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Biofilm and Food industry

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Abstract

Biofilms are communities of microorganism that are formed on solid or fluid interfaces and are designed to protect the individual cells, such as bacteria, from the environment. It is a natural tendency of microorganisms to attach to wet surfaces, to multiply and to embed themselves in a slimy matrix composed of extracellular polymeric substances (EPS) that they produce, forming a biofilm. The driving force in bacterial community development is the self-organization and cooperation among cells, rather than the competitive natural selection of individual microorganisms. Outbreaks of pathogens associated with biofilms have been related to the presence of *Listeria monocytogenes*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Salmonella* spp. *Staphylococcus* spp. and *Escherichia coli* O157:H7 on food contact surfaces. The main strategy to prevent biofilm formation is to clean and disinfect regularly before bacteria attach firmly to surfaces.

Keywords: Biofilms, EPS.

Introduction

Biofilms are communities of microorganism that are formed on solid or fluid interfaces and are designed to protect the individual cells, such as bacteria, from the environment. It is a natural tendency of microorganisms to attach to wet surfaces, to multiply and to embed themselves in a slimy matrix composed of extracellular polymeric substances (EPS) that they produce, forming a biofilm. They are problematic in particular food industry sectors such as brewing, dairy processing, fresh produce, poultry processing and red meat processing (Chen, Rossman, & Pawar, 2007). Bacterial biofilms are integrated, multi-species communities of cells that adhere to almost any surface and are fundamental to the ecology and biology of bacteria. Biofilms constitute a protected mode of growth that allows survival in a hostile environment. The structures that form in biofilm contain channels in which nutrients can circulate, and cells in different regions of the biofilm exhibit different patterns of gene expression. The complexity of the biofilm structure and metabolism has led to the analogy of biofilm to tissue of higher organism. These sessile biofilm communities can give rise to non-sessile individuals, planktonic bacteria that can rapidly multiply and disperse

Microbial adhesion

Microbial adhesion and biofilm formation are major concerns in the control of biofilm. Bacteria rapidly adapt to their extracellular conditions to survive in diverse environmental conditions forming communities including biofilms. Adhered microorganisms, embedded in biofilms or microorganisms hiding in cracks or crevices may escape of cleaning and disinfecting procedures and be a source of recontamination of food products during processing; because of this, a major part of the pre-requisite of a Good Hygienic Practices Programme of a food manufacturing plant is therefore to ensure that

microbial biofilms do not form or are efficiently removed. In the native physiological state in vivo, microorganism demonstrated that the process of contamination of surface following a successive chain, including an initial microbial adhesion, strength of the binding of the attached microorganisms through exopolymer production, growth of attached microorganisms and continued secretion of exopolymers and localized detachment of biofilm organisms caused by occasionally high fluid shear or other detachment forces operative, allowing the colonization of closer surfaces. Adhered and biofilm-forming microorganisms may also have other adverse effects in the colonizated surface such as decreasing heat transfer or causing corrosion. The mechanism of attaching to surfaces follows an organized sequence starting with the deposition of specific adhesive protein which binds to the surface reversibly. A successive deposition of cells creates a strong binding by cell to cell cohesion and cell-binding-proteins. Cell adhesion molecules involved in the process are first hydrolyzed by extracellular enzymes. Bacterial adhesion is directly related to protein adsorption.

Bacterial adhesion to surfaces is due to the influence of surface roughness.Since the report in 1940 for Heukelekian H. et al, has been known that the surface characteristics are an important factor for the bacterial adhesion and development and this central research area for the control of bacterial biofilm related disease. The adhesion of bacteria to a surface depends on a number of microbiological, physical, chemical, and material-related parameters; especially the surface topography is a parameter influencing bacterial adhesion. The contact with a solid surface induces the expression of a bacterial enzyme, which catalyzes the formation of exo-polysaccharides that promote colonization and protection. Thus, the modification of surfaces can to reduce attachment surfaces to limit the adhesion of microorganism e.g. electropolishing of stainless-steel. Several parameters or measures have been used to characterize the material surface based on two-dimensional characteristics such as the Ra (roughness average), Rt (is the maximum peak to valley height in the sample length), and Rz values (the average maximum profiler height. Amongst the most widely used is the surface roughness Ra value (which is the arithmetical mean deviation of the profile) and an Ra value of 0.8 μm or less has been recommended for dairies and, in general, for food contact surfaces. Although widely used, the Ra value will typically not characterize features of the surface such as soft or sharp topography or the presence of scratches or porosities. During recent years, scanning electron microscopy (SEM) and atomic force microscopy (AFM) have been used to give a three-dimensional visualization of the surface topography including AFM determination of three-dimensional topographical parameters in the nanometer range.Through the recommendation of a minimum Ra value of 0.8 μm, a number of studies have evaluated if further reductions in numbers of adhering bacteria can be obtained by using even smoother surfaces with lower Ra values. However, experiments in milk showed no significant difference between adhesion on surfaces with Ra 0.4 and 0.035 μm (Barnes *et al* 1999). Similar tendencies were found in a study of elements and base metal, where no significant differences were found in bacterial adhesion.

Specialized attachment structures/surface properties of the cell. Cell surface hydrophobicity and the presence of extracellular filamentous appendages may influence the rate and the extent of microbial attachment. The hydrophobicity of the cell surface is important inadhesion because hydrophobic interactions tend to increase with an increasing non-polar nature of one or both surfaces involved, i.e., the microbial cell and the adhesion surface (Donlan, 2002). The ability of bacteria to attach to each other and to surfaces depends in part on the interaction of hydrophobic domains. Many cells produce extracellular filamentous appendages. These may, therefore, play a role in the attachment process. In fact, their radius of interaction with the surface is far lower than that of the cell itself. A number of such structures are known to exist – flagella, pili or fimbrae, prothecae, stalks and hold-fast (Harbron & Kent, 1988). Flagella, when existent, are responsible for the motility of bacteria. These are very fine threads of the protein flagellin with a helical structure extending out from the cytoplasm through the cell wall. Flagella may have a diameter between 0.01 and 0.02 mm, and a length of up to 10 mm. It is possible that the flagellum itself may form an adhesive bond with the adhesion surface (Harbron & Kent, 1988). The primary function of flagella in biofilm formation is assumed to be in transport and in initial cell–surface interactions (Sauer & Camper, 2001). Flagella-mediated motility is believed to overcome repulsive forces at the surface of the substratum and, as a consequence, a monolayer of cells forms on the adhesion surface . Pili or fimbriae are found on many Gram-negative bacteria . They are fine, filamentous appendages, also of protein, 4–35 nm wide and up to several micrometers long (Harbron & Kent, 1988). These structures are usually straight, and are not involved in motility. Their only known general function is to make cells more adhesive, since bacteria

with pili can adhere strongly to other bacterial cells and inorganic particles (Harbron & Kent, 1988). Nevertheless, they are not always involved in the attachment process even if they are present .According to certain researchers, pili and pilus-associated structures have been shown to be important for the adhesion to and colonization of surfaces, probably by overcoming the initial electrostatic repulsion barrier that exists between the cell and the substratum. Prosthecae and stalks form a third group of attachment structures. These occur in several types of microorganisms. They may occur at one or more sites on the cell surface, and are filiform or blunt extensions (commonly 0.2 mm) of the cellwall and membrane (Harbron & Kent, 1988). At the end of a prosthecae or stalk is usually found an adhesive disk, or hold-fast. The stalk and hold-fast structure is quite often used by diatoms to attach to a surface (Harbron & Kent, 1988).

Extracellular polymeric substances (EPS)

EPS are responsible for binding cells and other particulate materials together (cohesion) and to the surface (adhesion) (Allison, 2003; Characklis & Wilderer, 1989; Sutherland, 2001). The general composition of bacterial EPS comprises polysaccharides, proteins, nucleic acids, lipids, phospholipids, and humic substances. Biofilms form a gel phase where microorganisms live inside . The EPS matrix acts as a barrier in which diffusive transport prevails over convective transport .A function frequently attributed to EPS is their general protective effect on biofilm microorganisms against adverse conditions. As an example, it has frequently been observed that biofilm cells can tolerate high concentrations of biocides (Foley & Gilbert, 1996; Simo˜es & Vieira, 2009; Simo˜es, Pereira, & Vieira, 2005). This is supposed to be due mainly to physiological characteristics of biofilm bacteria, but also to a barrier function of EPS (Simo˜es et al., 2005). The EPS matrix delays or prevents antimicrobials from reaching target microorganisms within the biofilm by diffusion limitation and/or chemical interaction with the extracellular proteins and polysaccharides Moreover, within the EPS matrix the molecules required for cell–cell communication and community behavior may accumulate at concentrations high enough to be effective .The role of EPS components other than polysaccharides and proteins (fundamental structural elements of the biofilm matrix determining the mechanical stability of biofilms) remains to be established . Bacterial alginates represent an example of the few EPS which have been studied in detail, however, under the aspects of their relevance as a general virulence factor in infection processes of plants, animals, and man as well as in terms of their potential commercial exploitation (Wingender et al., 1999). Lipids and nucleic acids might significantly influence the rheological properties and thus the stability of biofilms . The extracellular DNA is required for the initial establishment of biofilms by Pseudomonas aeruginosa, and possibly for biofilms formed by other bacteria that specifically release DNA.

The driving force in bacterial community development is the self-organization and cooperation among cells, rather than the classical ‘‘competitive’’ natural selection of individual microorganisms (Daniels et al., 2004; Davies et al., 1998;)This concept becomes particularly apparent when examining bacterial biofilm communities. Cell– cell signalling has been demonstrated to play a role in cell attachment and detachment from biofilms (Daniels et al., 2004; Donlan, 2002). Bacteria are considered to be far from solitary microorganisms, and in fact are colonial by nature and exploit elaborate Extracellular polymeric substances (EPS).

EPS are responsible for binding cells and other particulatematerials together (cohesion) and to the surface (adhesion). The general composition of bacterial EPS comprises polysaccharides, proteins, nucleic acids, lipids, phospholipids, and humic substances . According to researchers, proteins and polysaccharides account for 75–89% of the biofilm EPS composition, indicating that they are the major components. Biofilms form a gel phase where microorganisms live inside .The EPS matrix acts as a barrier in which diffusive transport prevails over convective transport. A function frequently attributed to EPS is their general protective effect on biofilm microorganisms against adverse conditions. As an example, it has frequently been observed that biofilm cells can tolerate high concentrations of biocides (Foley & Gilbert, 1996; Simo˜es & Vieira, 2009; Simo˜es, Pereira, & Vieira, 2005). This is supposed to be due mainly to physiological characteristics of biofilm bacteria, but also to a barrier function of EPS (Simo˜es et al., 2005). The EPS matrix delays or prevents antimicrobials from reaching target microorganisms within the biofilm by diffusion limitation and/or chemical interaction with the extracellular proteins and polysaccharides. Moreover, within the EPS matrix the molecules required for cell–cell communication and community behavior may accumulate at concentrations high enough to be

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Cell–cell communication

The driving force in bacterial community development is the self-organization and cooperation among cells, rather than the classical ‘‘competitive’’ natural selection of individual microorganisms (Daniels et al., 2004; Davies et al., 1998) This concept becomes particularly apparent when examining bacterial biofilm communities . Cell– cell signalling has been demonstrated to play a role in cell attachment and detachment from biofilms (Daniels et al., 2004; Donlan, 2002). Bacteria are considered to be far from solitary microorganisms, and in fact are colonial by nature and exploit elaborate systems of intercellular interactions and communications to facilitate their adaptation to changing environments .The successful adaptation of bacteria to changing natural conditions is dependent on their ability to sense and respond to the external environment and modulate gene expression accordingly (Daniels et al., 2004). Quorum sensing is based on the process of autoinduction . The process of quorum sensing provides a mechanism for self-organization and regulation of microbial cells . It involves an environmental sensing system that allows bacteria to monitor and respond to their own population densities. The bacteria produce a diffusible organic signal, originally called an auto-inducer (AI) molecule, which accumulates in the surrounding environment during growth High cell densities result in high concentrations of signal, and induce expression of certain genes and/or physiological changes in neighbouring cells. A response to chemical signals in the process of cell communication is a concentration dependent process, where a critical threshold concentration of the signal molecule must be reached before a physiological response is elicited Oligopeptides and N-acylhomoserine lactones (AHL) are major AI molecules involved in intra-specific communication in Gram-positive and Gram-negative bacteria, respectively, whereas boronated diester molecules (AI-2) are involved in inter-specific communication among both Gram-positive and Gram-negative bacteria AHL (AI-1) are the best characterized molecules.

Quorum sensing systems are known to be involved in a range of important microbial activities. These include extracellular enzyme biosynthesis, biofilm development, antibiotic biosynthesis, biosurfactant production, EPS synthesis and extracellular virulence factors in Gram-negative bacteria.

Outbreaks of pathogens associated with biofilms have been related to the presence of *Listeria monocytogenes, Yersinia enterocolitica, Campylobacter jejuni, Salmonella spp. Staphylococcus spp. and Escherichia coli* O157:H7 Foodborne pathogens can enter the milk processing equipment by direct contact with contaminants in the dairy farm environment (e.g. faecal contamination and udders of infected animal) and also through the water used in the milking machines.These contaminating microorganisms can form biofilms that are difficult to eradicate and can act as a harbour and/or substrate for other microorganisms less prone to biofilm formation, increasing the probability of pathogen survival and further dissemination during food processing. Post-pasteurization contaminations of milk products are mainly due to the filling machines. Biofilms that can develop on the sides of gaskets may also be a source of post-pasteurization contamination Environmental surfaces such as floors and walls may also be indirect sources of contamination e.g. transference to the food products by vectors such as air, people and cleaning systems

Approach for biofilm mitigation – biofilm prevention

Ideally, preventing biofilm formation would be a more logical option than treating it. However, there is presently no known technique that is able to successfully prevent or control the formation of unwanted biofilms without causing adverse side effects. The main strategy to prevent biofilm formation is to clean and disinfect regularly before bacteria attach firmly to surfaces (Simo˜es et al., 2006). Biofilm detectors were already developed to monitor the surface colonization by bacteria and allow the control of bifilms in the early stages of development developed a mechatronic surface sensor able to detect biofilms in the early stages of

development. This sensor was also able to detect the presence of cleaning products in a surface, identify when it was biologically and chemically cleaned and measure the rate of cleaning. Other preventive strategies attempted to identify materials that do not promote or can even suppress biofilm formation .This study ranked different materials according to their biofilm growth propensity concluding that there is hardly any material that does not allow biofilm formation .Moreover, biofilm formation may vary with the microbial species present and with the environmental conditions (Simo˜es, *et al*. 2009). The research conclusions by different scientists indicated a higher significance of surface defects/roughness on the ease of surface cleaning rather than the surface finishing type.Inhibition of biofilm formation by limitation of the carbon source is a virtually impossible procedure, as ultra-pure water systems have been found to support the formation of biofilms Another approach is to supply the microorganisms with growth factors, so surface attachment is no more a benefit for them .Several attempts have been made to avoid biofilm formation by the incorporation of antimicrobial products into surface materials by coating surfaces with antimicrobials a reduction in infection rate using silicone rubber implants with covalently coupled quaternary ammonium coatings. Other authors reported biofilm formation inhibition by coating surfaces with silver. These studies focused on biomedical applications but the approaches may also be useful in the dairy industry if restricted to some parts of the process equipment such as valves, dead ends or where biofilms are more prone to form and difficult to control. The possibility of carry over of antimicrobials into food products is a concern when coatings release antimicrobial products. The surface pre-conditioning with surfactants has potential to prevent bacterial adhesion. Nonionic and anionic surfactants were evaluated in preventing the adhesion of P. aeruginosa to stainless steel and glass surfaces. The surfactants give more than 90% inhibition of adhesion. Some surfactants affects the development of flagella, demonstrating significant changes in the bacteria attachment ability in the presence of surfactants.

Cleaning and disinfection

In the dairy industry the classical operations of cleaning and disinfection are essential parts of milk production. The efficiency with which these operations are performed greatly affects the final product quality . Generally, disinfectants do not penetrate the biofilm matrix left on a surface after an ineffective cleaning procedure, and thus do not destroy all the biofilm living cells (Simo˜es *et al*., 2006). Therefore,cleaning is the first step and of utmost importance to improve the sanitation of the processing equipment It is important to effectively remove food debris and other residues that may contain microorganisms or promote microbial growth. The use of high temperature can reduce the need for the application of physical forces such as water turbulence and scrubbing Chemical products commonly used for cleaning are surfactants or alkali products, used to suspend and dissolve food residues by decreasing surface tension, emulsifying fats, and denaturing proteins.Cleaning-in-place (CIP) procedures are usually employed in milk processing lines. An independent quality control system to monitor the cleaning results for a dairy plant can be integrated in the Hazard Analysis Critical Control Points (HACCP) program. Evaluation of biofilm sanitation should be part of the HACCP development plan in order to control those biofilms prevalent in the processing areas. Moreover, impairing the formation of biofilms can be achieved through a better knowledge of the mechanisms that contribute to their formation, development and maintenance (Simo˜es *et al*, 2007).

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