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Bacterial Biofilms on Food Contact Surfaces – a Review

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This review will discuss some of the basic concepts concerning biofilm formation, development and control in the food industry. Biofilm formation process on food contact surfaces can have a detrimental effect on the microbial status of food. The presence of biofilm on abiotic materials can contaminate the product through direct contact. As a consequence, food spoilage is likely to occur that may lead to reduced shelf life and increased risk of food poisoning from pathogens. Bacteria colonizing food processing surfaces are extremely difficult to eradicate. Biofilms can tolerate antimicrobial agents at concentrations of 10–1000 times that needed to inactivate genetically equivalent planktonic bacteria. A better understanding of bacterial adhesion process is needed for the production of microbiologically-safe and good-quality products in the food industry.

INTRODUCTION

Bacteria attach to available surfaces in industrial environments, and can develop into extensive biofilm. Biofilm consists of a complex consortium of microorganisms enmeshed within an extracellular matrix. Biofilms are considered to have a heterogenous structure consisting of microcolonies [Costerton *et al*., 1995; Costerton, 1999; Sutherland, 2001].

Food-processing environments provide a variety of conditions, which might favour the formation of biofilm, for instance: presence of moisture, nutrients and inocula of microorganisms from the raw materials [Bower *et al*., 1996]. Such a biofilm is a potential source of contamination of foods that may lead to spoilage or transmission of foodborne pathogens [Gunduz & Tuncel, 2006]. Moreover, when a biofilm detaches from the abiotic surface individual microorganisms can easily be spread [Poulsen, 1999].

Investigated microflora forming biofilms include *Salmonella* spp., *Klebsiella* spp., *Pseudomonas* spp., *Campylobacter* spp., *Escherichia coli* and *Listeria* spp. These bacteria are of special significance in ready-to-eat and minimally-processed food products, where microbiological control is not conducted in the terminal processing step [Herald & Zottola, 1988b; Cabanes *et al*., 2002; Faille *et al*., 2002; Gunduz & Tuncel, 2006; Kim *et al*., 2006]. In addition, bacterial colonization of food processing equipment is the main concern of damage to metal surfaces (pitting and corrosion) and breakdown of plastics. Biofilms also contribute to decreased heat transfer, lost sensor sensitivity and filters plugging [Mittelman, 1998]. To control these problems, it has been recognised that the greater understanding of the interactions between microorganisms and food-processing equipment is required.

MECHANISMS OF BIOFILM FORMATION

Bacterial colonization of solid material is a multiple-step process involving physicochemical and biological factors. Biofilm formation could be described as a five-step process [Costerton *et al*., 1995; Liu & Tay, 2001; Jefferson, 2004; van Houdt & Michiels, 2005], including:

1. an initial reversible attachment of planktonic microorganisms to solid surfaces;
2. transition of reversible to irreversible attachment by production of extracellular polymers (EPS) by the bacteria;
3. early development of biofilm architecture;
4. development of microcolonies into mature biofilm;
5. dispersion of cells from biofilm into the surrounding environment.

Particular stages of *Bacillus megaterium* biofilm formation process on stainless steel surface (type 304L) are depicted in Figure 1 (author's unpublished data).

Formation of bacterial biofilms begins with the attachment of single cells to abiotic surfaces. This process is time dependent and can roughly be divided into two phases: the reversible and the irreversible one [Marshall *et al*., 1971; Poulsen, 1999]. The reversible adhesion is initiated when bacteria arrive to the surface within a certain distance (2 to 50 nm) via van der Waals and electrostatic forces [Miron *et al*., 2001]. Electrostatic interactions tend to facilitate the repulsion because most bacteria and inter surfaces are negatively charged [Carpentier & Cref, 1993]. *Stenotrophomonas maltophilia* is one

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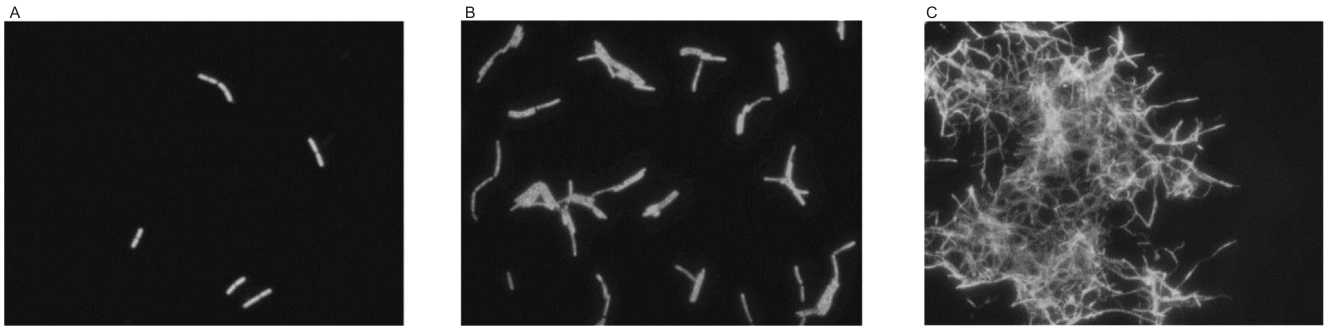

FIGURE 1. Fluorescence microscopic views of stages of *Bacillus megaterium* biofilm formation on stainless steel surface (type 304L) (A - single bacteria cells; B - microcolonies + single bacteria cells; C - mature biofilm) [author's unpublished data].

exception to this rule. The positive surface charge of this microorganism can promote reversible adhesion to negatively--charged material such as Teflon [Jucker *et al*., 1996].

The interactions in the irreversible phase (the step where binding to the surface finishes) are various short-range forces including dipole-dipole, hydrophobic, ion-dipole, ion-ion, covalent bonds and hydrogen interaction [Marshall *et al*., 1971; Zottola, 1994]. The direct contact with the abiotic material is due to the production of surface appendages by the bacteria such as flagella, fimbriae, pili and extracellular polymers [Kumar & Anand, 1998; Cunliffe *et al*., 1999; Liu & Tay, 2001]. In a few species in which the irreversible stages of adhesion have been studied using a modern molecular technique, cells that have committed to adhesion upregulated specific adhesion genes within a few minutes of their attachment to the surface [Dunne, 2002]. The attached cells mostly upregulate all of the genes that produce enzymes involved in the synthesis of EPS itself, including a pivotal sigma factor that turns on EPS synthesis and several cell envelope changes unrelated to the EPS itself [Costerton, 1999].

Once bacteria have irreversibly attached to the surface, the process of biofilm maturation begins. The development of the biofilm and the detachment or the release of cells (either individually or in groups) can be regulated by population density-dependent gene expression controlled by cell-to-cell signaling molecules such as acylated homoserine lactones (AHLs) for Gram-negative bacteria and specific peptides for Gram-positive bacteria [Davies *et al*., 1998].

The irreversibly attached cells grow and divide by using nutrients present in the conditioning film and the surrounding fluid environment. This leads to the formation of microcolonies, which enlarge and coalesce to form a layer of cells covering the surface. During this step, the anchored cells also synthesize additional extracellular polymers that help in the attachment process of the cells to the surface and protect the cells aggregate from the fluctuations of the environment [Kumar & Anand, 1998; Jefferson, 2004]. The biofilm maturation is a slow process and reaches a few millimeters thick in a matter of days depending on the culture conditions [Dunne, 2002].

As the biofilm ages, the attached bacteria, in order to survive and colonise new niches, detach and disperse from the biofilm. The cells get detached individually or are sloughed off [Kumar & Anand, 1998]. Sloughing is a discrete process whereby periodic detachment of relatively large particles of biomass from the biofilm occurs [Applegate & Bryers, 1991]. The released microorganisms may be transported to newer locations and again restart the biofilm formation process [Marshall, 1992].

FACTORS AFFECTING THE ADHESION OF MICROORGANISMS TO SOLID SURFACES

In the process of biofilm formation, properties of solid material, hydrophobicity of both material and cell surface, production of extracellular polymeric substances, and environmental factors play an important role in reversible or irreversible adhesion and microcolony formation to mature biofilm structure [Prigent-Comabaret *et al*., 2000; Lindsay *et al*., 2000; Liu & Tay, 2001; Cabanes *et al*., 2002; Czaczyk & Myszka, 2007].

Solid surface properties

Properties of abiotic surfaces are key factors for biofilm formation, because they influence initial cell attachment [Shi & Zhu, 2009]. Investigators have focused on surface roughness believed to be important in reversible adhesion process [Howell & Behrends, 2006; Scardino *et al*., 2006]. Surface roughness is a distance measurement between the peaks and valleys on the material's surface. Howell & Behrends [2006] noticed that the extent of microbial attachment was correlated with surface roughness. Also studies of Jones *et al*. [1999] demonstrated that surface defects were associated with a significant increase in bacterial adhesion. This phenomenon is likely to be due to the fact that: (i) a rough surface has a greater surface area, and (ii) depressions in the roughened material provide more favourable sites for colonization [Mitik-Dineva *et al*., 2008, 2009]. Moreover roughened surfaces form a harborage to protect bacteria from shear forces in the food fluid.

The conditioning of solid material also plays a significant role in the rate of microbial attachment. The abiotic material could be covered by a film of organic molecules such as proteins from milk, pork, beef and even EPS synthesised by bacteria [Dunne, 2002; Shi & Zhu, 2009]. McGuire & Swartzel [1989] demonstrated that primary adhesion of bacteria to stainless steel and Teflon was enhanced in the presence of surface-associated milk proteins. According to Hood & Zottola [1997], the material conditioned by diluted milk was better for attachment of pathogens than the whole milk. It was as-

sumed that some molecules, like bovine serum album (BSA), inhibited cells attachment process to solid surfaces [Hood & Zottola, 1997].

Hydrophobicity/hydrophilicity

Hydrophobic/hydrophilic interactions have been suggested as being responsible for a wide range of adherence phenomena [Hood & Zottola, 1995; Czaczyk *et al*., 2008]. Metal surfaces have a high surface energy, are negatively charged, and hydrophilic as shown by water contact angles, while polymers such a Teflon have a low surface energy, are less electorostatically-charged and hydrophobic [Faille *et al*., 2002]. The hydrophobicity of a metal surface has been determined mainly by contact angle measurement [Liu *et al*., 2004]. Depending on the hydrophobicity of material, bacteria adhere differently to surfaces with different hydrophobicities. The adherence of *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Escherichia coli* was found to be greater when the abiotic material surface free energy was close to that of the bacteria [Vuong & Otto, 2002; Liu & Zhao, 2005; Pereni *et al.*, 2006]. Several research groups have reported that hydrophobic materials are more resistant to bacterial adhesion than the hydrophilic surfaces [Benito *et al*., 1997; Flint *et al*., 1997; Cunliffe *et al*., 1999; Fuster-Valls, *et al*., 2008]. In contrast, Baker [1984] observed no difference between hydrophilic glass and polystyrene plates in adhesion of freshwater bacteria. Moreover, Bos *et al*. [2000] demonstrated that microbial attachment process happened at the hydrophilic region of the hydrophilic-hydrophobic interface of the stainless steel material.

Biosynthesis of extracellular polymers

EPS molecules are regarded as the major factor influencing the microbial biofilm formation process. The extracellular substances promote irreversible adhesion phase. EPS molecules strengthen the interactions between the microorganisms and thus they determine the cells microcolony formation process on solid surfaces [Costerton *et al*., 1995; Branda *et al*., 2005]. EPS are an integral part of biofilm and their thickness varies from 0.2 to 1.0 μm [Fleming & Wingender, 2001; Branda *et al*., 2005]. Recent reports suggest that mostly extracellular proteins and exopolysaccharides are responsible for the architecture and morphology of the biofilm matrix [Langille *et al*., 2000].

EPS synthesised by microbial cells vary greatly in their composition and hence in their chemical and physical properties [Sutherland, 2001]. Exopolysaccharides are composed of either homopolysaccharides or heteropolysaccharides. Microbial homopolysaccharides are composed of only one monosaccharide's type: D-glucose or L-fructose. Homopolysaccharides contain three distinct groups: α-D-glucans, β-D-glucans and fructans [Monsan *et al*., 2001; Sutherland, 2001]. Extracellular proteins are compounds with molecular masses between 10 and 200 kDa. They contain from 40% to 60% of hydrophobic amino acids. Amino acids with hydroxyl groups and L- and D-glutaminosyl residues are mostly detected constituents of exogenous proteins in bacteria. The absence of sulfuric amino acids in this layer was noticed as well [Ton–That *et al*., 2004; Czaczyk & Myszka, 2007]. The EPS synthesis depends on many environmental determinants, such as carbon and/or nitrogen supply, pH value, cultivation temperature, oxygen limitation and stage of cells growth [Shu & Lung, 2004; Czaczyk *et al*., 2008; Myszka & Czaczyk, 2009].

Other factors

Bacterial formation process on abiotic surfaces varies among species and with environmental conditions (nutrient level, pH and temperature) [Donian, 2002]. Different models have been proposed in laboratory experiments, in which starvation is the trigger for adhesion mechanism, while other research suggest that bacterial colonization process is enhanced when nutrients availability is high [Costerton *et al*., 1995; Sanin *et al*., 2003; Myszka *et al*., 2007]. Depending on the system applied, starvation and nutrient availability may induce the particular stages of biofilm development process. Nutrient-rich condition sets the stage for initial surface colonization, while starvation promotes the biofilm maturation process of *Proteus vulgaris* and *Pseudomonas areuginosa* cells [Myszka *et al*., 2007; Myszka & Czaczyk, 2009].

The concentration of phosphate in a medium is needed for basic cellular processes improving bacteria colonization mechanism [McEldowney & Fletcher, 1986]. The inorganic phosphate is responsible for synthesis of polyphosphate kinase. This enzyme is involved in *quorum sensing* and rhamnolipid production, both of which are important factors for biofilm maturation. In strains lacking a functional *ppk* gene, which encodes polyphosphate kinase, the concentrations of *quorum sensing* autoinducers are reduced by 50%, and the synthesis of rhamnolipids is significantly reduced. The *ppk* mutants have a biofilm phenotype composed of a thin cells layer (20% of wild-type thickness) and their biofilms lack the three-dimensional structure [Rashid *et al*., 2000].

Several studies have shown that both pH and temperature values are important for bacteria extensive adhesion to stainless steel surface. *Pseudomonas aeruginosa* and *Listeria monocytogenes* cells show the maximal adhesion on abiotic materials at neutral pH and a temperature of 30ºC [Herald & Zottola, 1988a; Busalmen & de Sanchez, 2001]. In *Yersinia enterocolitica* the adhesion is enhanced on solid surfaces at pH 8–9 and a temperature of 30ºC [Herald & Zottola, 1988b]. Linsay *et al*. [2000], have demonstrated that the attachment of *Bacillus* spp. to stainless steel surface is promoted at 36––44ºC and at pH 4 and 10.

RESISTANCE TO ANTIMICROBIAL AGENTS

Removal or inactivation of bacteria from abiotic surfaces by washing with water or treatment with disinfectant or sanitizers is not always achieved because the cells are enmeshed in biofilms or otherwise protected against exposure to antimicrobials [Kim *et al*., 2006].

Several mechanisms have been proposed to explain high resistance of biofilms, including restricted penetration of antimicrobial agents into biofilm structure, slow growth owing to nutrient and oxygen limitation, expression of genes involved in the general stress response and emergence of the biofilm-specific architecture [Stewart, 2002; Ito *et al*., 2009]. However,

since combinations of these factors are involved in most biofilm studies, it is difficult to fully understand the mechanisms of biofilm resistance to antimicrobials.

Biofilm matrix may act as a diffusion barrier, preventing biocides reaching their targets. Biofilm matrix limits penetration of antimicrobials [Drenkard, 2003]. Alginate, produced by *Pseudomonas aeruginosa* has been studied for its ability to trap antimicrobial agents. This ability appears to be related to the anionic nature of the exopolymer. Cationic molecules can thus be retained within the matrix and prevented from acting upon the biofilm bacteria. Alginate has also been shown to bind positively-charged biocides and inhibit their activity. A recent study found that diffusion of biocides through biofilm of *Pseudomonas aeruginosa* was delayed. However biocides eventually penetrated through to the distal regions of the biofilm in a sufficient concentration to kill microorganisms [Suci *et al*., 1994].

Killing the bacteria by many biocides is a growth-dependent action. The bacteria located in a biofilm periphery have better access to nutrients and oxygen than bacteria located deeper in a biofilm population. Establishment of microenvironments within biofilms leads to slow growth or disorders of bacteria metabolism. Most antimicrobial agents target some type of macromolecular production. These agents would affect bacteria in which macromolecular synthesis is arrested [Stewart, 2002]. Bacteria in the non-growing zones of the biofilm are well positioned to survive antimicrobial challenge and are less susceptible than in the biofilm in which all of the bacteria grow at the intermediate rate [Xu *et al*., 2000].

The slow growth and altered metabolic activity apparent in biofilms suggest that biofilm bacteria are in a stationary--phase state. The hallmark feature of this bacteria growth phase is the activity of *rpoS* , the stationary-phase sigma factor instrumental in regulating stress response factors. The fact that *rpoS* plays also a role in *quorum sensing* provoked a study on the later stages of biofilm development, where high cell density and *quorum sensing* are both contributing factors [Anderl *et al*., 2000]. The analysis of *Escherichia coli* biofilms revealed the upregulation of nearly 50% of all *rpoS*-regulated genes. In the work carried out by Schembri *et al*. [2003], an *rpoS* mutant failed to form a biofilm.

The expression of efflux pumps in biofilms might also lead to antimicrobial resistance in the surface-attached communities. It was found that two of the best-studied Resistance--Nodulation-Division (RND) efflux pumps in *Pseudomonas aeruginosa*, MexAB-OprM and MexCD-OprJ were involved in biofilm resistance to biocides [Zhang & Mah, 2008]. The MexAB-OprM efflux pump is produced at low levels of *mexA-mexB-opr M* gene operon in all strains of *Pseudomonas aeruginosa*. The MexCD-OprJ efflux pump is detected and overproduced in the so-called *nfxC* and *nfxD* mutants [De Kievit *et al*., 2001]. These efflux systems are responsible for extruding antimicrobials from inside the cell. Expression of efflux pumps is induced during exposure to sublethal doses of a wide range of biocides. The efflux systems are characterised by a three-component organization: a membrane fusion protein that is associated with the cytoplasmatic membrane, a transporter protein that exports substrate across the inner membrane, and an outer membrane protein (OMP) that facilitates the passage of the substrate across the outer membrane. The channel traverses the inner and outer membranes, whereas substrates are extruded directly from the cytoplasm into the external medium [Zgurskaya & Nikaido, 2000; Drenkard, 2003].

ELIMINATION OF MICROBIAL BIOFILMS FROM ABIOTIC SURFACES

In industrial practice it is necessary to develop a complete and cost-effective cleaning programme, which will inhibit accumulation of both particulates and bacterial cells present on abiotic surfaces [Dunsmore *et al*., 1981; Pontefract, 1991]. However, an inappropriate cleaning procedure may lead to biofilm development and increase the risk of spoilage and pathogenic microflora transmission. Biofilms can be removed from food contact surfaces by adopting different physical and chemical methods. Table 1 lists the major physical and chemical agents eliminating bacterial biofilms from abiotic surfaces.

Physical methods

The most common physical method employed to remove biofilms is manual cleaning of the surface using scrubbers. According to Qian *et al*. [1999], the scraping methods followed by ultrasonic technique can remove the thick layer of attached *Escherichia coli* cells (in this study, at sonication time of 100–150 kHz).

Among the physical methods, raising temperature of water can control biofilm formation. However temperature at which this occurs differs with the type of microorganisms present in biofilms. Studies show water with a very high tem-

TABLE 1. Major physical and chemical agents eliminating biofilms from abiotic surfaces.

Agent/technique	Biofilm	References
Scraping and ultrasonic technique	*Escherichia coli*	Qian *et al*. [1999]
Very high temperature of water	*Pseudomonas aeruginosa*	Wirtanen & Matilla-Sandholm [1993]; Burfoot *et al*. [2009]
Low electrical current with antibiotics	*Escherichia coli*	Caubet *et al*. [2004]
Chlorine	*Salmonella* spp.	Gelians *et al*. [1984]
Peroxygen	*Salmonella* spp.; *Listeria monocytogenes*	Harkonen *et al*. [1999]
Quaternary ammonium compounds	*Listeria monocytogenes*	McCarthy [1992]

perature such as 125°C applied for 30 min to be the most effective in eliminating microbial communities, however 3-day-old biofilms with EPS were found to be difficult to remove completely even at this temperature [Wirtanen & Matilla-Sandholm, 1993; Burfoot *et al*., 2009].

Recently, low electrical currents in combination with antibiotics have been successively employed for the removal of *Escherichia coli* biofilm [Caubet *et al*., 2004]. The bioelectric effect is due to transport of additional biocide ions through biofilm structure. Even though the antibiotic moved into the cell and target site much quickly in potentially lethal concentrations, it was still dependent on the rate of growth and metabolism of bacteria for its antimicrobial activity [Kumar & Anand, 1998; Caubet *et al*., 2004]. These data suggested also that biofilm age and activity were the limiting factors in the antibiotic effectiveness [Drenkard, 2003; Caubet *et al*., 2004].

Chemical methods

The major type of chemical sanitizers used in the food industry are: halogens, peroxygens, acids and quaternary ammonium compounds (QAC). The effectiveness of chemical agents is limited by the presence of soil, water hardness and temperature of applications [Kim & Frank, 1995]. The proper method for eliminating biofilms from abiotic surface must break up or dissolve the EPS matrix associated with the biofilm so that sanitizing agents can gain access to the cells [Chmielewski & Frank, 2003].

Chlorine is commonly used as a sanitizer due to its oxidizing and disinfecting value [De Beer *et al*., 1994]. Ronner & Wong [1993] found that chlorine was able to remove *Salmonella* EPS material from stainless steel better than a quaternary ammonium compound. Gelians *et al*. [1984] showed that extending the contact time for chlorine sanitizers from 5 to 30 min would greatly improve efficacy of chlorine. It was demonstrated on the example of *Pseudomonas* spp. biofilms on a stainless steel surface [Gelians *et al*., 1984]. Chlorine dioxides and chloramines are also applied as sanitizers in the food industry. Samrakandi *et al*. [1997] observed that monochloramine was better able to penetrate bacterial biofilm than chlorine, but chloramines require longer contact time to be effective.

Peroxygen sanitizers are a broad-spectrum agents. They are both bactericidal and active against endospores [McDonell & Russel, 1999]. Peroxide-based sanitizers were found to be more effective against *Listeria monocytogenes* and *Salmonella* spp. biofilms than chlorine [Harkonen *et al*., 1999].

Quaternary ammonium compounds are cationic surfactant sanitizers and also display cleaning activity [McEldowney & Fletcher, 1987]. They are often used as a foam, which provides longer contact times on surfaces. QAC are effective against Gram-positive and Gram-negative bacteria. These agents are often recommended for floors, walls and storage containers surfaces that can be sanitized for a long contact time, and for surfaces which do not require rinsing before production process [Chmielewski & Frank, 2003]. McCarthy [1992] demonstrated that 5-min exposure of 400 ppm of QAC inactivated *Listeria monocytogenes* biofilms.

PREVENTING BIOFILM FORMATION

Methods for preventing biofilm formation on food contact surfaces include applying various kinds of disinfectants, modifying surface hydrophobicity and designing food industry equipment. Some surfaces impregnated with antimicrobial agents may prevent microbial colonization for as long as the antimicrobial agents are being released from these materials. Antifoulant paints, which are used to protect the hull of ships from fouling, may also be used to control microbial adhesion on food contact surfaces. For example, antifoulant paints containing silver ions have been successfully applied to control mixed biofilms containing *Legionella pneumophila* [Gu *et al.,* 2001; Silvestry-Rodriguez *et al.,* 2008]. It has been reported that the applications of lactic acid bacteria cultures, their cell-free supernatants or some metabolites (bacteriocins), prevented the adhesion of microorganisms to abiotic and biological surfaces [Tuomola *et al.,* 2000]. Several studies have demonstrated that the adsorption of biologically-active molecules (nisin, lysozyme) on solid surfaces may inhibit the initial bacterial adhesion [Bower *et al*., 1998]. Zeraik & Nitschke [2010] observed the reduction of *Listeria monocytogenes* and *Staphylococcus aureus* attachment process to polystyrene surface after its conditioning by surfactin.

In recent years, food packaging materials containing active compounds have gained practical significance in the control of spoilage microorganisms and foodborne pathogens on food surfaces. These active antimicrobial substances incorporated in the packing materials migrate to the food surface and eliminate microbial contamination [Le Magrex-Debar *et al.,* 2000]. Hydrophilic materials such as glass or stainless steel are known to reduce microbial adhesion and therefore they are often used as food contact surfaces. It is caused by low interfacial energy characteristic for such materials, and the fact that the cells and surfaces are similarly charged. The low-affinity contact surfaces may be obtained by attaching to them some passivating compounds. Microbial passivation of the surface can be obtained by non-covalent attachment of some proteins (*e.g.* albumin) at the surface or treatment the materials with surface-active or non-ionic compounds. Protein films that are formed on surfaces can profoundly alter the properties of the material [Barnes *et al.,* 1999]. The proper design of food contact equipment plays an important role in preventing biofilm formation. Materials which are used as food contact surfaces should be smooth (without any cracks, scratches and topographical defects), and resistant to corrosion and damage. However the rate of bacterial adhesion to smooth and slippery surfaces is slower only at the beginning of the process [Bogusławska-Wąs *et al*., 2007]. Therefore, in the food industry the more important in equipment design is the cleanability of surfaces [Bower *et al.,* 1996; Kumar & Anand, 1998; Branda, 2005].

According to Aminov [2010], additional possible approach to prevent biofilm formation process on food contact surfaces is application of antimicrobial peptides from marine animals and plants that mimic bacterial signal molecules. These peptides were observed to disturb the cells' communication process and thereby block off the biofilm's maturation process on solid materials [González & Keshavan, 2006].

In the food industry, Hazard Analysis and Critical Control Point (HACCP) approach is one of the most reasonable paths to follow to reduce and/or eliminate bacterial biofilms [Bryan, 1990; Havelaar, 1994]. The HACCP system is used to manage the CCP(s) identified, as well as to prevent or reduce specified food safety hazards from the food processing plant, as determined during hazard analysis. Hazard analysis determines the strategy ensuring hazard control by combining the Good Manufacturing Practice (GMPs), Good Hygienic Practices (GHPs) and Good Agricultural Practices (GAPs). The HACCP system improves product safety by anticipating and preventing health hazards before they occur. The attention to the detail of mostly cleaning and hygiene procedures, the treatment and formulation of food products are needed to control any biofilms and to prevent their potential to cause harm to consumer [Bryan, 1990].

CONCLUSIONS

The problem of bacterial attachment process to food contact surfaces has not been dissolved yet. The growth of biofilms in food processing environments leads to the increased opportunity for microbial contamination of the processed product. This increases the risk of reduced shelf life and disease transmission. To better understand and control biofilms in food processing plants, research must progress in an area of methods for the study of biofilms. A more reliable technique for direct observation of biofilms in food processing environments or an effective experimental system that simulates the food processing environments, are one of the most pressing challenges to modern microbiology. This technique should improve the examination of all factors affecting the biofilm formation process and identification which of these factors are particularly involved in maintaining biofilm architecture. A better understanding of the mechanisms of biofilm development process on food contact materials will help in the eradication of the attached microflora.

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