all surfaces on which biofilm is expected to have formed.

Nelson (1990) and Charlton and others (1990) in 1990 isolated *Listeria* spp. throughout dairy processing plants, on processing and packing equipment and especially in wet, difficult-to-clean environments such as conveyor belts and drains. A survey of milk processing plants in California in 1987 (Brocklehurst and others 1987) showed that of the 156 plants sampled, 46 plants or 29.5% were positive for Listeria. Surveys of meat processing facility produce similar findings with *Listeria* spp. found on various wet environments, particularly in drains, on conveyor belts, and on ceilings that collect condensate. Meat processing equipment, such as frankfurter casing strippers, conveyor belts and rollers, slicer blades, and packaging equipment, are difficult to clean and are wet for extended periods of time, and therefore provide ideal conditions for biofilm development and good harborage for *Listeria* spp. (Nickelson and others 1999). *Listeria* is found in vegetable processing facilities, as it is brought into the plant with soil and raw product. The potential hazard was demonstrated by outbreaks of listeriosis associated with broccoli and coleslaw (Hines 1999, Kuntz 1995). As with meat and dairy processing, *Listeria* survives and grows in the wet, cold temperatures present in vegetable processing and storage environments (Saguy 1992). Although *L. monocytogenes* has been isolated from suspected biofilm-forming growth niches in many food processing facilities, direct evidence that the presence of pathogen-containing biofilms leads to disease outbreaks is lacking. Most likely, the growth of *L. monocytogenes* in food plant biofilms increases the general contamination level in the food plant and is indicative of unsatisfactory cleaning/sanitizing procedures. Such conditions ultimately put exposed product at risk. Recent outbreaks of listeriosis and salmonellosis have implicated post processing contamination of

cheese, milk, hotdogs and ice cream as a contributing factor (Brocklehurst and others 1987, Hedberg and others 1992).

Pseudomonas spp.

Pseudomonads are ubiquitous spoilage organisms. They are found in food processing environments including drains and floors, on fruits, vegetables, meat surfaces and in low acid dairy products (Brocklehurst and others 1987, Criado and others 1994, Hood and others 1997, Piette and Idziak 1991). *Pseudomonas* spp. produce copious amounts of EPS and has been shown to attach and form biofilms on stainless steel surfaces (Barnes and others 1999). They coexist within biofilms with *Listeria, Salmonella* and other pathogens (Bagge and others 2001, Fatemi and Frank 1999, Jeong and Frank 1994).

Bacillus spp.

Bacillus is found throughout dairy processing plants (Oosthuizen and others 2001). *Bacillus* survives heat processing and accumulates on pipelines and joints in the processing environment (Jeong and Frank 1994). If hot fluid continuously flows over a surface for over 16 h, *Bacillus* and other thermoduric bacteria may form a biofilm (Frank 2000).

Salmonella spp.

Salmonella can be isolated from poultry processing equipment especially in the slaughter and evisceration area (Helke and others 1994, Joseph and others 2001). The poultry processing operation is a wet environment and therefore ideal for biofilm formation. There is little information on the presence of *Salmonella* in biofilms in food processing environments. However, various studies (Helke and Wong 1994, Jones and Bradshaw 1997, Joseph and others 2001) show that *Salmonella* can attach and form biofilms on surfaces found in food processing plants, including plastic, cement, and stainless steel.

Biofilm Removal and Control

Nutrient and water limitation, equipment design, and temperature control are important in biofilm control. Unfortunately, it is often not possible to reduce water availability, improve equipment design, or reduce operating temperatures, so biofilm control efforts most often focus on effective cleaning of potential growth sites (Frank 2000). Biofilms will eventually form in wet areas even with minimal nutrients, but the presence of nutrients enhances growth. Once biofilms are allowed to form, cleaning the surface becomes more difficult because of the presence of adherent EPS.

Cleaning procedures should effectively remove food debris and other soils that may contain microorganisms or promote microbial growth. Most cleaning regimens include removal of loose soil with cold or warm water followed by the application of chemical agents, rinsing, and sanitation. (Frank 2000). Cleaning can be accomplished by using chemicals or combination of chemical and physical force (water turbulence or scrubbing). High temperatures can reduce the need for physical force. Chemical cleaners suspend and dissolve food residues by decreasing surface tension, emulsifying fats, and peptizing proteins. The mechanism by which cleaning agents remove EPS associated with biofilms has not been determined.

Cleaning

Most chemical cleaning agents used in the food processing industry are alkali compounds that act as detergents for fat and protein. They can be used in combination with sequestrant or chelators and anionic wetting agents (compatible with acid or alkali cleaners). Many situations require the occasional use of acid cleaners to clean surfaces soiled with precipitated minerals or having a high food residue/mineral content (such as milkstone). Nonionic wetting agents are used in some formulations since they are good emulsifiers and control foaming. Sequestrants, such as sodium phosphate derivatives, are often required to chelate minerals depending on water hardness. Chlorine compounds added to alkali help peptize protein. Cleaning compounds must be formulated with care, as many components are incompatible or most effective if applied separately (Flemming and others 2000, Lewis 1980). Lewis (1980) suggests that surfaces such as glass, ceramic, and stainless steel should be cleaned with alkali or nonionic detergents; additionally, stainless steel should be cleaned with alkali or acid detergents. For plastics, alkali cleaners and nonionic detergents are recommended. Manufacturers recommend that detergent solutions be applied at temperatures between 40 and 90 °C depending on soil type and the risk of redeposition. Jackson (1985) recommended that solution temperatures above 70 °C be used to clean milk pipelines and above 77 °C to clean pasteurizer and heat exchanger systems. Caution should be taken when using hot cleaning solutions, since soils high in carbohydrates and proteins may cook onto or attach more firmly to the surfaces. On the other hand, if solutions are not sufficiently hot, soil can redeposit. The cleaning process can remove 90% or more of microorganisms associated with the surface, but cannot be relied upon to kill them. Bacteria can redeposit at other locations and, given time, water and nutrients can form a biofilm. Therefore, sanitation in addition to cleaning must be implemented (Gibson and others 1999).

In most food processing plants, food contact surfaces are cleaned and sanitized daily; however, many environmental surfaces such as storage tank and pump exteriors, walls, and ceilings are cleaned infrequently. This infrequent cleaning provides the opportunity for biofilm formation if moisture is present. An effective cleaning procedure must break up or dissolve the EPS matrix associated with the biofilm so that sanitizing agents can gain access to the viable cells. Little is understood about the effectiveness of cleaning processes as currently used in the food industry to remove biofilm in this manner. Wirtanen and others (1996), Gibson and others (1999), and Schwach and Zottola (1984) provided evidence that mechanical and chemical treatments can destroy biofilms. The removal of *Bacillus* biofilm was influenced by flow rate, time, and temperature of cleaning and the presence of chelators in the cleaning solution. Wirtanen and others (1996) found that alkali cleaning, especially with chelators such as EDTA, was more effective than acid cleaning in removing biofilm. Superheated water (125 °C, 30 min) was the most effective cleaning method even though it did not completely remove a 3-d old biofilm (Wirtanen and others 1996). However, Gibson and others (1999) found that alkali and acid cleaners were ineffective in removing *Pseudomonas* and *Staphylococcus aureus* biofilms on stainless steel, as they obtained only a 1-log reduction of microorganisms. In comparison, Dunsmore (1981) observed a 3-log reduction of bacteria from a milk-soiled stainless steel surface on which biofilm had not formed. These studies suggest that prolonged cleaning with alkali cleaners containing chelators is necessary to remove biofilm. It can also be concluded that sanitizer application is essential to inactivate microorganisms remaining on the surface after cleaning (Dunsmore 1981, and others 1981).

Sanitizing

The major types of sanitizers used in the food industry are halogens, peroxygens, acids, and quaternary ammonium compounds. Effectiveness of chemical sanitizers is limited by the presence of soil, water hardness, temperature of applications, and ability to physically contact the surviving microorganisms (Gibson and others 1999, Kim and Frank 1995).

Chlorine is commonly applied as a sanitizer due to its oxidizing

and disinfecting power (DeBeer and others 1994). Its most toxic form, hypochlorous acid (HOCL) is generated from hypochlorite ion at pH 4 to 7. Schwach and Zottola (1984) found that sanitation with 150 ppm chlorine was not sufficient to remove a *Salmonella* biofilm matrix (EPS material) from stainless steel even though the cells were apparently killed. De Beer and others (1994) noted that chlorine could not fully penetrate a *Pseudomonas–Klebsiella* mixed biofilm (400 um thick) after 1 h of exposure. They suggested that the biofilm matrix itself inactivated the chlorine. LeChevallier and others (1988) reported that biofilms in drinking water distribution systems were not inactivated with a residual chlorine of 5 ppm. It took 15 to 20 ppm residual chlorine to control biofilm fouling of reverse osmosis membranes. Chlorine is less effective on older *Listeria* biofilms (Lee and others 1991) and on abraded stainless steel and mineral resin surfaces with a high bacterial load (Frank and Chmielewski 1997), as well as on ropy lactic acid bacteria biofilms (Makela and others 1991). Ronner and Wong (1993) found that chlorine and anionic sanitizers were better able to remove *Listeria* and *Salmonella* EPS material from stainless steel than quaternary ammonium compound (QAC) and iodine. Gelinas and others (1984) suggested that increasing the contact time for chlorine sanitizers from 5 to 30 min would greatly improve the efficacy of chlorine, as demonstrated with *Pseudomonas* biofilm on stainless steel. Chlorine is readily inactivated by organic material, so the presence of soil and biofilm may significantly reduce its effectiveness. Chlorine dioxide and chloramines are also used as sanitizers in the food industry. LeChevallier and others (1988) and Samrakandi and others (1997) found that monochloramine was better able to penetrate bacterial biofilm than chlorine, but chloramines require longer contact time for effectiveness.

QACs are cationic surfactant sanitizers and also have cleaning activity (McEldowney and Fletcher 1987). They are often applied as a foam, which provides longer contact times on surfaces such as pipes, walls, and ceilings than does water application. QAC is effective against gram positive and gram negative bacteria, molds, and yeast (Carsberg 1996). It is noncorrosive, nonirritating, and its activity is unaffected by organic load. QAC is not recommended for use in processing plants that use starter cultures because the residues inhibit these cultures. McCarthy (1992) demonstrated that 400 ppm QAC for 5 min contact time was required to inactivate *L. monocytogenes* biofilm on chitin, while work by Frank and Koffi (1990) showed that *Listeria* biofilm treated with 800 ppm of QAC for 20 min was not completely inactivated. Quaternary ammonium compounds are often recommended for floors, walls, and storage containers, surfaces which can be sanitized for long contact times, and for surfaces that do not require rinsing before production (nonfood contact surfaces) (Giese 1991).

Peroxygen sanitizers include hydrogen peroxide and peracid compounds. Hydrogen peroxide is a broad-spectrum sanitizer. It is both bactericidal and active against bacteria endospores (McDonnell and others 1999). Peracetic acid (PAA) is the most widely used of the peracid sanitizers. PAA is a more potent biocide than hydrogen peroxide and is often more effective than chlorine, since it maintains activity with an organic load (McDonnell and Russell 1999). Peracid sanitizers are often used for cold disinfection because of their activity at low temperatures. Peroxide based sanitizers were found to be more effective against *L. monocytogenes* and *Salmonella* spp. in a biofilm matrix than was hypochlorite (Harkonen and others 1999). However, this study reported that *L. innocua* showed resistance to the peroxide sanitizer . Fatemi and Frank (1999) determined the efficacy of various sanitizers on *Listeria/Pseudomonas* biofilms attached to stainless steel surfaces in the presence of milk soil. They found that peracetic acid was more effective than chlorine in inactivating *L. monocytogenes* in the milk-*Pseudomonas* biofilm. Richards (1999) and Makela and others (1991) reported that PAA and QAC were more effective in inactivating *Listeria* biofilm and ropy lactic acid bacteria in dairy plants than hypochlorite. However, Rossoni and Gaylarde (2000) showed that hypochlorite was more effective in inactivating a mixed culture of *E. coli, P. fluorescens*, and *S. aureus* attached to stainless steel than peracetic acid. In general, peracetic acid has been found to be effective against biofilm bacteria and is advantageous to use if the biofilm contains food residues.

Acid-anionic sanitizers such as phosphoric, sulfamic, and acid blends are applied at a pH below 3 (Giese 1991). They are fast acting on yeast and viruses but slower acting on bacteria. Anionic sanitizers have good wetting ability, are relatively unaffected by organic load or hard water, are noncorrosive, and can solubilize mineral films. They are often used in clean-in-place (CIP) systems, though not always on a daily basis. Gelinas and others (1984) showed that when an anionic sanitizer was applied at temperatures above 20 °C, its efficacy was greatly improved . Anionic sanitizers are neutralized by alkali cleaner residue and by cationic surfactants (Carsberg 1996, Giese 1991). Frank and Koffi (1990) found acid anionic sanitizers to be ineffective against *L. monocytogenes* biofilms.

Sanitizer selection should be based on whether or not a biofilm is likely to be present and the organic load likely to associated with the biofilm. All approved sanitizers work well in biofilm-free low organic load systems.

Equipment design

Ideally, equipment should be designed to prevent the accumulation of soil and allow for ease of cleaning, so that biofilms will not develop. Equipment must be fabricated using appropriate materials. Proper layout of the processing equipment as well as process automation and installation of CIP system may minimize cleaning problems (Giese 1991, Jackson 1985). Unfortunately, such designs are sometimes either not practical or not implemented. Cleaning problems often occur at dead ends and where gaskets must be used, such as pumps and joints. Such locations may not receive sufficient exposure to cleaning and sanitizing chemicals to remove soil and kill microorganisms. Surviving microorganisms are then provided with sufficient nutrients to form a biofilm that resists subsequent cleaning and sanitation. Generally, food processing plants employing well designed equipment with effective cleaning programs will not have biofilm formation on food contact surfaces. In fact, a survey by Gibson and others (1999) found that biofilm formation within processing plants occurred only on environmental surfaces such as drains and walls, not on food contact surfaces even though there was bacterial attachment to those surfaces.

Formation of viable aerosols is often a by-product of cleaning. Aerosolization provides a means of dispersal of microorganisms present in biofilms. Aerosols are formed during the washing and spraying of surfaces and drains, or when biofilms dry and release dust particles (Kang and others 1989). High pressure, low volume water is usually used to rinse surfaces; however, flow above a pressure of 17.2 bars has been shown to not enhance biofilm removal (Gibson and others 1999). Flooding of a floor drain produced an aerosol that increased airborne microflora for 40 min (Kand and others 1990). Spurlock and Zottola (1991) demonstrated that 210 min after aerosol generation, *L. monocytogenes* was still detectable in the air.

Biofilm detection

Various methods are used to detect and monitor the microbial load on surfaces in food processing plants. The conventional methods include plating of swabbing solution, contact plates, and the dipstick technique. In general, these methods are inexpensive and easy to use. In the swab plating method, moistened swabs or sponges are used to remove microflora from the surfaces. The sample liquid is then plated onto plate count agar or a selective

medium and incubated, then colonies are enumerated and identified if desired. The advantage of this method is that with selective media, specific bacteria, yeast, and mold can be isolated and identified. The major disadvantages are that the method is time consuming. In addition, microorganisms may be selectively removed from the surface (Chae and Schraft 2001, Wirtanen and others 1993). Contact plating directly samples a surface by pressing a plate of solidified agar against the surface. This method is simpler than swabbing, but it is not possible to sample irregular or rough surfaces, the very types of surfaces likely to harbor biofilms. The limitation of the method depends on how much pressure is applied to the agar, contact time, presence of soil, and if the agar picks up the bacterial contaminant (Elliot 1980). In addition, microorganisms do not quantitatively adhere to the agar surface upon application, again resulting in selection for a specific microflora or underestimating microbial numbers on the sampled surface.

ATP bioluminescence test is a rapid biochemical method for estimating total ATP collected by swabbing a surface. Total ATP is related to the amount of food residues and microorganisms collected by the swab. ATP from microbial cells and food residues react with the luciferin-luciferase, resulting in emission of light, the intensity of which is related to the amount of ATP. A result can be obtained in 5 to 10 min. ATP bioluminescence is a good method for rapid determination of cleaning effectiveness, since both food residues and microorganisms are detected. Since the test is rapid, immediate corrective action can be taken. The ATP bioluminescence test cannot detect low levels of microorganisms; for example, more than 10^3 bacteria or 10 yeast cells must be collected by the swab to have positive results (Verran and Jones 2000). There is no practical method for quantitative determination of biofilm microorganisms in the food industry environment. This is because swabs and sponges do not quantitatively detach firmly adherent microflora. However, swab and sponge sampling provide useful information on the extent of microbial growth on a surface and on the extent to which cleaning has been effective.

Consequences of biofilm development

Growth of biofilms in food processing environments leads to an increased opportunity for microbial contamination of the processed product. This increases the risk of reduced shelf life and disease transmission. Microorganisms within biofilms are protected from disinfectants (Frank and Koffi 1990, McCarthy 1992, Ronner and Wong 1993), increasing the likelihood of survival and subsequent contamination of food. EPS associated with biofilms that is not removed by cleaning provides attachment sites and nutrients for microorganisms newly arrived to the cleaned system (Hood and Zottola 1997). Wong (1998) reported that undesirable microorganisms such as *Lactobacillus curvatus* and *Lactobacillus fermentum* persisted on milk residues in cheese processing plants even after repeated cleaning, subsequently contaminating products. Reduction in the efficiency of heat transfer (Mittelman 1998) can occur if biofilms become sufficiently thick at locations such as plate heat exchangers. Some microorganisms in biofilms catalyze chemical and biological reactions causing corrosion of metal in pipelines and tanks.

Conclusions

Microorganisms on wet surfaces have been observed to aggregate and grow into microcolonies, form 3-dimensional structures and communal relationships, resulting in a complex biofilm. However, much of what we know about biofilms has been learned from studies of nutrient-limited natural environments and simulations of these environments that are unlike what is often found in the food industry. The significance of biofilms in food processing is not well understood because of a lack of direct observation of biofilms in this environment and a lack of research using model systems that closely simulate the food system environment. Foodborne pathogens and spoilage organisms can attach to and produce EPS on many food contact and environmental surfaces. Pathogenic bacteria can coexist within a biofilm with other environmental microflora; an example of this is *L. monocytogenes* surviving in *Pseudomonas* biofilms. Biofilms are difficult to remove from food processing surfaces and environments due to the production of EPS materials and the difficulties associated with cleaning complex processing equipment and processing environments. Therefore, biofilm control relies on the implementation of effective cleaning and sanitizing procedures and on a design of processing equipment and the food processing environment that allows easy and thorough soil removal. Effectiveness of biofilm removal can be monitored using ATP-bioluminescence for rapid results or plate count procedures for sensitive results.

References

Adams J, Mclean R. 1999. Impact of rpoS deletion on *Escherichia coli* biofilms. Appl Environ Microbiol 65:4285-7.

Allison D, Sutherland I. 1987. The role of exopolysaccharide in adhesion of freshwater bacteria. J Gen Microbiol 133:1319-27.

Bagge D, Hjelm M, Johansen C, Huber I, Gram L. 2001. *Shewanella putrefaciens* adhesion and biofilm formation on food processing surfaces. Appl Environ Microbiol 67(5):2319-25.

Barnes LM, Lo MF, Adams MR, Chamberlain AH. 1999. Effect of milk proteins on adhesion of bacteria to stainless steel surfaces. Appl Environ Microbiol 65(10):4543-8.

Bishop PL. 1997. Biofilm structure and kinetics. Wat Sci Tech 36(1):287-94.

Blackman IC, Frank J. 1996. Growth of *Listeria Monocytogenes*as a biofilm on various food processing surfaces. J Food Prot 59(8):827-39.

Bos R, Mei HC, Gold J, Busscher HJ. 2000. Retention of bacteria on a substratum surface with micro-patterned hydrophobicity. FEMS Microbiol Lett 189(2):311-5.

Boulange-Peterman, L 1996. Processes of bioadhesion on stainless steel surfaces and cleanability: a review with special reference to the food industry. Biofouling 10(4):275-300.

Boulange-Peterman, L, Barroux B, Bellon-Fontaine M-N. 1993. The influence of metallic wettability on bacterial adhesion. J Adhesion Sci Technol 7(3):221-30.

Boulange-Petermann L, Rault J, Bellon-Fontaine M-N. 1997. Adhesion of *streptococcus thermophilus* to stainless steel with different surface topography and roughness. Biofouling 11(3):201-16.

Bower CK, McGuire J, Daeschel MA. 1996. The adhesion and detachment of bacteria and spores on food contact surfaces. Trends Food Sci Technol 7:152-7.

Briandet R, Meylheue T, Maher C, Bellon-Fontaine MN. 1999. *Listeria monocytogenes* Scott A: cell surface charge, hydrophobicity, electron donor and acceptor characteristics under different environmental growth conditions. Appl Environ Microbiol 65(12):5328-33.

Brocklehurst TF, Zaman-Wong CM, Lund BM. 1987. A note on the microbiology of retail packs of prepared salad vegetables. J Appl Bacteriol 63:409-15.

Bryers JD. 1987. Biologically active surfaces: processes governing the formation and persistence of biofilms. Biotechnol Prog 3(2):57-68.

Butler JL, Stewart JC, Vanderzant C, Carpenter ZL, Smith GC. 1979. Attachment of microorganisms to pork skin and surfaces of beef and lamb carcasses. J Food Prot 42(5):401-6.

Carpentier B, Cerf O. 1993. Biofilm and their consequences with particular reference to hygiene in the food industry. J Appl Bacteriol 75:499-511.

Carr JH, Anderson RL, Favero MS. 1996. Comparison of chemical dehydration and critical point drying for the stabilization and visualization of aging biofilm present on interior surfaces of PVC distributuion pipe. J Appl Bacteriol 80:225-32.

Carsberg H. 1996. Selecting your sanitizers. Food Qual 2(3):35-6, 61-2.

Chae MS, Schraft H. 2001. Cell viability of *Listeria monocytogenes* biofilms. Food Microbiol 18:103-12.

Charlton BR, Kinde H, Jensen LH. 1990. Environmental survey for *Listeria* species in California milk processing plants. J Food Prot 53(3):198-201.

Costerton JW, Cheng KJ, Geesey GG, Ladd T, Nickel JC, Dagupta M, Marrie T. 1987. Bacterial biofilm in nature and disease. Ann Rev Microbiol 41:435-84.

Costerton JW, Irvin RT. 1981. The bacterial glycocalyx in nature and disease. Ann Rev Microbiol 83:299-324.

Cox LJ, Kleiss T, Cordier JL, Cordellana C, Konkel P, Pedrazzini C, Beumer R, Siebenga A. 1989. *Listeria* spp. in food processing, nonfood and domestic environments. Food Microbiol 6:49-61.

Cramton SE, Gerke C, Schnell NF, Nichols WW, Gotz F. 1999. The intercellular adhesion (*ica*) locus is present in *Staphylococcus aureus* and is required for biofilm formation. Infec Immunol 67(10):5427-33.

Criado MT, Suarez B, Ferreiros CM. 1994. The Importance of bacterial adhesion in the dairy industry. Food Technol 48:123-6.

Cunliffe D, Smart CA, Tsibouklis J, Young S, Alexander C, Vulfuson EN. 2000. Bacterial absorption to thermoresponsive polymer surfaces. Biotechnol Lett 22(2):141-145.

Dalton HM, March PE. 1998. Molecular genetics of bacterial attachment and biofouling. Environ Biotechnol 9(3):252-5.

Davey ME, O'Toole GA. 2000. Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 64(4):847-67.

DeBeer D, Srinivasan R, Stewart PS. 1994. Direct measurement of chlorine penetration into biofilms during disinfection. Appl Environ Microbiol 60(12):4339-44.
Dhir V, Dodd CER. 1995. Susceptibility of suspended and surface attached *Salmonella enteritidis* to biocide and elevated temperatures. Appl Environ Microbiol 61(5):1731-8.
Dunsmore DG. 1981. Bacteriology control of food equipment surfaces by cleaning systems. I. detergent effects. J Food Prot 44(1):15-20.
Dunsmore DG, Thomson MA. 1981. Bacteriological control of food equipment surfaces by cleaning systems. II. sanitizer effects. J Food Prot 44(1):21-7.
Elliot RP. 1980. The microbiology of sanitation. In: Katsuyama AM, Strachan JP, editors. Principles of food processing sanitation. Washington, D.C.: Food Processors Institute. p 51-5.
Fatemi P, Frank JF. 1999. Inactivation of *Listeria monocytogenes*/*Pseudomonas* biofilms by peracid sanitizers. J Food Prot 62(7):761-5.
Flemming H-C, Griebe T. 2000. Control of biofilms in industrial waters and processes. In: Walker J, Surman S, Jass J, editors. Industrial biofouling. Chicester, N.Y.: Wiley. P 125-141.
Fletcher, M 1976. The effects of proteins on bacterial attachment to polystyrene. J Gen Microbiol 94:400-4.
Frank J, Chmielewski R. 2001. Influence of surface finish on the cleanability on stainless steel. J Food Prot 68(8):1178-82.
Frank J, Koffi R. 1990. Surface-adherence growth of *Listeria monocytogenes* is associated with increased resistance to surfactant sanitizers and heat. J Food Prot 53(7):550-4.
Frank JF. 2000. Control of biofilm in the food and beverage industry. In: Walker J, Surman S, Jass J, editors. Industrial Biofouling. Chicester, N.Y.: John Wiley & Sons Ltd. p 205-224.
Frank JF, Chmielewski RA. 1997. Effectiveness of sanitation with quaternary ammonium compound or chlorine on stainless steel and other domestic food-preparation surfaces. J Food Prot 60(1):1-6.
Gelinas P, Goulet J, Tastayre GM, Picard GA. 1984. Effect of temperature and contact time on the activity of 8 disinfectants- a classification. J Food Prot 47(11):841-7.
Gibson H, Taylor JH, Hall KE, Holah JT. 1999. Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms. J Appl Microbiol 87:41-8.
Giese J 1991. Sanitation: the key to food safety and public health. Food Technol 45(12):74-80.
Gilbert P, Evans DJ, Evans E, Duguid IG, Brown MRW. 1991. Surface characteristics and adhesion of *Escherichia coli* and *Staphylococcus epidermidis*. J Appl Bacteriol 71:72-7.
Harkonen P, Salo S, Mattila-Sanholm T, Wirtanen G, Allison DG, Gilbert P. 1999. Development of a simple *in vitro* test system for the disinfection of bacterial biofilm. Wat Sci Technol 39(7):219-25.
Hedberg CW, Korlath JA, D'Aoust J-Y, White KE, Schell WL, Miller MR, Cameron DN, MacDonald KI, Osterholm MT. 1992. A multistate outbreak of *Salmonella javiana* and *Salmonella oranienburg* infections due to consumption of contaminated cheese. JAMA 268(22):3203-7.
Heilmann C 1996. Molecular basis of intracellular adhesion in the biofilm forming *Staphylococcus epiderrmidis*. Mol Microbiol 20(5):1083-91.
Heilmann C, Gerke C, Perdreau-Remington F, Gotz F. 1996. Characterization of *TN917* insertion mutants of *Staphylococcus epidermidis* affected in biofilm formation. Infec Immunol 64(1):277-82.
Helke DM, Somers EB, Wong ACL. 1993. Attachment of *Listeria monocytogenes*and *Salmonella typhimurium* to stainless steel and buna-N in the presence of milk and individual milk components. J Food Prot 56(6):479-84.
Helke DM, Wong ACL. 1994. Survival and growth characteristics of *Listeria monocytogenes* and *Salmonella typhimurium* on stainless steel and buna-N rubber. J Food Prot 57(11):963-8, 974.
Herald PJ, Zottola EA. 1988. Scanning electron microscopic examination of *Yersinia enterocolitica* attached to stainless steel at selected temperatures and pH values. J Food Prot 51(6):445-8.
Hines E. 1999. *Listeria* control strategies: a brief history of *Listeria monocytogenes*. Food Qual 5(4):32-4.
Holah JT, Thorne RH. 1990. Cleanability in relation to bacterial retention on unused and abraded domestic sink materials. J Appl Bacteriol 69:599-608.
Hood S, Zottola E. 1997. Adherence to stainless steel by foodborne microorganisms during growth in model food systems. Int J Microbiol 37:145-53.
Hood SK, Zottola EA. 1997. Isolation and identification of adherent gram- negative microorganisms from four meat processing facilities. J Food Prot 60(9):1135-8.
Hyde FW, Alberg M, Smith K. 1997. Comparison of fluorinated polymers against stainless steel, glass and polypropylene in microbial biofilm adherence and removal. J Ind Microbiol Biotechnol 19:142-9.
Jackson AT. 1985. Cleaning of Food Processing Plant. In: Thorne S, editor. Developments in Food Preservation-3, vol. 3. New York: Elsevier Science Publ. Co. p 95-125.
Jeong DK, Frank JF. 1994. Growth of *Listeria monocytogenes* at 10 °C in biofilms with microorganism isolated from meat and dairy processing environments. J Food Prot 57(7):576-86.
Jones CR, Adams MR, Zhdan PA, Chamberlain AHL. 1999. The role of surface physiochemical properties in determining the distribution of the autochthonous microflora in mineral water bottles. J Appl Microbiol 86:917-27.
Jones K, Bradshaw SB. 1997. Synergism in biofilm formation between *Salmonella enteritidis* and a nitrogen-fixing strain of *Klebsiella pneumoniae*. J Appl Microbiol 82:663-8.
Joseph B, Otta SK, Karunasagar I, Karunasagar I. 2001. Biofilm formation by Salmonella spp. on food contact surfaces and their sensitivity to sanitizers. Int J Food Microbiol 64(3):367-72.
Kang Y-J, Frank JF. 1990. Characteristics of biological aerosols in dairy processing plants. J Dairy Sci 73:621-6.
Kang Y-J, Frank JF. 1989. Biological aerosols: a review of airborne contamination and its measurement in dairy processing plants. J Food Prot 52(7):512-24.
Kim KY, Frank JF. 1994. Effect of growth nutrients on attachment of *Listeria monocytogenes* to stainless steel. J Food Prot 57(8):720-6.
Kim KY, Frank JF. 1995. Effect of nutrients on biofilm formation by *Listeria monocytogenes* on stainless steel. J Food Prot 58(1):24-8.
Kjelleberg S, Humphrey B, Marshall K. 1983. Initial phases of starvation and activity of bacteria at surfaces. Appl Environ Microbiol 46(5):978-84.
Kumar CG, Anand SK. 1998. Significance of microbial biofilms in food industry: a review. Int. J Food Microbiol 42:9-27.
Kuntz LA. 1995. New bugs on the block. Food Prod Design 5(5):113-21.
Lawrence JR, Korber DR, Hoyle BD, Costerton JW, Caldwell DE. 1991. Optical sectioning of microbial biofilms. J Bacteriol 173(20):6558-67.
Lazarova V, Pierzo V, Fontvielle D, Manem J. 1994. Integrated approach for biofilm characterisation and biomass activity control. Wat Sci Technol 29(7):345-54.
LeChevallier MW, Cawthon CD, Ramon RG. 1988. Inactivation of biofilm bacteria. Appl Environ Microbiol 54(10):2492-9.
Lee S-H, Frank J. 1991. Inactivation of surface adherent *Listeria monocytogenes* hypochlorite and heat. J Food Prot 54(1):4-6.
Lewis KH. 1980. Cleaning, disinfection and hygiene. In: Silliker JH, Elliot RP, Baird-Parker AC, Bryan FL, Christain JHB, Clark DS, Olson JC, Roberts TA, editors. Microbial Ecology of Foods, vol. 1. New York: Academic Press. p 232-58
Lindberg LE, Holmbom BR, Vaisanen OM, Weber A, Salkinoja M-L. 2001. Sugar composition of biofilms produced by paper mill bacteria. Appl Microbiol BioTechnol 55(5):638-43.
Lunden JM, Miettinen MK, Autio TJ, Korkeala HJ. 2000. Persistent *Listeria monocytogenes* strains show enhanced adherence to food contact surface after short contact time. J Food Prot 63(9):1204-7.
Mafu AA, Roy D, Goulet J, Magny P. 1990. Attachment of *Listeria monocytogenes* to stainless steel, glass, polypropylene and rubber surfaces after short contact time. J Food Prot 53(9):742-6.
Makela PM, Korkeala HJ, Sand EK. 1991. Effectiveness of commercial germicide products against the ropy slime-producing lactic acid bacteria. J Food Prot 54(8):632-636.
McCarthy SA. 1992. Attachment of *Listeria monocytogenes* to chitin and resistance to biocides. Food Technol 46(12):84-8.
McDonnell G, Russell AD. 1999. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 12(1):147-79.
McEldowney S, Fletcher M. 1987. Adhesion of bacteria from mixed cell suspension to solid surfaces. Arch Microbiol 148:57-62.
McLean RJC, Whiteley M, Stickler D, Fuqua WC. 1997. Evidence of autoinducer activity in nauturally occurring biofilms. FEMS Microbiol Lett 154:259-63.
Mittelman MW. 1998. Structure and functional characteristics of bacterial biofilms in fluid processing operations. J Dairy Sci 81:2760-4.
Nelson J. 1990. Where are *Listeria* likely to be found in dairy plants? Dairy Food Environ San 10(6):344-5.
Netting J. 2001. Sticky situation. Sci News 160:28-30.
Nickelson N, Schmidt C. 1999. Taking the hysteria out of *Listeria*: the mechanics of *Listeria* and the strategies to find it. Food Qual 5(4):28-35.
Noterman S. 1994. The significance of biofouling to the food industry. J Food Technol 48(7):13-4.
Oh D-H, Marshall DL. 1995. Destruction of *Listeria monocytogenes* biofilms on stainless steel using monolaurin and heat. J Food Prot 57(3):251-5.
Oosthuizen MC, Steyn B, Lindsay D, Brozel VS, Holy AV. 2001. Novel method for the proteomic investigation of dairy-associated *Bacillus cereus* biofilm. FEMS Microbiol Lett 194:47-51.
Pesci EC, Milbank JBJ, Pearson JP, McKnight S, Kende AS, Greenberg EP, Iglewski BH. 1999. Quinolone signaling in the cell-cell communication system of *Pseudomonas aeruginosa*. Proc Nat Acad Sci 96(20):11229-34.
Piette JPG, Idziak ES. 1991. Role of flagella in adhesion of *Pseudomonas Fluorescens* to tendon slices. Appl Environ Microbiol 57(6):1635-9.
Pratt LA, Kolter R. 1998. Genetic analysis of Escherica coli biofilm formation:roles of flagella, motility, chemotaxis and type I pili. Mol Microbiol 30(2):285-93.
Richards R. 1999. Efficacy of sanitizers on *Listeria monocytogenes* biofilms. Food Austr 51(12):624-5.
Ronner A, Wong A. 1993. Biofilm development and sanitizer inactivation of *Listeria Monocytogenes* and *Salmonella Typhimurium* on stainless steel and buna-N rubber. J Food Prot 56(9):750-8.
Rossoni EM, Gaylarde CC. 2000. Comparison of sodium hypochlorite and peracetic acid as sanitising agents for stainless steel food processing surfaces using epifluorescence microscopy. J Food Microbiol 61:81-5.
Saguy I. 1992. Simulated growth of *Listeria monocytogenes* in refrigerated foods stored at variable temperatures. Food Technol 46(3):69-71.
Samrakandi MM, Roques C, Georges M. 1997. Influence of tropic conditions on expolysaccharide production: bacterial biofilm susceptibility to chlorine and monochloramine. Can J Microbiol 43(8):751-8.
Sandu C, Singh RK. 1991. Energy increase in operation and cleaning due to heat-exchanger fouling in milk pasteurization. Food Technol 45(12):84-91.
Sanford BA, Thomas VL, Mattingly SJ, Ramsay MA, Miller MM. 1995. Lectin-biotin assay to slime present in *in-situ* biofilm produced by *Staphylococcus epidermis* using transmission electron microscopy. J Ind Microbiol 15:156-61.
Schwach TS, Zottola E. 1984. Scanning electron microscopic study on some effects of sodium hypochlorite on attachment of bacteria to stainless steel. J Food Prot 47(10):756-759.
Sinde E, Carballo J. 2000. Attachment of *Salmonella* spp. and *Listeria monocytogenes* to stainless steel, rubber and polytetrafluorethylene: the influence of free energy and the effect of commercial sanitizers. Food Microbiol 17:439-47.
Smoot LM, Pierson MD. 1998. Effect of environmental stress on the ability of *Listeria monocytogenes* Scott A to attach to food contact surfaces. J Food Prot 61(10):1293-8.
Smoot LM, Pierson MD. 1998. Influence of environmental stress on the kinetics and strength of attachment of *Listeria monocytogenes* Scott A to buna-n rubber and stainless steel. J Food Prot 61(10):1286-92.
Sorongon M, Bloodgood R, Burchard R. 1991. Hydrophobicity, adhesion and surface-exposed proteins of gliding bacteria. Appl Environ Microbiol 57:3193- 9.
Spurlock AT, Zottola EA. 1991. The survival of *Listeria monocytogenes* in aerosols. J Food Prot 54(12):910-2, 916.
Stanley P. 1983. Factors affecting the irreversible attachment of *Pseudomonas aeruginosa* to stainless steel. Can. J Microbiol 29:1493-9.
Stewart PS, Camper AK, Handran SD, Huang CT, Warnecke M. 1997. Spatial distri-

bution and coexistence of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in biofilms. Microbiol Ecol 33:2-10.
Sutherland IW. 1983. Microbial exopolysaccharides- Their role in microbial adhesion in aqueous systems. Crit Rev Microbiol 10(2).
Vatanyoopaisarn S, Nazli A, Dodd CER, Rees CED, Waites WM. 2000. Effect of flagella on initial attachment of *Listeria monocytogenes* to stainless steel. Appl Environ Microbiol 66(2):860-3.
Verran J, Jones M. 2000. Problems of biofilm in the food and beverage industry. In: Walker J, Suramn S, Jass J, editors. Industrial Biofouling. Cichester, N.Y.: John Wiley. p 145-73.
Wimpenny J, Manz W, Szewzyk U. 2000. Heterogeneity in biofilms. FEMS Microbiol Rev 24:661-71.
Wimpenny JWT, Kinniment SL, Scourfield MA. 1993. The physiology and biochemistry of biofilm. In: Denyer SP, Gorman SP, Sussman M, editors. Microbial biofilms: formation and control. London: Blackwell Scientific. p 51-94
Wirtanen G, Husmark U, Matilla-Sandholm T. 1996. Microbial evaluation of the biotransfer potential from surfaces with *Bacillus* biofilms after rinsing and cleaning procedures in closed food-processing systems. J Food Prot 59(7):727-33.
Wirtanen G, Matilla-Sandholm T. 1993. Epifluorescence image analysis and cultivation of foodborne biofilm bacteria grown on stainless steel surfaces. J Food Prot 56(8):678-83.
Wong ACL. 1998. Biofilm in food processing environments. J Dairy Sci 81:2765-70.
Zhang TC, Bishop PL. 1994. Structure, activity and composition of biofilm. Wat Sci Technol 29(7):335-44.
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The authors are with the Dept. of Food Science and Technology, Center for Food Safety, Univ. of Georgia, Athens, GA 30602. Direct inquiries to author Frank (E-mail: cmsjoe@arches.uga.edu).