# Antibiofilm Strategies in the Food Industry

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**Abstract** Biofilms in food processing plants represent not only a problem to human health but also cause economic losses by technical failure in several systems. In fact, many foodborne outbreaks have been found to be associated with biofilms. Biofilms may be prevented by regular cleaning and disinfection, but this does not completely prevent biofilm formation. Besides, due to their diversity and to the development of specialized phenotypes, it is well known that biofilms are more resistant to cleaning and disinfection than planktonic microorganisms. In recent years, a considerable effort has been made in the prevention of microbial adhesion and biofilm formation on food processing surfaces and novel technologies have been introduced. In this context, this chapter discusses the main conventional and emergent strategies that have been employed to prevent bacterial adhesion to food processing surfaces and thus to efficiently maintain good hygiene throughout the food industries.

## 1 Introduction

Food processing environments provide a diversity of favorable conditions for biofilm formation such as the presence of nutrients and moisture and the inocula of microorganisms from raw products. Hence, while totally undesirable, biofilms are formed in all food processing surfaces such as plastic, glass, metal, wood, etc. "Dead zones," like cracks, corners, joints, and gaskets, are places where biofilm can remain after cleaning. In addition, biofilms provide a protective environment, in which exopolymeric substances (EPS) lead to a significantly higher tolerance of biofilm cells to many stresses including disinfectants or sanitizers than to free-floating cells or planktonic cells (Gilbert et al. 2001). These biofilms are potential

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sources of contamination with the consequent spoilage of foods as well as the transmission of foodborne microorganisms. Moreover, when a biofilm detaches from the surface, individual microorganisms can easily be spread, contaminating the surrounding environment and causing cross and post-processing contamination. In addition, biofilms are often responsible for the interference of mechanical locks in the process of heat transfer, as well as for the increased rate of corrosion on surfaces. In drinking water systems, for instance, biofilms can clog pipes, leading to decreases in speed and capacity, which means increased energy usage. Similarly, biofilm formation in heat exchangers and cooling towers can reduce heat transfer and efficiency. Moreover, the ability of bacteria to persist in biofilms on the metal surfaces of processing facilities can also cause corrosion of the surface due to acid production by bacteria. From the above-mentioned, it can be concluded that biofilms in food industries can cause serious health problems and large economic losses.

Many food safety problems can be avoided if good manufacturing practices codified in 21 CFR 110 are followed (FDA 2004). In fact, most of the problems are due to inefficient hygienic practices among employees, language barriers, ineffective training of employees, the existence of biofilms in niche environments, ineffective use of cleaning agents/disinfectants, lack of sanitary equipment design, reactive instead of routine maintenance, ineffective application of sanitation principles, contamination of raw materials with microorganisms, allergens and/or toxins, post-processing contamination microorganisms, allergens and/or toxins, incorrect labeling or packaging, older equipment (more difficult to clean), corrosion of metal containers/equipment/utensils, and contamination with cleaner/sanitizer residues (FDA 2004). However, it is generally accepted that the main problem of the food industry is the survival of foodborne pathogens or microorganisms that cause food spoilage, due to inadequate disinfection of instruments or surfaces that come in contact with food resulting in the formation of biofilms. Biofilms are problematic mainly in food industry sectors such as dairy processing, brewing, fresh produce, poultry processing, and red meat processing (Frank et al. 2003; Jessen and Lammert 2003; Somers and Wong 2004; Chen et al. 2007). These industries are the principal reservoirs for *Salmonella, Campylobacter, Listeria, Yersinia enterocolitica*, and *Staphylococcus aureus* worldwide, which transmit disease to consumers when the contaminated products are inappropriately cooked (Farber and Peterkin 1991; Dewanti and Wong 1995; Kim et al. 2008).

Since biofilms are a great concern in the food industry, many studies have been performed in order to find an efficient strategy to their control and eradication. However, the most important antibiofilm approach will always be to prevent microbial adhesion and biofilm formation by regular cleaning and disinfection of surfaces.

## 2 Main Foodborne Pathogens

Illnesses caused by ingestion of contaminated food include a broad range of diseases and are a rising global public health concern. The contamination of food can be caused by microorganisms or chemicals, can take place at every step in the process from food production to consumption, and might be a consequence of environmental contamination (such as pollution of soil, air, or water). Although the main clinical presentation of foodborne illnesses consists of gastrointestinal symptoms, they may also have gynecological, neurological, immunological, and other symptoms. Multiorgan breakdown and even cancer can be caused by the intake of contaminated food and is associated with a substantial burden of disability and mortality (WHO 2013). According to the European Food Safety Authority (EFSA 2012), 5,262 foodborne outbreaks were reported in the European Union in 2010, leading to a large amount of human infections and hospitalizations, and causing 25 deaths. The majority of outbreaks that occurred in 2010 were caused by *Salmonella*, viruses, *Campylobacter*, and bacterial toxins. Besides these microorganisms, *Listeria monocytogenes* and *Escherichia coli* are also among the main foodborne pathogens responsible for severe human infections. Moreover, all mentioned bacteria are known to form biofilms on food contact equipment and food surfaces, causing financial losses and severe health problems (Kumar and Anand 1998; Chae and Schraft 2000; Wirtanen et al. 2000).

*L. monocytogenes* is a facultative intracellular bacterium that is ubiquitous in the environment and pathogenic to humans, since it causes listeriosis—a predominately foodborne illness that has a higher mortality rate in comparison with other foodborne diseases (EFSA 2012). This bacterium is commonly found in diverse foodstuffs as well as in animal feed, soil, water, plants, sewage, and fecal matter (Moltz and Martin 2005; Tompkin 2002). Moreover, ready-to-eat food, uncooked meat products, vegetables, poultry, and soft cheeses have all been reported as vehicles of listeriosis (Teixeira et al. 2007b; Conter et al. 2009; Jadhav et al. 2012), with ingestion of contaminated food being the main route of transmission for humans (Dussurget 2008). Contamination of food by *L. monocytogenes* may happen through several distinct routes, such as staff equipment, uncooked materials, or contact surfaces (Møretrø and Langsrud 2004; Teixeira et al. 2008). Nevertheless, as far as commercial foodstuff is concerned, contamination by these bacteria is not frequently a consequence of flaws in cleaning and disinfection, but it is due to cross-contamination in the post-processing environment (Ryser and Marth 2007; Latorre et al. 2010). This typically takes place in spaces where organic remains accumulate and biocidal compounds have reduced access (slicers, joints, cutting equipment, etc.), which are favorable for continuous biofilm development and provide an opportunity for some strains to become dominant and persevere at the food plant (Verghese et al. 2011).

*Salmonella* spp. are a group of food contaminant organisms with significant importance in the food industry. Although there are currently more than 2,500 identified serotypes of *Salmonella*, *Salmonella enterica* serovars Enteritidis and

Typhimurium most commonly cause human disease. It is believed that some salmonellosis outbreaks were due to the inexistent or deficient cleaning and disinfection of surfaces and tools (e.g., Ellis et al. 1998; Reij and Aantrekker 2004; Giraudon et al. 2009; Podolak et al. 2010). In fact, several studies have shown that these bacteria are able to colonize various food contact surfaces (e.g., Teixeira et al. 2007a; Oliveira et al. 2006; Rodrigues et al. 2011), and it was also reported that *Salmonella* adhere and form biofilms in food processing facilities (Joseph et al. 2001). Moreover, it has already been well established that the antimicrobial efficiency of diverse biocidal agents is inferior against these biofilms than for their respective planktonic cells. Accordingly, nine disinfectants usually applied in the food industry and efficient against planktonic *Salmonella* cells revealed a variable efficiency against biofilms, with products containing 70 % ethanol being most efficient (Møretrø et al. 2009). Previous studies have also pointed out that, in comparison to *Salmonella* planktonic cells, biofilms were more resistant to trisodium phosphate (Scher et al. 2005), chlorine, and iodine (Joseph et al. 2001).

*Campylobacter* spp. are foodborne pathogens with the ability to colonize different inert surfaces (Kusumaningrum et al. 2003; Sanders et al. 2007; Shi and Zhu 2009) and are also frequently isolated from poultry and poultry processing. *Campylobacter jejuni* has been the most predominant strain found in such environments (Deming et al. 1987; Sanders et al. 2007) and, consequently, several studies have been performed in order to understand the behavior of this bacterium (Trachoo et al. 2002; Dykes et al. 2003; Hanning et al. 2008). One of the main findings was that, although *C. jejuni* does not readily form a biofilm, it does form mixed biofilms with enterococci (Trachoo and Brooks 2005), within which it gains a higher tolerance to various chemical biocides (Trachoo and Frank 2002). The fact that *C. jejuni* adhesion and colonization of surfaces is eased by a preexisting biofilm (Hanning et al. 2008) highlights the importance of intensifying the control of biofilms, especially in poultry environments where these bacteria are more commonly found.

*E. coli* O157:H7 is among the most severe foodborne pathogens, with outbreaks related mainly to ingestion of undercooked meat (Proctor et al. 2002), but also with other contamination routes such as drinkable (Swerdlow et al. 1992) and leisure water (Ackman et al. 1997). The adhesion and biofilm formation ability of *E. coli* O157:H7 on diverse food contact surfaces existent in the meat industry has been investigated, and it was observed that these bacteria adhered to and developed biofilms on such materials, even at low temperatures (Dourou et al. 2011). It was also found that the adhesion of these bacteria was affected by the existence of other microbes on the surfaces (Klayman et al. 2009; Marouani-Gadri et al. 2009). As an example, a study conducted by Habimana and coworkers (2010) showed that *E. coli* O157:H7 cells were entrenched and enclosed in an *Acinetobacter calcoaceticus* biofilm, which is in agreement with several other reports that demonstrated multispecies biofilms enhanced the chances for pathogens to flourish in food processing environments (Habimana et al. 2010; Stewart and Franklin 2008).

*Bacillus* spp. and especially *Bacillus cereus* are associated with food spoilage (Andersson et al. 1995; Janneke et al. 2007). Since *B. cereus* is ubiquitous in the environment, contamination by this bacterium is quite unavoidable in food industry facilities. As an example, over 12 % of the microbial biofilms found in a commercial dairy plant corresponded to *B. cereus* (Sharma and Anand 2002). In addition, this bacterium produces spores that can endure a large range of adverse conditions and promptly attach to food contact surfaces, due to their highly hydrophobic character (Lindsay et al. 2006). *B. cereus* is responsible for two kinds of gastrointestinal diseases, diarrheal and emetic, and the outbreaks associated with this bacterium have been related to the ingestion of several different food items, such as meat, fish, vegetables, rice, milk, cheeses, pasta, and foodstuff with sauces (puddings, roasted, and salads). Moreover, between 1998 and 2008, 1,229 foodborne outbreaks reported in the USA were caused by this bacterium as well as by *Clostridium perfringens* and *S. aureus* (Bennett et al. 2013).

## 3 Antibiofilm Strategies in the Food Industry

Microbial adhesion to food processing surfaces is a rather fast process, and therefore, cleaning and disinfection of such surfaces is often not sufficient to prevent the adhesion of microorganisms. In fact, cleaning only removes approximately 90 % of bacteria from surfaces and does not kill them (Srey et al. 2013), so disinfection is crucial. Nevertheless, an adequate frequency of disinfection should be carefully determined to avoid accumulation of both particulates and bacterial cells present on abiotic surfaces. The main strategy to prevent biofilm formation is to avoid bacterial adhesion by choosing the correct materials and performing the appropriate cleaning methods. In this context, it is of utmost importance to use materials that do not promote or even suppress biofilm formation. Antimicrobial agents should be applied to walls, ceilings, and floors. Surfaces should have modified physicochemical properties or be impregnated with biocides or antimicrobials to minimize bacterial colonization (Rogers et al. 1995). Hydrophobic surfaces are more prone to biofilm formation than hydrophilic ones. It is also essential that equipment design is smooth and does not contain faults like crevices, corners, cracks, gaskets, valves, and joints, which are vulnerable areas for biofilm accumulation and not easily accessible to sanitizers. Cleaning and disinfection should be performed regularly before bacteria firmly attach to surfaces. To this end, cleaning-in-place (CIP) procedures have been used and sometimes include physical methods, such as mechanical brushing, chemical agents, such as detergents, and biological agents, like enzymes to obtain a biofilm-free industrial environment (Kumar and Anand 1998). Even with these procedures microorganisms can remain on surfaces. Thus, Good Manufacturing Practice (GMP), Good Hygienic Practices (GHPs), Good Agricultural Practices (GAPs), and Hazard Analysis and Critical Control Points (HACCP) have been established for controlling food quality and safety (Myszka and Czaczyk 2011). The HACCP system has the advantage of improving product safety by anticipating and preventing health hazards before they occur. Nevertheless, adhesion and biofilm formation on food processing surfaces and food spoilage and contamination still occur. In recent years several physical and chemical methods have been developed to avoid/control biofilm formation and will be discussed below.

# 4 Current Approaches

## *4.1 Chemical Disinfection*

To obtain an efficient disinfection, surfaces should be properly cleaned. However, disinfection can be affected by environmental conditions such as temperature, pH, concentration, contact time, soiling and type of surface or medium to be disinfected, and the presence of organic substances including fat, carbohydrates and protein-based materials (Møretrø et al. 2012). Disinfectants may also differ in their ability to kill target microorganisms. There is a wide range of chemical disinfectants, which can be divided according to their mode of action: oxidizing agents including chlorine-based compounds, hydrogen peroxide, ozone and peracetic acid, surface-active compounds including quaternary ammonium compounds (QACs) and acid anionic compounds, and iodophores (van Houdt and Michiels 2010). Chlorine-based compounds, such as hypochlorite, are widely used in the food industry because chlorine has a broad spectrum of activity, acts fast, and is usually cheap. This compound has been shown to be highly effective against biofilms (Toté et al. 2010; da Silva et al. 2011) and has greater efficacy in low pH than alkaline pH environments (Araújo et al. 2011).

Disinfectants containing hydrogen peroxide or peracetic acid are regarded as environmentally friendly because they decompose into oxygen and water (or acetic acid). Hydrogen peroxide affects the biofilm matrix, has been found to be effective against biofilm cells and is widely used in disinfectants (Robbins et al 2005; Shikongo-Nambabi et al. 2010). Hydrogen peroxide-based disinfectives also have a broad spectrum of activity and act fast. Peracetic acid has the advantage of being relatively stable in the presence of organic compounds compared to other disinfectant types. Several studies have reported its efficacy against biofilms. For instance, Cabeça et al. (2008) showed that 0.50 % w/v peracetic acid reduced 24 h-old *L. monocytogenes* biofilms by 5 log. Similarly, Frank and coworkers (2003) demonstrated that 2.0 mL/L peracetic acid reduced *L. monocytogenes* biofilms more than 6 log on stainless steel in the presence of fat, protein, and soil after 10 min of exposure.

Ozone is regarded as an environmentally friendly disinfectant as it rapidly disintegrates into water and oxygen. Unfortunately, its instability can cause it to react and disintegrate before reaching the target organism. However, ozone is a potent antimicrobial agent, which can be used against bacteria, fungi, viruses, protozoa, and bacterial and fungal spores (Khardre et al. 2001).

Quaternary ammonium compounds (QACs) are active against a range of vegetative bacteria and can be used over a wide temperature so they are widely used in the food industry. However, they are usually not used in CIP because of foaming and their activity is reduced in the presence of hard water. Also, their degradability in the environment is slow and residues may contribute to resistance development in bacteria.

Disinfectants based on alcohols are effective against a wide range of microorganisms and are relatively robust in the presence of organic material. However, their use is limited due to safety reasons (health and flammability) and their relatively high price. Alcohols are therefore mainly used for hand disinfection and on equipment that does not stand in water (Møretrø et al. 2012).

Due to the abovementioned reasons, a disinfectant must be carefully chosen according to the type of application and some aspects must be taken into account: the disinfectant must be environmentally friendly and economical; should be safe to use (nontoxic and nonallergenic), have no negative impact on surface materials (corrosiveness, staining and reactivity), be stable during storage and over a wide range of pH and temperatures, be robust to environmental factors (soil, hard water, and dilution), and have a broad spectrum of activity (Møretrø et al. 2012). Furthermore, it is of the outmost importance to know the mode of growth of the target organisms (i.e., planktonic, adhered, or biofilm). The efficacy of the disinfectant is strongly dependent on this factor because cells within a biofilm are more tolerant to antimicrobial agents than their planktonic counterparts. Wirtanen and Mattila-Sandholm (1992) showed that increased biofilm age may also lead to enhanced resistance against disinfectants and biocides. Usually, to obtain a good sanitary effect, when there is a biofilm present, it is necessary to combine an extensive mechanical action, such as scrubbing or scraping, with the use of cleaning and sanitizing agents. Chemical disinfectants react with the exopolymeric matrix of biofilms, which enhances the mechanical biofilm removal. Otherwise, chemical disinfectants can kill planktonic bacterial cells, while the exopolymeric matrix remains unaffected. Thus, chemical and mechanical treatment can have a synergistic effect in biofilm removal.

## 4.2 *Physical Methods*

The most commonly used physical method to remove biofilms is the manual cleaning of surfaces using scrubbers. Pressure washing is another approach currently being used that consists of rinsing surfaces with hot or cold water, the application of a detergent for the required contact time, and rinsing the surface before the application of a disinfectant. Usually, water is applied at 125 °C for 30 min and this method is considered as very effective in eliminating microbial communities. However, Wirtanen and Matilla-Sandholm (1993) verified that 3-day-old biofilms were difficult to completely remove even at this temperature. Kiskó and Szabó-Szabó (2011) also observed that hot water was not sufficient to eliminate *Pseudomonas aeruginosa* and *Pseudomonas stutzeri* biofilms from surfaces. The disadvantage of this method is that hot water denatures proteins and increases the adhesion properties of equipment, which can aid in the formation of biofilms, so it is not advisable. In order to be more efficient in biofilm removal, this method should be combined with chemical disinfection.

Ultrasounds, the application of electrical fields and super-high magnetic fields have been identified as newer physical methods for biofilm control. These approaches will be addressed below.

## 5 Emergent Approaches

### *5.1 Ultrasons*

Ultrasonication has been reported as an efficient biofilm removal method. This technique is particularly useful in surface decontamination where the inrush of fluid that accompanies cavitational collapse near a surface is nonsymmetric (Chemat et al. 2011). The particular advantage of ultrasonic cleaning in this context is that it can reach crevices that are not easily reached by conventional cleaning methods. The use of ultrasound allows the destruction of a variety of fungi, bacteria, and viruses in a much reduced processing time when compared to thermal treatment at similar temperatures (Chemat et al. 2011). However, by itself, this technique doesn't eliminate all the bacteria in food industries and thus it is recommended to be used in combination with other treatment techniques (Srey et al. 2013). In fact, it has been postulated that ultrasound induces cavitation within the biofilm, which increases transport of solutes, as antimicrobial agents, through the biofilm or outer bacterial membranes (Carmen et al. 2005). Thus, there is a synergistic effect between ultrasound and other antimicrobial agents. For instance, the combination of ultrasound and ethylenediaminetetraacetic acid (EDTA), and ultrasound and enzymes showed a higher efficacy in removing biofilms. Baumann and coworkers (2009) also showed a significant effect on biofilm removal on stainless steel food contact surfaces by combining the use of ozonation and sonication.

### *5.2 Electrical Methods*

Electrical methods for controlling bacterial adhesion have received special attention and are regarded to be environmental friendly because they use "electrons" as the nontoxic reaction mediator. These methods can be divided into current and potential applications, and each application can be conducted in the cathodic, anodic, and block (or alternating) modes (Hong et al. 2008). Electrical methods have been applied in some studies to prevent bacterial adhesion and to detach adhered bacteria, but it was verified that the removed bacteria could again accumulate on the surface and thus the problem of surface contamination continues. Besides, according to Wagnera et al. (2004), when an anodic current or potential is applied, the inactivated bacteria tend to remain on the surface providing new sites for bacterial adhesion. Thus, the control of bacterial adhesion through the exclusive application of anodic current is still limited. In order to try to overcome these limitations, Hong and colleagues (2008) investigated the specific role of electric currents in bacterial detachment and inactivation when a constant current was applied in the cathodic, anodic, and block modes. These authors observed that the application of cathodic current promoted the detachment of adhered bacteria by electrorepulsive forces, but bacteria remaining on the surface were still viable. On the other hand, the anodic current inactivates most of the remaining bacteria. Thus, these authors concluded that the best electrical strategy for reducing bacterial adhesion consists of the application of a block current.

Flint and coauthors (2000) observed that it may be possible to disrupt the attachment of thermo-resistant streptococci to stainless steel by applying a small voltage. In fact, when a voltage of 9 V and a current of 40 mA were applied to a suspension of *S. thermophilus* held between stainless steel electrodes, attachment to the cathode was reduced, whereas attachment to the anode was inhibited. This may result from the disruption of the electrical bilayer on the substrate.

An approach using electrical current to enhance the activity of antimicrobials against established biofilms has also been proposed. Blenkinsopp et al. (1992) found that three common industrial biocides (glutaraldehyde, a quaternary ammonium compound and kathon) exhibited enhanced action when applied against *P. aeruginosa* biofilms within a low strength electric field with a low current density.

Concerning its mode of action, it has been suggested that the mechanism of antibacterial activity of electrical current results from the oxidation of enzymes and coenzymes, membrane damage leading to the leakage of essential cytoplasmic constituents, and toxic substances (e.g., $H_2O_2$, oxidizing radicals, and chlorine molecules) produced as a result of electrolysis and/or a decreased bacterial respiratory rate (del Pozo et al. 2009).

### *5.3 Electrolyzed Water*

Electrolyzed water (EW) has been used in the food industry as a novel disinfecting agent. This process was shown to be more efficient than water and chlorine solutions as a sanitizer of meats, some fresh products, cutting boards, and utensils. EW is generated in a cell containing inert positively charged and negatively charged electrodes separated by a septum (membrane or diaphragm) (Al-Haq et al. 2005). By electrolysis, a dilute sodium chloride solution dissociates into acidic electrolysed water (AEW; pH between 2 and 3, oxidation–reduction potential of N1100 mV, and an active chlorine content of 10–90 mg/L), and basic electrolyzed water (BEW; pH between 10 and 13 and oxidation–reduction potential of −800 to −900 mV) (Hricova et al. 2008). Neutral electrolyzed water (NEW; pH 7–8) is produced by adding hydroxyl ions to AEW or by using a single chamber (Hricova et al. 2008). AEW has been determined to have a strong bactericidal effect on several pathogenic food bacteria such as *L. monocytogenes* (Park et al. 2004), *C. jejuni* (Park et al. 2002), *E. coli* O157:H7 (Park et al. 2004), *S.* Enteritidis (Koseki et al. 2003) and others, having more antimicrobial effect than BEW. Thus, according to Møretrø et al. (2012), a combination of BEW and AEW is more efficient than AEW alone. AEW has also been demonstrated to have an antibiofilm effect, namely, to inactivate *L. monocytogenes* biofilms on stainless steel surfaces. Treatment with acidic EO water for 30–120 s reduced the bacteria population by 4.3–5.2 log CFU/coupon (Ayebah et al. 2005). NEW is advantageous because it does not promote corrosion of processing equipment or irritation of skin and is stable because chlorine loss is significantly reduced at pH values of 6–9 (Len et al. 2002). In general, electrolysed water is considered environmental friendly because it is generated from water and a dilute salt solution and reverts to water after use.

## *5.4 Antimicrobial Materials*

Numerous efforts have been made in order to impede microbial adhesion and biofilm development by altering surface physicochemical properties (Rodriguez et al. 2007), integrating antimicrobial compounds into materials, and/or coating surfaces with biocides (Gottenbos et al. 2001). As a result, a large variety of materials and products are now available to be applied in the food industry, household, and for personal use (e.g., conveyor belts, refrigerators, cutting boards, and boxes for transport of food). Nevertheless, it is highly important to notice that all these materials and products must be seen as an extra contamination obstacle and not as a substitute for correct sanitary procedures (Kampmann et al. 2008; Møretrø et al. 2006).

One of the main biocidal agents incorporated in materials is triclosan, which can be applied in plastic polymers and has Microban® as a trade name (http://www.microban.com). Although a vast amount of products available nowadays contain this antimicrobial agent, there is evidence that its efficacy may not be satisfactory. Accordingly, although a plastic enclosing 1.5 g/kg triclosan had restrained *S. typhimurium* growth in an agar plate assay, when beef was vacuum sealed using the same material, no effect was observed on *S. typhimurium* development on meat compared to the control after up to 14 days incubation at different temperatures (Cutter 1999). Moreover, when Rodrigues et al. (2011) compared *Salmonella* Enteritidis adhesion on silestones (quartz surfaces incorporating Microban®, used as kitchen bench stones) and on other food contact surfaces without antimicrobial treatment, no significant effect was found. Although the results concerning biofilm formation highlighted a potential bacteriostatic activity of this antibacterial agent, all materials tested did not support food safety, revealing that these surfaces imply a cautious use and a correct sanitation when applied in food processing areas (Rodrigues et al. 2011). Furthermore, some worries have been associated with the wholesale application of triclosan in the household area, mainly because of the concern about expansion of resistant bacteria (Levy 2001; Webber et al. 2008).

### 5.5 Surface Coatings and Surface Modifications

Since stainless steel is one of the most commonly used materials in the food industry and food processing areas, several modifications have been made in order to prevent microbial colonization: coating with antimicrobial compounds; implantation of ions to lower surface energy; creation of bioactive surfaces (e.g., immobilized enzymes); production of diamond-like carbon surfaces; coating with a molecular brush (steric hindrance); development of silica surfaces to create either a hard glass-like surface or a hydrophilic anionic surface; or integration of polytetrafluoroethylene (PTFE) into the surfaces. Zhao and coworkers (2005a) reported a decrease of 94–98 % in *E. coli* adhesion to Ag-PTFE-coated stainless steel, in comparison to titanium surfaces, silver coating, or uncoated stainless steel. Moreover, these same researchers also produced surfaces with particular energies known to avoid biofouling by using coatings of PTFE, nickel, copper, and phosphorus (Zhao et al. 2005b; Zhao and Liu 2006).

Titanium dioxide ($TiO_2$) and, more recently, nitrogen-doped titanium dioxide (N-$TiO_2$) coatings are other possible forms to enhance food contact surface performance in terms of better hygiene and easier sanitation. When Rodrigues et al. (2013) compared *L. monocytogenes* viability on N-$TiO_2$ coated and uncoated stainless steel and glass, satisfactory results were found on the coated surfaces since, for most conditions tested, survival rates decreased below 50 %. Nevertheless, no successful disinfection was accomplished, since the required bacterial reduction of at least 3 log was not achieved (Rodrigues et al. 2013). Thus, N-$TiO_2$ coating still requires more investigation and enhancement in order to become a really useful tool against microbial contamination of food contact surfaces. In fact, new surface coatings and different disinfectant agents are regularly investigated worldwide, but these data have yet to be transferred to the industry due to several reasons, such as process consistency, charges, product quality and safety, and maintenance (Goode et al. 2013).

In work dealing with biofilm control, microparticles ($CaCO_3$) coated with benzyldimethyldodecylammonium chloride have successfully repelled biofilm formation (Ferreira et al. 2010), and various researchers have shown that silver coatings prevented biofilm formation (Hashimoto 2001; Knetsch and Koole 2011). Furthermore, passive coatings of organic polymers are also a promising approach to prevent microbial contamination. Due to the propensity of some plastics to microbial degradation, efforts have been made to integrate inhibitors into these materials. Price and coworkers (1991) have shown that, compared to a control polymer, a significant decrease of attachment and viability of *Klebsiella pneumoniae*, *S. aureus*, and *P. aeruginosa* was achieved on an ethylene vinyl acetate/low-density polyethylene product containing a low-solubility commercial quaternary amine complex. Although further studies are needed, this seems to be a promising application to control microbial contamination on food contact surfaces. Nevertheless, it is also important to note that not all antimicrobial coatings tested so far have shown efficacy. For example, a polystyrene surface coated with antimicrobial fullerene-based nanoparticles was created aiming to prevent biofilm formation by *Pseudomonas mendocina*, but it actually enhanced biofilm development (Lyon et al. 2008). This demonstrates that antibacterial nanomaterials can lose their efficacy when applied as coatings.

Another possible way to avoid biofilm formation is by steric hindrance, or blocking, of bacterial adhesion by means of a "molecular brush," which involves coating a surface with an inert material that physically prevents bacterial adhesion. Namely, polyethylene glycol (PEG) is the most investigated molecular brush that controls protein adsorption to materials (Jönsson and Johansson 2004). Although the prevention of protein adsorption by a molecular brush has generally been established, its usefulness in preventing microbial attachment is somehow controversial. In fact, Wei and coworkers (2003) have reported that stainless steel coated with PEG inhibited the adsorption of b-lactoglobulin, but did not inhibit the adhesion of *Pseudomonas* sp. and *L. monocytogenes* cells. A possible explanation for these observations may be related with the particular nature of the PEG layer used, as well as the complexity of bacterial adhesion, since protein interactions are not the only aspect that influences it.

## 5.6 Natural Compounds

Recently, the emergence of antibiotic-resistant strains and the reluctance of consumers toward the use of chemical products, such as biocides, have led to a search for natural alternative products. The use of biocides as sanitizers in the food industry has associated concerns such as biocide biodegradability, their risk to human health, and their environmental impact (Cappitelli et al. 2006). The use of substances obtained from plants is preferred since they may have been used in traditional medicine for a long time, they are generally considered to be safe by consumers, and are not known to cause harm to the environment (Leonard et al. 2010). Essential oils (EOs) or their constituents are one of the more promising and natural alternative antimicrobial agents. EOs are volatile, natural, complex compounds characterized by a strong odor and are obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots). Concerning their mode of action, they pass through the bacterial cell wall and cytoplasmic membrane, disrupt the structure of the different layers of polysaccharides, fatty acids, and phospholipids, and permeabilize them (Bakkali et al. 2008).

Oliveira et al. (2012) evaluated the antibacterial potential of EOs from *Cinnamomum cassia* bark and *Melaleuca alternifolia* and *Cymbopogon flexuosus* leaves against planktonic and sessile cells of *E. coli* (EPEC) and *L. monocytogenes*. These authors observed that all of the EOs and combinations tested possessed antibacterial activity against planktonic cells; however, the EO of *C. cassia* was the most effective antibiofilm agent. Jadhav et al. (2013) also observed the inhibitory effect of the essential oil obtained from yarrow (*Achillea millefolium*) against planktonic cells and biofilms of *L. monocytogenes* and *Listeria innocua* isolates obtained from food processing environments.

Other natural compounds are biosurfactants that are surface-active compounds of microbial origin and have attracted attention due to their low toxicity and high biodegradability, when compared to synthetic surfactants (Nitschke et al. 2005; Banat et al. 2010). The adsorption of biosurfactants to a solid surface can modify its hydrophobicity and thus bacterial adhesion and consequently biofilm formation. One study investigated whether surfactin from *Bacillus subtilis* and rhamnolipids from *P. aeruginosa* could reduce the adhesion and/or disrupt the biofilms of some foodborne pathogenic bacteria (Gomes and Nitschke 2012). It was observed that after 2 h contact with surfactin at 0.1 % concentration, the preformed biofilms of *S. aureus* were reduced by 63.7 %, *L. monocytogenes* by 95.9 %, *S.* Enteritidis by 35.5 %, and the mixed culture biofilm by 58.5 %. Concerning the effect of rhamnolipids, it was observed that, at a concentration of 0.25 %, they removed 58.5 % of the *S. aureus* biofilm, 26.5 % of *L. monocytogenes*, 23.0 % of *S.* Enteritidis, and 24.0 % of the mixed species biofilm. Nevertheless, although the replacement of synthetic surfactants by biosurfactants would provide advantages such as biodegradability and low toxicity, their use has been limited by their relatively high production cost, as well as scarce information on their toxicity in humans (Rodrigues 2011).

## *5.7 Enzymes*

Enzymes are biological catalysts, i.e., substances that increase the rate of chemical reactions without being used up. In other words, enzymes are proteins capable of lowering the activation energy of a chemical reaction; their action relies on the possibility of interacting with the substrate to be transformed, via its active site (Glinel et al. 2012).

Concerning their mode of action, enzymes immobilized on a material surface and in contact with a biological environment may act against biofilm in various ways. Enzymes may impair the initial step of surface colonization by microorganisms by cleavage of proteins and carbohydrates; these types of enzymes are called adhesive-degrading enzymes. Enzymes may also have a biocidal effect when they compromise the viability of living organisms growing on surfaces. In the first category, enzymes such as proteases can impede microbial adhesion by hydrolyzing peptidic bonds (Rawlings et al. 2006), while glycosidases specifically break ester bonds of polysaccharides, which are the main constituents of microbial adhesives (Moss 2006). van Speybroeck et al. (1996) reported the use of an enzymatic preparation comprised of exopolysaccharide-degrading enzymes, particularly the colanic acid-degrading enzymes, derived from a strain of *Streptomyces* for the removal and/or prevention of biofilm formation on surfaces.

Molobela et al. (2010) tested proteases (savinase, everlase, and polarzyme) and amylase (amyloglucosidase and bacterial amylase novo) activity on biofilms formed by *P. fluorescens* and on extracted EPS. They observed that everlase and savinase were the most effective enzymatic treatments for removing biofilms and degrading the EPS.

Enzymes have also been used as antibiofilm coatings. In this case, they can be either covalently grafted onto solid substrates or incorporated into polymer matrices to produce antibacterial coatings and it is thought that enzymes impair one or several "bricks" of the biofilm construction (Glinel et al. 2012). Yuan and coworkers (2011) tested a coating composed by coupling lysozyme on a PEG layer against two different bacterial species, Gram-negative *E. coli* and Gram-positive *S. aureus*. These authors observed that more than 90 % of *S. aureus* and ~80 % of *E. coli* that adhered to lysozyme-functionalized surfaces were damaged within 4 h. In addition, these coatings showed long-term activity since the antibacterial effect against *S. aureus* was retained after a contact time of ~36 h. However the effect faded over time for *E. coli*. This result was probably due to the fact that lysozyme is more active toward peptidoglycans present in the Gram-positive bacterial wall than toward the double membrane of the Gram-negative cell wall. It can be concluded that, as the structural composition of EPS varies even among bacteria of the same species, the mode of action and the consequent efficiency of enzymes will also be variable.

Therefore, enzymes constitute an important alternative for biofilm removal in the food industry. Though, it must be noted that enzymes, as coatings, may contribute to the unwanted degradation of substances surrounding the surface coating. In addition, enzymes that produce biocidal substances have to be approved by the appropriate legislative body before being implemented.

## *5.8 Quorum-Sensing Interfering Molecules*

Quorum sensing (QS) or cell-to-cell communication is employed by a diverse group of bacteria, including those commonly associated with food. Through the mechanisms of QS, bacteria communicate with each other by producing the signaling molecules known as autoinducers and are consequentially able to express specific genes in response to population density. Since several types of signaling molecules have been detected in different spoiled food products, disrupting the QS circuit can potentially play a major role in controlling microbial gene expression related to human infection and food spoilage (Bai and Rai 2011). QS inhibitors can be developed in order to target synthesis of the cell signaling molecules themselves or to block these signaling systems (Bai and Rai 2011).

QS systems appear to be involved in all phases of biofilm formation. They regulate population density and the metabolic activity within the mature biofilm to fit the nutritional demands and resources available. Furthermore, bacteria within biofilms have markedly different transcriptional programs from planktonic bacteria of the same strain (Asad and Opal 2008).

The relation between QS and biofilm formation in food-related bacteria has been observed by several authors. However, according to Bai and Rai (2011), though signaling molecules have been detected in biofilms, their precise role in the different stages of biofilm formation is still not clear.

Kerekes and coauthors (2013) investigated the effect of clary sage, juniper, lemon, and marjoram essential oils and their major components on the formation of bacterial and yeast biofilms and on the inhibition of AHL mediated QS and verified that the compounds tested seemed to be good candidates for prevention of biofilm formation and inhibition of the AHL-mediated QS mechanism.

Furanones are one of the most studied QS inhibitors and it was demonstrated that they were able to control multicellular behavior induced by autoinducer-1 (Manefield et al. 2002) and autoinducer-2 (Ren et al. 2004) in Gram-negative microorganisms.

### *5.9 Bacteriophages*

Bacteriophages (phages) are viruses that infect bacteria and can be found in the same biosphere niches as their bacterial hosts (Kutter and Sulakvelidze 2005). They were originally found by Harkin in 1896 and were applied in the cure of microbial infections previous to antibiotic discovery. The application of phages to control biofilms can be a practicable, natural, harmless, and greatly specific way to deal with numerous microorganisms implicated in biofilm formation (Kudva et al. 1999). In fact, phages and their endolysins have already been used to stop biofilm development by *L. monocytogenes* and *E. coli* (Gaeng et al. 2009; Sharma et al. 2005). Accordingly, a *L. monocytogenes* phage (ATCC 23074-B1) was effectively used for biofilm eradication (Hibma et al. 1997), and a synergistic effect of an alkaline disinfectant and a phage has been described for the eradication of *E. coli* O157:H7 biofilms grown on stainless steel (Sharma et al. 2005). Moreover, Lu and Collins (2007) produced a phage that expresses a biofilm-degrading enzyme, which attacked both biofilm bacteria and matrix, leading to more than 99.9 % elimination of the biofilm cells.

A study conducted by Sillankorva and coworkers (2008) showed that the phage phiIBB-PF7A can be an outstanding natural agent regarding its ability to lyse *P. fluorescens* biofilm cells in a very short period of time. This same phage was also applied to control a *P. fluorescens* and *Staphylococcus lentus* mixed biofilm and led to a remarkable decline in the attached bacterial cells (*P. fluorescens*).

Moreover, it was also shown that phages can be effective in both monoculture and mixed-culture biofilms and competently reach and lyse their specific host, despite the coresidence of a nonvulnerable species (Sillankorva et al. 2010). When Briandet and coworkers (2008) investigated the dispersion and response of phages within biofilms, it was observed that phages were able to penetrate distinct biofilm complexes. In addition, these authors found that, in general, phages within biofilms are immobilized, reproduced, and released by a lytic cycle, connecting with their specific binding sites on the hosts. Moreover, Tait et al. (2002) reported that phages and bacteria were able to progressively coexist in biofilms, and therefore recommended a combination of phages and polysaccharide depolymerases and disinfectant for improved biofilm control. On the other hand, Brooks and Flint (2008) have suggested that it may be productive to look for phages in biofilm samples from food industry facilities and to apply them against microbial communities found in the same environment. Moreover, since phages are likely to be highly host specific, this approach should not represent any danger to other fractions of the production, even though the application of a phage mixture would likely to be required due to arising host resistance.

Although it is already known that infection of biofilm cells by phages is highly dependent on several factors, such as their chemical composition, phage concentration, temperature, media, and growth stage (Sillankorva et al. 2004; Chaignon et al. 2007), there is much more to explore and explain. Since dairy foodstuffs are highly vulnerable to contamination by bacterial biofilms, the dairy industry has become the leader of exploiting phages as an antibacterial approach (Thallinger et al. 2013), and it is expected that the development of highly efficient and inexpensive methods of genetic material treatment and DNA sequencing will accelerate the finding and creation of engineered phages.

## 6 Conclusions

Due to the ability of foodborne pathogens to form biofilms on diverse food contact surfaces, leading to a continuous contamination of food, prevention and elimination of biofilms are significant concerns for the food industry. Currently, the best practical ways to prevent biofilm development consists of a successful application of hygienic and sanitation compounds, appropriated sanitation, and a good operation of the process line. Although much progress has been made in this area, out-of-date prevention means are still being applied. Nevertheless, given the ability of bacteria to become resistant and consequently to endure approaches that used to be efficient, new methods of elimination for these microbial communities are continuously required. However, a lot more is still left to discover about the effect of antibacterial compounds on biofilms and their subsequent recovery reaction. This, together with an improved knowledge about the mechanisms involved in biofilm formation on food contact surfaces is of utmost importance towards the goal of achieving a novel, highly effective, cheaper, and ecological tactic to assure food safety.

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