Bacterial Biofilms: Formation, Properties and Prevention in Food Industry

Kefyalew CHIRKENA
Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Near East University, Nicosia, TRNC, yonkef@gmail.com

Beyza ULUSOY
Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Near East University, Nicosia, TRNC, beyza.ulusoy@neu.edu.tr

Canan HECER
Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Near East University, Nicosia, TRNC, canan.hecer@neu.edu.tr

Geliş tarihi/Received: 30.04.2019 Kabul tarihi / Accepted: 17.06.2019

Abstract

Biofilms are communities of microorganisms that are able to attach each other and any surface by bacterial adherence. The ability of bacterial biofilms to attach to each other and surfaces offers them advantages, such as protection from harsh environmental situation, increased availability of nutrients for growth, increased binding of water molecules, reduced possibility of dehydration and transferring of genetic materials. They are characterized by the production of an excessive network of highly hydrated extracellular polymeric substances facilitating the initial bacterial attachment to a surface, formation of micro colony and biofilm structure. The biofilms formation on the food contact surface, in particular are caused by different pathogens such as *Pseudomonas*, *Enterococcus* species, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* species, which in turn cause contamination of foods. The consequences of this situation always become the fundamental problem of food safety, hygiene and quality.

Keywords*: Biofilms, food industry, adherence, foods, microorganisms*

Bakteriyel Biyofilmler:
Gida İşletmelerinde Oluşumu, Özellikleri ve Önlenmesi

Öz

Biyofilmler, bakteriyal aderans (adezyon, tutunma) suretiyle birbirine ve herhangi bir yüzeye bağlanabilen mikroorganizma topluluklarıdır. Bakteriyel biyofilmlerin birbirine ve yüzeylere yapışma kabiliyetleri, zorlu *çevresel* koşullardan korunma, büyüme için besinlerin kullanılabilirliğinin arttırılması, su moleküllerinin bağlanmasının arttırılması, dehidrasyon olasılığının azaltılması ve genetik malzemelerin aktarılması gibi avantajlar elde etmelerini sağlar. Bakteriyel biyofilmler, bir yüzeye ilk bakteri yapışmasını, mikro koloni ve biyofilm yapısının oluşumunu kolaylaştıran, y*üksek* oranda hidratlanmış hücre dışı polimerik maddeler ağının *üretimi* ile karakterize edilirler. Gıda temas yüzeyinde, özellikle *Pseudomonas*, *Enterococcus* türleri, *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli* ve *Salmonella* türleri gibi farklı patojenler nedeniyle biyofilm oluşumu gıdaların bulaşmasına neden olmaktadır. Bu durumun sonuçları her zaman gıda güvenliği, gıda hijyeni ve gıda kalitesinin temel sorunu olarak karşımıza çıkmaktadır.

Anahtar Kelimeler*: Biyofilm, gıda işletmeleri, yapışma, gıda, mikroorganizma*

INTRODUCTION

Biofilms are complex communities, a collective of one or more types, of microorganisms that can grow on many different surfaces or be associated with interfaces. Most microorganisms found in natural, clinical and industrial settings persist in association with surfaces. These microorganisms can include bacteria, fungi and protists (Davey and O'Toole, 2000). These microbial communities are often composed of multiple species that interact with each other and their environment. They are also defined as organized communities, collaborating among themselves and being attached to an inert or living surface contained in a self-produced polymeric matrix made principally of exopolysaccharide (Donlan, 2002). Bacterial biofilms, which are microorganisms, can also massively colonize much food processing equipment in the food industries and household surfaces in the bathroom and kitchens including, toilet, sinks and cutting boards. The formation of bacterial biofilms creates an alternative routine in which they can adopt a multicellular behavior that facilitates and persists in diverse environmental niches.

Biofilms are more likely to be an issue of food safety and hygiene in the food industry. Pathogenic bacteria form biofilms on food and food contact surfaces in the food industry, thereby increasing their ability to withstand the harsh environmental conditions, to have resistance to antimicrobial treatments and persist in the food processing environment (Annous *et al*., 2009). They are co- adhered by means of physical attachment and extra cellular polymeric substances and are able to adhere themselves to any surfaces. The adherence of these bacterial biofilms can also be seen on the pathological tissues and organs; and the surface of the pipelines to eventually form bacterial biofilms. Once biofilms are formed, susceptibility to various chemicals and environmental changes is greatly reduced and they cannot be removed completely. Since they are well protected by the biofilms matrix, even the high dosages of antimicrobials cannot even remove the infectious biofilms (Zhao *et al*., 2017).

The ability to stick to surfaces and to engage in multistep process leading to the formation of biofilms is almost ubiquitous among bacteria. Bacterial biofilms formation has substantial implications in fields ranging from industrial processes like oil drilling, paper production and food processing to health-related fields like medicine and dentistry. Therefore, the adherence of biofilms to surfaces in a variety of industries, particularly food industry, causes a persistent source of contamination. Some biofilms forming bacteria such as *Lactobacillus brevis*, *Lactobacillus lindneri*, and *Pediococcus pentosaceus* isolates were detected and identified on the surfaces as possible source of contamination in the microbrewery environment (Maifreni *et al*., 2015). In addition, biofilms can also cause vigorous problems in dairy, poultry and red meat processing, especially on equipment or its surfaces and floor that have been challenging the food processors and their sanitation teams. Bacterial biofilms have a potential hazard on the safety and quality of the food products.

The contamination of the products by the introduction of pathogenic biofilm forming microorganisms such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli, Pseudomonas* species, *Enterococcus* species and *Salmonella* species causes spoilage and deterioration of food products (Srey *et al*., 2013) which in turn brings a serious public health risk. An increased resistance of biofilms- associated organisms to antimicrobials agents also poses a serious problem to public health, especially causing infectious in patients with indwelling medical devices (Donlan, 2001). Since bacterial biofilms are ubiquitous in nature and cause a number of infectious diseases such as cystic fibrosis and chronic prostatitis (Donlan, 2002), severe damage to organs like lungs and prostate glands could be developed. On the other hand, extrinsic factors like nutrient concentration and shear conditions may also play a vital role in influencing the degree of the attachment and biofilms formation. Materials such as stainless steel, glass and rubber that make up food contact surfaces are suggested to have an impact on the level of attachment and biofilm formation (Srey *et al*., 2013).

In food industries, the formation of biofilms results in serious problems, since it can be a contamination source for the the food product, compromising the final product quality and consumer health due to the risk of foodborne diseases (Dalla Costa *et al*., 2017) and even it can lead to the rejection of food products. Furthermore, food industries can face financial losses due to the reduction in the shelf life of food products, increasing product spoilage, impairing the heat transfer processes, and increasing the corrosion rate of the surfaces (Winkelstroter, 2015). Therefore, the objective of this paper is to review the formation, properties and prevention of microbial biofilms in food processing industry.

Formation of Bacterial Biofilms in Food Industry

Several bacteria are capable of adhering themselves to biotic and a biotic surface by using the self-produced matrix of extracellular polymeric substance (EPS) and form biofilms which are composed of microbial communities characterized by cells that colonize surfaces and embedded in the matrix of EPS. However, the formation of bacterial biofilms requires coordinated chemical signaling among the cells to coordinate gene expression (Shrout *et al.*, 2011) and the enabling of specific functions so that the signaling process makes the bacteria, benefits and allows them to sense the presence of neighboring bacteria and respond to varying conditions.

Intercellular communication of bacterial cells is delivered by extracellular signaling molecules. This molecule enables every bacterial cell to estimate the total number of bacteria (Marić and Vraneš, 2007) and regulates specific process in the bacteria. The process by which this signal communication can be done is known as quorum sensing. Bacteria such as *Pseudomonas aeruginosa* (Shrout *et al*., 2011) has responded to such a phenomenon to control its genes in response to a critical concentration of self-produced extracellular communication molecules. Interactive bacterial communication via signaling molecules enables them to organize into a community so that biofilms function as a multicellular organism. A study carried out on *Staphylococcus epidermidis* (Marić and Vraneš, 2007) confirmed that bacteria lose ability to form biofilms if the genes responsible for synthesis of EPS matrix are inactivated. In the formation of biofilms, the mobility of bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio cholera* is important for establishing the connection between bacteria and surface on which biofilms are developed.

Bacteria in the biofilms aggregate to form a colony for metabolic cooperation. The formation of bacterial biofilms constitutes an efficient adaptive strategy, because it offers them different advantages such as protection from against harsh environmental situations, enhance their antimicrobial resistance, increased availability of nutrients for growth, increase binding of water molecules, reducing the possibility of dehydration and for transfer of genetic materials (Téllez, 2010). In this case, for example, *Escherichia coli* is a highly adaptive microorganism and it has the ability to form biofilms under certain conditions to withstand antimicrobial resistance.

Other biofilm forming bacteria like *Listeria monocytogenes,* which can survive under adverse environmental conditions, has good adhesion ability to attach to any materials' surface in the industry. The organism has a capacity to persist in the environment for years. As a matter of fact, scientific research has shown the presence of *Listeria monocytogenes* on the surface of equipment and utensils in dairy processing industries (Oliveira *et al*., 2010). The occurrence of foodborne diseases as well as sporadic cases caused by this bacterium can be attributed to its increased ability of surviving in food processing environments through biofilm formation. Similarly, *Streptococcus thermophilus* has the ability to form biofilms which play a vital role in persistence in dairy environments such as milk pasteurization and cheese manufacturing plants (Bassi *et al*., 2017) and even it has even been found to be attached to the heat exchangers in milk processing equipment. Some other bacteria such as *Flavobacterium*, *Moraxella*, *Acinetobacter*, *Pseudomonas* and *Achromobacter* which are normally found in water are also responsible for bacterial biofilm formation.

Bacterial biofilm formation may also be of significance of existence in food-related *Staphylococci*. In food industry, the strains were found to differ greatly in their abilities to form biofilms on polystyrene and stainless steel (Møretrø *et al*., 2003). The mode of growth of biofilm in *Staphylococcus aureus* is firmly regulated by complex generic factors. However, the mechanisms of biofilm formation in this species are poorly understood and studies on the expression profiles of genes involved in mechanisms of biofilm are still limited (Doulgeraki *et al*., 2017). *Pseudomonas* species is a well-known bacterium to cause spoilage of pasteurized milk to reduce the shelf life of the product in the food industry. By biofilm forming, the bacteria can even be able to grow on the stainless steel by adhering to the surface. It has been considered that bacterial biofilm formation ability varies between genera, species and strains of the bacteria and it can be influenced by other factors such that one type of bacteria can be a strong biofilm producer under certain environment and become weak in another environment.

Dynamic Process of Bacterial Biofilm Formation

Conditioning of Surface

The process of biofilm formation begins with the conditioning of the surface. The conditioning surface is the basis on which biofilms can grow and it can be composed of many particles, organic and inorganic. So, the accumulation of organic and inorganic materials on processing surfaces creates an environment where the organisms can adhere. Following the conditioning surface, the bacteria are able to attach and then retain on the surface (Moreira *et al*., 2017) where the bacteria begin to form micro-colonies and secrete the extracellular polymeric substance.

A study investigated (Gomes *et al*., 2017) the effect of surface conditioning with cellular components on *Escherichia coli* adhesion and biofilm formation in 96 agitated, well micro-titer plates and parallel plate flow chamber and different surface conditioning agents such as mannose, myristic and palmitic acid was used to evaluate the formation of biofilms. Hence, the study revealed that the number of biofilms formed in mannose was similar to the control for all tested concentrations; but lower number of biofilms was detected in myristic and palmitic acid. On the other hand, the study conducted (Siboni *et al*., 2007) on conditioning film and biofilms formation on ceramics tiles in marine environment had observed the buildup of conditioning film and development of biofilms in the given period of time.

In food processing environments, microorganisms along with organic and inorganic molecules like proteins from milk and meat get adsorbed in the surface forming a conditioning film/layer. The conditioning layer starts as a thin, resilient layer of microorganisms, any combination of spoilage and pathogenic bacteria that form on and coat the conditioning layer. This layer modifies substrata facilitating accessibility to bacteria. Surface charge, potential and tensions can be altered favorably by the interactions between conditioning layer and substrate. These organic and inorganic molecules and microorganisms are transported to the surface by diffusion (Garrett *et al*., 2008).

Biofilms Quorum Sensing

Bacterial cell to cell inter-communication system through coordinated chemical signaling has played a vital role in biofilm formation in the foodborne pathogens. Genetic materials expression in some bacterial species may be regulated by the system of stimulus called quorum sensing (Annous *et al*., 2009). Since many bacteria are well organized to regulate their cooperative activities and physiological processes through this mechanism, their ability to communicate and behave as a group for social interactions like a multi-cellular organism has provided significant benefits to them in host colonization, formation of biofilms, defense against competitors and adaptation to changing environments (Li and Tian, 2012).

Quorum sensing uses signaling molecules, auto inducers, which frequently produced by bacteria mainly at the stage of micro colony formation (Zhao *et al*., 2017) and easily diffuse throughout the cell membrane where the concentration of signaling molecules become higher following the larger number of bacteria present around the area. After the concentration of the signaling reaches a threshold level, it is recognized by the receptors found in the cytoplasm or cell membrane and activates gene expression involved in signal production (Toyofuku *et al*., 2016). However, it can synchronously be able to control and regulate specific genes that facilitate various groups of activities such as bioluminescence and the production of extra cellular enzyme.

Adhesion of Bacterial Cells

The growth of bacterial biofilms can be governed by a number of physical, chemical and biological processes. The attachment from cell to substrate is termed as adhesion (Garrett *et al*., 2008). Cell adhesion in the formation of biofilm is the attachment of microorganisms to the conditioned surface. The attachment can be reversible where the extracellular polymeric substance produced by bacteria reaches a certain level to generate the interaction between the bacteria and surface of attachment (Zhao *et al*., 2017). Some evidences showed that adhesion of bacterial cells takes place in reversible phase of biofilms formation where weak interaction with the substratum can be seen. This phase is dictated by physicochemical variables that define the interaction between bacterial cell surface and conditioned surface (Dunne, 2002). On the other hand, the cells can be evident for the irreversible attachment when the biofilms show development for direct contact with the surface commonly by flagella; and in this stage bacterial cell secretion of extracellular polymeric substance enhances adhesion of the cell to surface, such bacterial attachment needs strong disinfectant to be removed from the surface later.

The process of attachment may be active or passive and depends on the bacterial motility or the transportation of free floating cells by gravity, diffusion or fluid dynamics force from the surrounding fluid phase. However, the adhesion of bacterial cells is influenced by the availability of nutrients and their stage of growth take place among themselves. On the other hand, as Van Houdt and Michiels stated bacterial adherence can also be influenced by physical and chemical processes determined by Van der Waals, electrostatic and steric forces acting between the cells and attachment surfaces. Besides this, hydrophilic/hydrophobic and osmotic interactions also result in bacterial adhesion/attachment and detachment (Krasowska and Sigler, 2014).

The biofilm growth cycle encompasses bacterial adhesion at all levels starting with the physical attraction of bacteria to a substrate and ending with the eventual liberation of cell clusters from the biofilm matrix (Garrett *et al*., 2008). The adherence ability of bacterial biofilms depends on the surfaces they are adhered. A study conducted by Dias *et al*. revealed that the initial bacterial adhesion to polystyrene, which was evaluated by using micro-titer plate assay and crystal violet staining for biofilm quantification, showed varying level of adhesion of the diverse strains of different *Aeromonas* species. According to this work, 8 (57%) of the 14 strains of the bacterial species collected for the study, were classified as moderately adherent to polystyrene; but *Aeromonas bestriarum* and *Aeromonas salmonicida* had the highest and lowest adhesion abilities respectively. This may indicate that biofilm formation is more possibly influenced by cell to cell communication than the adhesion to the substratum. The ability of bacterial adhesion depends not only on the surface characteristics and the environment surrounding the microorganisms, but also on their phenotype and genotype.

Formation of Micro Colonies

Formation of micro colonies can be observed when nutrients present in the conditioning layer and the surrounding fluid environment make the irreversible bacterial cell attachment grow and divide. In the communal organization of biofilms, the formation of micro colonies comes first in a group of cells that seem to spontaneously aggregate and thereby nucleate the growth of the biofilms. Its formation takes place after bacteria are adhered to the physical surface/biological tissue. Bacterial multiplication in the biofilm starts as a result of chemical signals where cell divisions take place within the embedded exopolysaccharide matrix, which finally result in micro-colony formation (Jamal *et al*., 2015). Besides this, the formation of micro colonies results from concurrent accumulation and growth of microorganisms and is associated with the production of extracellular polymerase substance (EPS) which helps the strength of bond between the bacteria and the substratum (Chmielewski and Frank, 2003).

Biofilm Maturations

The formation of bacterial biofilms has been passed through different phases such as adhesion (including reversible and irreversible attachment), micro colony formation, maturation and dispersion (Marić and Vraneš, 2007). The process of biofilm maturation commences after the reversible attachment of bacteria to the surface. Biofilm maturation is the step where bacterial biofilms develop to a matured and organized structure and when micro colony can reach its maximum size and becomes thick (Srey *et al*., 2013).

Biofilm Dispersion

In the mature biofilms, there is a group of bacteria that actively exchange and share product materials to maintain biofilms. After biofilms get matured, dispersion takes place which is considered to be the final stage in the biofilm formation cycle. In this stage, the cells become revert to their planktonic form (Srey *et al*., 2013) and bacterial cells probably have better access to nutrients (Marić and Vraneš, 2007). Dispersion can be the result of several cues, such as changes in nutrient availability, oxygen fluctuations and increase in toxin products or other stress inducing conditions (Kostakioti *et al*., 2013). However, bacterial cells can be detached by disruptive factors such as catabolite repression, nutrient limitation and internal biochemical changes (extracellular polymeric substance or surface binding protein releasing).

Some researchers have verified that bacteria detach from the biofilm itself on their own to undergo rapid multiplication and dispersal after biofilm formation (Jamal *et al*., 2015). The detachment of bacterial cells is considered as the reason for the spread of pathogens to cause food borne infection (Zhao *et al*., 2017). However, dispersion of biofilm occurs either by detachment of new formed cells from growing cells or dispersion of biofilms aggregates due to flowing effect or quorum sensing (Jamal *et al*., 2015).

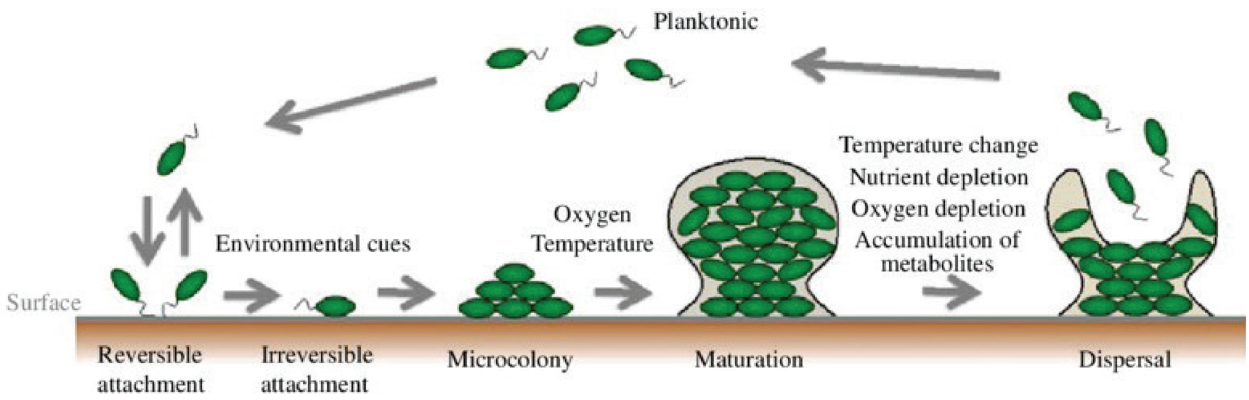

Figure 1: Stage of biofilm formation and environmental factors that influence its formation (Toyofuku *et al*., 2016)

Properties of Biofilms in Food Industry

A distinguishing feature of microbial biofilms is the presence of an adhesive matrix of highly hydrated extracellular polymeric substances comprised of polysaccharides, proteins, lipids, and nucleic acids. The physicochemical mechanisms of bacterial adhesion involve the superficial free energies and interaction surface theory in which adhesion is regarded as the interaction of Van der Waals forces and electronic phenomena (Bakker *et al*., 2004).

Mechanical properties of biofilms arise from the internal structural organization. Biofilms consist of bacteria and hydrated macromolecules in water, creating a complex fluid that does not behave as purely viscous or purely elastic. Characterization of their internal structure requires an assessment of physical properties of the biofilms (Billings *et al*., 2015). The physical characteristics of solid surfaces, where pathogenic biofilms are adhered, in the food processing industry are very important for biofilm formation as they can influence the initial cell attachment (Van Houdt and Michiels, 2010). In addition to this, cell surface property in the formation of biofilms has the major implication to various food industries where biofilms are creating problems by contaminating food and food contact surfaces.

Factors Affecting Biofilm Formation

Biofilm formation can be influenced by various factors throughout the whole stage of biofilm development.

Environmental Factors

Many bacteria are ubiquitous in the natural environment and they get together to attach to each other or to the contact surfaces to form biofilms, where they are contained within a self-produced extra cellular polymeric substance that enables them to protect themselves from harsh environmental conditions (Lebeer *et al*., 2007). It is well understood that the surrounding environmental factors under which bacteria grow and develop can largely affect the behavior of bacterial cell growth, resistance and toxin production. These factors may include temperature, pH, water activity, microbial communities and antimicrobials (Pagán and and García-Gonzalo, 2015).

Changes in pH and temperature can have a significant effect on the growth of bacterial biofilm (Garrett *et al*., 2008). The optimum temperature for microorganism is related with an increase in nutrient intake to facilitate the formation of biofilm. Inversely, temperature changes away from the optimal level can reduce the growth of bacteria because bacterial activities are sensitive to change in temperature. An investigation done on a total of 72 *Pseudomonas* species isolated from milk, dairy products and dairy plants, for their ability to produce biofilm onto polystyrene surfaces revealed that the biofilm formation levels of all the species depends on the length of time and the incubation temperature (Rossi *et al*., 2018).

Nutrient Conditions

The formation of biofilms vary under diverse nutrient conditions. They are plenty and dense in a nutrient-rich environment as it promotes the bacteria to form biofilm state; however, the depletion of these nutrients causes detachment of the biofilm's cells from surfaces. Nutrient concentration has an impact on biofilm formation (Rochex and Lebeault, 2007). The investigation done on biofilm formation of *Pseudomonas putida* at a constant temperature of 5^{o}C, 10^{o}C, 20^{o}C or 30^{o}C under rich and poor nutrient conditions indicated that the biofilm detached after it reached maturity at high temperature under the rich nutrient condition, but those at low temperature remain attached. In contrast, under the poor nutrient condition, the biofilm detachment occurred regardless of the temperature (Morimatsu *et al*., 2012).

Property of Bacterial and Abiotic Surface

Bacterial cell surface attachment which can be seen as physicochemical surface property of bacterial cells influences the formation of biofilms (Van Houdt and Michiels, 2010); so, the property of attachment surface is the determinant factors that affect the formation of biofilms potential together with bacterial cells. Flagellar motility of the bacteria is critical for cell to contact surface and biofilm formation. It is the influential bacterial cell to be attached to the surfaces for the development of the biofilm. Bacteria having flagella, such as *Escherichia coli* and *Vibrio cholera*, have formed biofilms to be attached to the food contact surfaces and cause fundamental problem in food industry. These observations indicate that flagella can affect adherence and biofilm formation through different mechanisms depending on the type of bacterium (Van Houdt and Michiels, 2010).

Hydrophobicity

It is widely accepted that bacterial surface hydrophobicity is a key factor in bacterial adhesion process and further biofilm development. Bacteria with hydrophobic properties are adhering to hydrophobic contact surfaces whereas those with hydrophilic characteristics would prefer to hydrophilic surfaces. However, those bacteria with hydrophobic properties are more adherent to the contact surfaces than those with hydrophilic behavior (Pagán and García-Gonzalo, 2015). In general, hydrophobic materials, such as plastic, are higher in promoting bacterial attachment than hydrophilic materials such as glass or metals.

The effect of cell surface hydrophobicity in microbial biofilm formation in bacteria such as *Pseudomonas* strains have different hydrophobicity capability to form biofilm. The bacterial cells that are more hydrophobic in nature always show high capability of biofilm formation and biodegradation. An investigation conducted to determine the biofilm formation and colonization of bacteria during the biodegradation of low density polyethylene (LDPE) films by *Bacillus amyloliquefaciens* strains BSM-1 and BSM-2 revealed that BSM-2 strain have the maximum capacity to form biofilm on the inert LDPE and exhibited higher cell surface hydrophobicity when compared to BSM-1 strain (Das and Kumar, 2013). On the other hand, a study conducted on drug resistant *Aeromonas* of 14 strains in the determination of bacterial contact angles and surface hydrophobicity with different reference liquids (water, formamide and α- bromonaphthalene) indicated that all the 14 *Aeromonas* species had hydrophilic surface characteristics (Dias *et al*., 2018).

Prevention of Bacterial Biofilms in Food Industry

Bacterial attachment to the surfaces in food processing is a fast process and for most applications it is not possible to clean and disinfect frequently enough to avoid the attachment. However, an adequate frequency of disinfection should carefully be determined to avoid biofilm maturation and buildup of absorbed organic material, which can influence the hygienic status of the material and the availability of nutrients (Van Houdt and Michiels, 2010). An effective preventive strategy reduces the likelihood of cross contamination within the food processing environment and of the food products (Phillips, 2016).

Chemical Methods of Prevention

Adequate chemicals such as oxidizing agents (hydrogen peroxide), surface active compounds (quaternary ammonium and acidic anionic compounds) and iodophores have been used as disinfectants to avoid the bacterial biofilm matrix in the food processing industries (Van Houdt and Michiels, 2010). Cleaning and sanitation of food processing surfaces with short intervals is an effective approach to prevent the sporulation of biofilms formed by vegetative *Bacillus* cells. On the other hand, strong alkaline cleaning agents are considered to be the most effective, especially against gram-positive bacteria such as *Staphylococcus aureus* (Furukawa *et al*., 2010). Another chemical compound to disinfect the surface is sodium hypochlorite. According to the explanation of (Srey *et al*., 2013), sodium hypochlorite used to be an effective disinfectant for biofilms inactivation. However, it is known to be more effective in low pH than alkaline pH environment. Acid pre-treatment, followed by the use of a concentrated leach solution, is another most effective for the removal of a multispecies biofilm. It removes calcium and magnesium carbonate crystals that are always found in dialysis biofilms (Marion-Ferey *et al*., 2003).

Physical Methods of Prevention

Bacterial biofilms are resistant to various physical forces such as shaving and washing action. Biofilms can withstand the physical/ environmental conditions like nutrient deprivation, pH changes, oxygen radicals, and chemicals such as antibiotics and disinfectants better than planktonic organisms (Jefferson, 2004). They are also resistant to phagocytosis, and the phagocytes that attempt an assault on the biofilm may actually do more harm to surrounding tissues than to the biofilm itself.

Physical treatments have been used as an alternative method for a particular sanitation of surface in the food industries. The authors showed that, for example, using ionizing radiation against biofilm cells of *Salmonella* species is effective, therefore it could be a useful sanitary treatment on a variety of foods and contact surfaces (Niemira and Solomon, 2005; Van Houdt and Michiels, 2010). Tuson and Weibel (2013) explained that the physical strategies for reducing the attachment of microbial have been inspired by natural materials like sharkskin and lotus leaves. The report of these authors showed that another system that designed to reduce bacterial attachment include the implementation of sharklet TM technology. The removal of biofilms can significantly be facilitated by the application of mechanical forces like brushing and scrubbing to the surface during cleaning (Wirtanen *et al*., 1996).

Biological Methods

The prevention of matrix formation by enzymes would seem to be an advantageous method of biofilm removal (Simões *et al*., 2010). Enzymes are specific in their activity to remove the matrix. It is probable that a mixture of enzymes would be more effective than when they appear individually (single enzyme); thus, various enzymes such as deoxy ribonucleases, glycosidases and proteases have significant disruptive effect on biofilms architecture and their maturation (Phillips, 2016; Sadekuzzaman *et al*., 2015). Torres *et al*., (2011) conducted a research on 17 commercial nonspecific enzymatic products to evaluate their ability in preventing and degrading biofilms. This study revealed that among these enzymatic products, pectinex smash was the most effective in preventing the adherence of bacterial biofilms and their formation; while others enzymatic products such as Viscozyme L, Pulpzyme, Termox and Bio-feed Beta L showed moderate effect.

Hurdle Technology

The implementation of hurdle technology is to achieve an effective treatment to reduce the foodborne pathogens in food through combing two or more control techniques. The combination treatment of sodium hypo chloride with UV radiation is the best way to hinder the effect of pathogens in foods. According to Srey *et al*. (2013) and De Queiroz and Day (2007), the combination of sodium hypo chlorite and hydrogen peroxide is more effective in removing of *Pseudomonas aeruginosa* biofilm from contact surfaces. Additionally, the combined effect of ozone and ultrasound is effective for the cell reduction of biofilms.

CONCLUSIONS and RECOMMENDATIONS

Bacterial biofilms are everywhere in nature. They probably represent the most important mechanism of microorganism attachment and colonization in nature and are structured communities of microorganisms organized in a complex structure that adheres to an inert or living surface. The formation of bacterial biofilms requires coordinated chemical signaling among the cells to adhere on food contact surface in the food industries and other food processing equipment. In food processing industries, different pathogenic microorganisms form biofilms that affect the quality and safety of food resulting in foodborne diseases which occupy a significant place in public health. To develop and plan cleaning and disinfection programs in order to reduce the occurrence of biofilms and monitor their efficacy is a concern of priority in food industry with respect to the preventive strategies to be employed in this regard. Therefore, to guarantee the quality and safety of food products, efforts should be in place for improving the cleaning and sanitizing programs to inactivate microorganisms and consequently prevent the formation of biofilms. In addition to this, it would be recommended to practice Hazard Analysis of Critical Control Points (HACCP) or Quality Assurance and Control to restrict possible areas open for biofilm formation.

REFERENCES

Annous, B. A., Fratamico, P. M., Smith, J. L. (2009). Scientific status summary: quorum sensing in biofilms: why bacteria behave the way they do. Journal of Food Science, 74(1): 24-37.

Bakker, D. P., Postmus, B. R., Busscher, H. J., van der Mei, H. C. (2004). Bacterial strains isolated from different niches can exhibit different patterns of adhesion to substrata. Appl. Environ. Microbiol., 70(6): 3758-3760.

Bassi, D., Cappa, F., Gazzola, S., Orrù, L., Cocconcelli, P. S. (2017). Biofilm formation on stainless steel by Streptococcus thermophilus UC8547 in milk environments is mediated by the proteinase PrtS. Appl. Environ. Microbiol., 83(8): e02840-16.

Billings, N., Birjiniuk, A., Samad, T. S., Doyle, P. S., Ribbeck, K. (2015). Material properties of biofilms—a review of methods for understanding permeability and mechanics. Reports on Progress in Physics, 78(3): 036601.

Chmielewski, R. A. N., Frank, J. F. (2003). Biofilm formation and control in food processing facilities. Comprehensive reviews in food science and food safety, 2(1): 22-32.

Dalla Costa, K. A., Ferenz, M., da Silveira, S. M., Millezi, A. F. (2017). Bacterial biofilm formation in different surfaces of food industries. Revista do Instituto de Latícinios Cândido Tostes, 71(2): 75-82.

Das, M. P., Kumar, S. A. N. T. O. S. H. (2013). Influence of cell surface hydrophobicity in colonization and biofilm formation on LDPE biodegradation. Int. J. Pharm. Pharm Sci., 5: 690-694.

Davey, M. E., O'toole, G. A. (2000). Microbial biofilms: from ecology to molecular genetics. Microbiol. Mol. Biol. Rev., 64(4): 847-867.

DeQueiroz, G. A., Day, D. F. (2007). Antimicrobial activity and effectiveness of a combination of sodium hypochlorite and hydrogen peroxide in killing and removing Pseudomonas aeruginosa biofilms from surfaces. Journal of Applied Microbiology, 103(4): 794-802.

Dias, C., Borges, A., Saavedra, M. J., Simões, M. (2018). Biofilm formation and multidrug-resistant *Aeromonas* spp. from wild animals. Journal of global antimicrobial resistance, 12: 227-234.

Donlan, R.M. (2001). Biofilm formation: a clinically relevant microbiological process. Clinical Infectious Diseases, 33(8): 1387-1392.

Donlan, R. M. (2002). Biofilms: microbial life on surfaces. Emerging Infectious Diseases, 8(9): 881-890.

Doulgeraki, A. I., Di Ciccio, P., Ianieri, A., Nychas, G. J. E. (2017). Methicillin-resistant food-related Staphylococcus aureus: a review of current knowledge and biofilm formation for future studies and applications. Research in microbiology, 168(1): 1-15.

Dunne, W.M. (2002). Bacterial adhesion: seen any good biofilms lately? Clinical Microbiology Reviews, 15(2): 155-166.

Furukawa, S., Akiyoshi, Y., Komoriya, M., Ogihara, H., Morinaga, Y. (2010). Removing Staphylococcus aureus and Escherichia coli biofilms on stainless steel by cleaning-in-place (CIP) cleaning agents. Food Control, 21(5): 669-672.

Garrett, T. R., Bhakoo, M., Zhang, Z. (2008). Bacterial adhesion and biofilms on surfaces. Progress in Natural Science, 18(9): 1049-1056.

Gomes, L., Moreira, J., Araújo, J. D., & Mergulhão, F. (2017). Surface conditioning with Escherichia coli cell wall components can reduce biofilm formation by decreasing initial adhesion.

Jamal, M., Tasneem, U., Hussain, T., Andleeb, S. (2015). Bacterial biofilm: its composition, formation and role in human infections. RRJMB, 4: 1-14.

Jefferson, K. K. (2004). What drives bacteria to produce a biofilm? FEMS Microbiology Letters, 236(2): 163-173.

Kostakioti, M., Hadjifrangiskou, M., Hultgren, S.J. (2013). Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harbor Perspectives in Medicine, 3(4): a010306.

Krasowska, A., Sigler, K. (2014). How microorganisms use hydrophobicity and what does this mean for human needs? Frontiers in Cellular and Infection Microbiology, 4, 112. DOI: 10.3389/fcimb.2014.00112

Lebeer, S., Verhoeven, T. L., Vélez, M. P., Vanderleyden, J., De Keersmaecker, S. C. (2007). Impact of environmental and genetic factors on biofilm formation by the probiotic strain Lactobacillus rhamnosus GG. Appl. Environ. Microbiol., 73(21): 6768-6775.

Li, Y. H., Tian, X. (2012). Quorum sensing and bacterial social interactions in biofilms. Sensors, 12(3): 2519-2538.

Maifreni, M., Frigo, F., Bartolomeoli, I., Buiatti, S., Picon, S., Marino, M. (2015). Bacterial biofilm as a possible source of contamination in the microbrewery environment. Food Control, 50: 809-814.

Marić, S., Vraneš, J. (2007). Characteristics and significance of microbial biofilm formation. Periodicum Bilogorum, 109: 115-121.

Marion-Ferey, K., Pasmore, M., Stoodley, P., Wilson, S., Husson, G. P., Costerton, J. W. (2003). Biofilm removal from silicone tubing: an assessment of the efficacy of dialysis machine decontamination procedures using an in vitro model. Journal of Hospital Infection, 53(1): 64-71.

Moreira, J. M. R., Gomes, L. C., Whitehead, K. A., Lynch, S., Tetlow, L. A., Mergulhão, F. J. (2017). Effect of surface conditioning with cellular extracts on Escherichia coli adhesion and initial biofilm formation. Food and Bioproducts Processing, 104: 1-12.

Møretrø, T., Hermansen, L., Holck, A. L., Sidhu, M. S., Rudi, K., Langsrud, S. (2003). Biofilm formation and the presence of the intercellular adhesion locus ica among Staphylococci from food and food processing environments. Appl. Environ. Microbiol., 69(9): 5648-5655.

Morimatsu, K., Eguchi, K., Hamanaka, D., Tanaka, F., Uchino, T. (2012). Effects of temperature and nutrient conditions on biofilm formation of Pseudomonas putida. Food Science and Technology Research, 18(6): 879-883.

Niemira, B. A., & Solomon, E. B. (2005). Sensitivity of planktonic and biofilm-associated Salmonella spp. to ionizing radiation. Appl. Environ. Microbiol., 71(5): 2732-2736.

Oliveira, M. M. M. D., Brugnera, D. F., Alves, E., Piccoli, R. H. (2010). Biofilm formation by Listeria monocytogenes on stainless steel surface and biotransfer potential. Brazilian Journal of Microbiology, 41(1): 97-106.

Pagán, R., García-Gonzalo, D. (2015). Influence of environmental factors on bacterial biofilm formation in the food industry: A review. Journal of Postdoctoral Research, 3 (6): 3-13

Phillips, C. A. (2016). Bacterial biofilms in food processing environments: a review of recent developments in chemical and biological control. International Journal of Food Science and Technology, 51(8): 1731-1743.

Rochex, A., Lebeault, J.M. (2007). Effects of nutrients on biofilm formation and detachment of a Pseudomonas putida strain isolated from a paper machine. Water Research, 41(13): 2885-2892.

Rossi, C., Serio, A., Chaves-López, C., Anniballi, F., Auricchio, B., Goffredo, E., Paparella, A. (2018). Biofilm formation, pigment production and motility in Pseudomonas spp. isolated from the dairy industry. Food Control, 86: 241-248.

Sadekuzzaman, M., Yang, S., Mizan, M. F. R., Ha, S. D. (2015). Current and recent advanced strategies for combating biofilms. Comprehensive Reviews in Food Science and Food Safety, 14(4): 491-509.

Shrout, J. D., Tolker-Nielsen, T., Givskov, M., Parsek, M. R. (2011). The contribution of cell-cell signaling and motility to bacterial biofilm formation. MRS Bulletin, 36(5): 367-373.

Siboni, N., Lidor, M., Kramarsky-Winter, E., Kushmaro, A. (2007). Conditioning film and initial biofilm formation on ceramics tiles in the marine environment. FEMS Microbiology Letters, 274(1): 24-29.

Simões, M., Simões, L. C., Vieira, M. J. (2010). A review of current and emergent biofilm control strategies. LWT-Food Science and Technology, 43(4): 573-583.

Srey, S., Jahid, I. K., Ha, S. D. (2013). Biofilm formation in food industries: a food safety concern. Food Control, 31(2): 572-585.

Téllez, S. (2010). Biofilms and their impact on food industry. VISAVET Outreach Journal. https://www.visavet.es/en/articles/biofilms-impact-food-industry.php

Torres, C. E., Lenon, G., Craperi, D., Wilting, R., Blanco, Á. (2011). Enzymatic treatment for preventing biofilm formation in the paper industry. Applied Microbiology and Biotechnology, 92(1): 95-103.

Toyofuku, M., Inaba, T., Kiyokawa, T., Obana, N., Yawata, Y., Nomura, N. (2016). Environmental factors that shape biofilm formation. Bioscience, Biotechnology and Biochemistry, 80(1): 7-12.

Tuson, H. H., Weibel, D. B. (2013). Bacteria–surface interactions. Soft Matter, 9(17): 4368-4380.

Van Houdt, R., Michiels, C. W. (2010). Biofilm formation and the food industry, a focus on the bacterial outer surface. Journal of Applied Microbiology, 109(4): 1117-1131.

Winkelstroter, L.K. (2015). Microbial Biofilms: The Challenge of Food Industry. Biochemistry and Molecular Biology Journal, 1(1:5): 1-3 DOI: 10.21767/2471-8084.100005.

Wirtanen, G., Husmark, U., Mattila-Sandholm, T. (1996). Microbial evaluation of the biotransfer potential from surfaces with Bacillus biofilms after rinsing and cleaning procedures in closed food-processing systems. Journal of Food Protection, 59(7): 727-733.

Zhao, X., Zhao, F., Wang, J., Zhong, N. (2017). Biofilm formation and control strategies of foodborne pathogens: food safety perspectives. RSC Advances, 7(58): 36670-36683.