Formation, architecture and functionality of microbial biofilms in the food industry.

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Abstract

Recent publications on biofilm formation, architecture and function were reviewed. Biofilm formation begins with organic material, then cell conditioning of a surface. Environmental conditions and microorganisms then influence the establishment of the biofilm architecture. This in turn supports the function of the biofilm which enhances microbial survival, reproduction and contamination of new areas. In the food industry, ‘true’ biofilms are usually found on closed surfaces such as pipe works where liquid flows over a solid surface. On open surfaces, fouling will affect microbial retention, survival and transfer potential but is less likely to support the development of a true biofilm. Each aspect of biofilm formation is complex with a myriad of influencing factors, which we are only just beginning to elucidate. Much more research needs to be carried out in all aspects of these areas to understand these elegant biofilm and fouling systems if they are ever to be controlled.

Introduction

The preparation and processing of food is considered an important route for cross contamination of pathogenic bacteria in food products [1-4].Within nature, as well as in food processing, cells living freely in bulk solution usually become attached to a surface, and if retained, can then form a biofilm. The formation, architecture and function of biofilms are complex phenomena influenced by surface properties, microbiological and environmental factors which will be related to the specific industrial setting in which they are found (Figure 1).

Biofilms are defined as matrix-enclosed bacterial populations that are attached to a surface, an interface and/or to each other [5]. It not surprising that more than 99% of all the planets bacteria live in a biofilm since microorganisms gain considerable advantages from being part of a community [6]. Microorganisms are living organisms with a vast range of physiologically and metabolically varied species that enables them to colonise, adapt and utilise almost any situation they encounter. Thus, a biofilm may be a small or large-scale entity and in the food processing environment these may be a few micrometres or several millimetres in thickness [7]. In the food industry, large-scale biofilms or fouling may occur on such items as heat exchangers or may form on enclosed surfaces when they are in contact with a wet product; an example of this is in pipework. Closed or 'true' biofilms usually occur under conditions of continuous or intermittent flow and are considered to have well developed stacked structures with pore channels. Under static conditions, it has been shown that biofilms with different architecture and functionalities occur [8,9].

Smaller scale biofilms or biofouling may also occur in the food industry on open surfaces. Open surfaces are exposed, with food being handled or prepared on them and in these situations flow is absent. On an open surface, organic soiling, which may also compromise microorganisms along with the food material, is a major issue in the food processing industries, causing a range of biofouling and microbiological problems [10-12]. The term biofilm is often used to describe cells and organic material retained on a surface; these do not have the characteristic, classic biofilm 'mushroom' type morphology. This type of biofouling is common to a regularly cleaned surface, where material may accumulate, but not possess the morphology of a traditional biofilm.

Biofilm formation, architecture and function is dependent on a wide range and combination of surface morphologies (chemistry, topography, physicochemistry), environmental conditions (pH, nutrient availability, temperature, host proteins/adhesins, fluid dynamics) and microbiological factors (Gram negative/positive, microbial shape, structure, molecular composition, species, physicochemistry, growth phase, age, presence of flagella, pili, capsules or exopolymeric substances) [13]. However, to cover all these factors is beyond the scope of this review. This article will give a brief summary of recent work on three aspects of the biofilm; formation, architecture and function.

Biofilm Formation

Biofilm formation is a complex process regulated by the diverse characteristics of the surroundings. Perhaps one of the most important factors that influence biofilm formation are the surface properties and deposition organic material. Prior to the onset of biofilm formation, initial cell attachment, adhesion, retention and proliferation must occur. However, before a cell can bind to a surface, the surface is conditioned by adsorbing molecules from the surrounding environment.

The chemical, topographical and physicochemical properties of the surface affect initial organic material adsorption and distribution [14-17]. The type and amount of organic material adsorbed onto the surface will then, in turn alter the surface properties [18]. Indeed, it has been demonstrated that a pristine surface only remains as such for one exposure, being subsequently irreversibly altered by organic material [18]. When stainless steel surfaces where repeatedly cleaned thirty times without soiling, organic material was still found to become built up on the surfaces [18]. Further, the biochemical structure, adsorption and distribution of the conditioning film or organic material is dependent on the type of food processing being carried out [19,20], adding an additional level of complexity to the surface (Figure 2). The composition of organic material that might be found in the fish industry (muscle proteins troponin, tropomyosin, and myosin, and the lipid binding protein apolipoprotein) [21], will vary from that deposited on surfaces in the dairy industry (α-casein, β-casein, κ-casein, and α-lactalbumin) [22]. This in turn will affect cell retention [9,10] and thus subsequent biofilm architecture, function [23], surface hygiene and cleanability [12,24]. Although most cleaning procedures remove gross organic material and microorganisms, there is concern regarding organic material that is retained on surfaces, especially in surface features [18] because of its influence on the subsequent adhesion of microorganisms and possibly increase the retention of organic soil (Figure 3). An example is *Shewanella putrefaciens*, a spoilage bacterium of marine fish, some vacuum-packed meats and chicken that was found to adhere readily to stainless steel disks. In this scenario, it was demonstrated that bacterial adhesion was facilitated by the formation of an initial conditioning film of tryptone soya broth [25]. While it might immediately be thought that biofouling might automatically enhance cell retention and reduce surface hygiene [10,12,24], the adherence of fish protein layers to surfaces has been shown to provide a steric barrier towards bacterial adhesion [20] and thus reduce cell retention. Therefore the influence of organic material or conditioning film on cell retention is a complex issue with sometimes unexpected outcomes. The adsorption of organic material onto a surface may therefore be considered to be of particular importance in the subsequent development of biofilm architecture.

Biofilm architecture

In this article, biofilm architecture is defined as the complex design of the biofilm structure. Once a surface has become conditioned with organic material, biofilm formation can take place, the architecture of which, will be dependent on a number of environmental and microbiological influences. In the majority of environments, biofilms are not usually found in a monoculture but are consortial. Biofilm associated bacteria may sense the growth of the same or other microbial species attached to the surface, either directly through physical contact or indirectly by sensing the proximity of fellow organisms in a process known as quorum sensing [26]. Exopolymeric substances (EPS) are also an important constituent of biofilm formation at surface liquid interfaces. Bacteria and other microorganisms produce extracellular matrix components which help them adhere to surfaces. However, the chemical composition of EPS matrix may differ, depending on the medium in which the biofilm is grown, for instance, it was demonstrated that the EPS of a biofilm grown in tryptic soy broth was more complex than a biofilm grown in meat thawing-loss broth [27].

In recent years, it has been demonstrated that cells grown in different nutrients resulted in different biofilm morphologies. For example, a meat *Salmonella* spp. grown in meat thawing-loss broth demonstrated a “cloud-shaped” morphology in a mature biofilm, whereas when grown in tryptic soy broth, biofilms appeared “reticular-shaped” [27]. However, it has also been revealed that some bacteria, for example *L. monocytogenes* were unable to form thick, multilayer biofilms when related to the fish or meat industries [30,31]. In the true sense of biofilm architecture for a number of food pathogens, mature biofilms are generally described as a collection of clusters or knitted chains (*L. monocytogenes*) [8], may be ball shaped (*Listeria monocytogenes*) [8], mushroom shaped (Staphylococcal) [28] or honeycombed shaped (*Vibrio cholerae*) [29]. In multi-species biofilms, alterations in biofilm architecture have also been confirmed when microorganisms have been co-cultured from fresh cut food processing facilities or in raw milk [32,33]. When thirteen Gram negative species were isolated from two fresh produce processing facilities, the strong biofilm producing strains of *Burkholderia caryophylli* and *Ralstonia insidiosa* exhibited 180% and 63% increase in biofilm biomass, and significant thickening of the biofilms when co-cultured with *E. coli* O157:H7. This has a subsequent effect on biofilm function since it can be suggested that when bacteria interact synergistically in biofilm formation, there is a potential for the increased survival of such pathogenic bacteria as *E. coli* O157:H7 in fresh produce processing environments [32].

A number of studies which concentrate on specific environmental elements that may influence biofilm architecture have also been carried out. One of the most investigated parameters is that of temperature which has been shown to produce increased biofilm production with a variety of *L. monocytogenes* or *S. enterica* strains that were found in a food production environment [33-36]. pH and biocides have also been shown to have a significant effect on biofilm architecture; the food pathogens *E. coli, L. monocytogenes* or *S. enterica* serovar Typhimurium demonstrated that increased biofilm production was correlated to the most acidic, or most alkaline growth conditions tested [37-41]. In contrast to the above findings, others have established that there was no consistent relationships between biofilm-forming ability and capacity to withstand stress exposures (acid, alkaline, heat and high hydrostatic pressure treatments) using verocytotoxigenic *Escherichia coli* strains [42]. The effect of the surrounding media has also been demonstrated to affect biofilm architecture, whereby enhanced biofilm production by *L. monocytogenes* was observed early in biofilm maturation in nutrient poor media [34].

Further work has been carried out with the emphasis on bacterial serotypes rather than on environmental factors and their influence on biofilm architecture, whereby differences between the biofilm forming capacity were found to exist between different *Salmonella enterica* serovars taken from different stages of the poultry farm environment [36]. This work demonstrated that certain farm isolates were capable of forming biofilms under laboratory conditions, whereas laboratory grown strains were not [36].

As might be expected, the role of hydrodynamics has a significant effect on biofilm architecture. Yazdi and Ardenaki [43] investigated in a micro channel the influence of fluid flow on the dynamics of motile microorganisms and their aggregation. They showed that vortical structures promoted cell aggregation and triggered biofilm streamer formation. Further, using *E. coli* it was demonstrated that biofilms adapted their architecture in order to cope with hydrodynamic conditions and nutrient availability [44]. It was found that until a certain thickness was reached, nutrient availability dictated biofilm architecture but when a critical thickness was exceeded, mechanical resistance to shear stress (i.e. biofilm cohesion) became more important [44].

Biofilm Function

Biofilm functionality may be defined in this review as the manner in which the biofilm operates. Biofilm function, is highly dependent on environmental and microbiological factors. The function of the biofilm is thought to be developed in order to primarily provide defence for the cells against harmful conditions and allow further cell colonisation of available surfaces [14]. The build-up of the biofilm structure and extracellular matrix provides protection from physical factors and from predators, as well as potentially providing a diffusion barrier against different chemical compounds (such as antibiotics, biocides, and disinfectants) [45], for example, a complex three-dimensional microscopic structure of a *L. monocytogenes* biofilm demonstrated a high resistance to benzalkonium chloride [46].

Bacteria in biofilms communicate through signalling molecules and use quorum-sensing to optimize their virulence factors and survival [47]. Quorum sensing is widely recognized as an efficient mechanism to regulate expression of specific genes responsible for communal behaviours in bacteria [48]. Greater antimicrobial resistance has been demonstrated in *S.*

aureus biofilms when compared to planktonic cells with bacteria isolated from the fish industries [49]. The age of a biofilm has also been suggested to influence both biofilm architecture and function, where an increase in *E. coli* O157:H7 population was observed as storage time progressed on surfaces encountered in meat processing plants [50] and aged *L. monocytogenes* biofilms demonstrated resistance to desiccation [23].

Although not often recognised, on an open surface, similarities between organic/microbial fouling and a biofilm can be made whereby a complex, heterogeneous matrix of organic material encloses the bacterial population attached to a surface (Figure 4) and the presence of organic material may protect the bacteria from cleaning agents in much the same way. Exposure to conditioning films has also been suggested to affect the function of the biofilm by significantly increasing the survival of *L. monocytogenes* [23], or to be one of the main reasons for disinfection failure [51].

Conclusions

Microbial biofilms are elegant systems that provide an impressive survival trait for microorganisms in food consortia. Each of the biofilm aspects (formation, architecture and function) invole numerous contributing factors which need to be investigated in order to understand these systems further. However, influencing and contributing factors are specific for each food-processing environment and setting. What is also clear is that the definition of a biofilm encompasses many different forms of microbial and organic material consortia. Whatever the setting, these sophisticated systems require much further investigation before we can truly begin to really understand or control them.

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Figure Legends

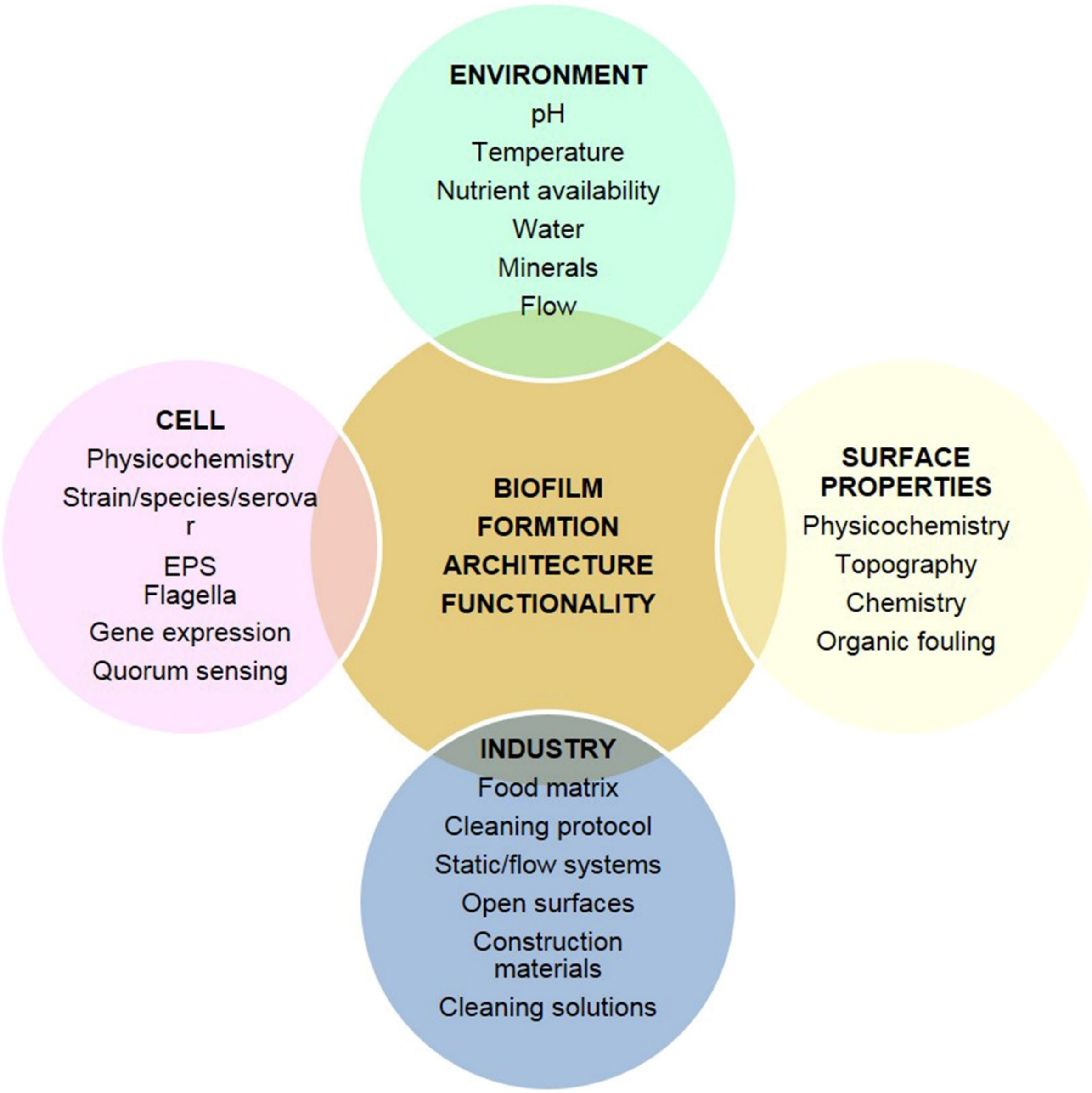

Figure 1 A complex interplay of factors results in biofilm formation, architecture and hence functionality which are related to the specific industrial food setting in which they are found.

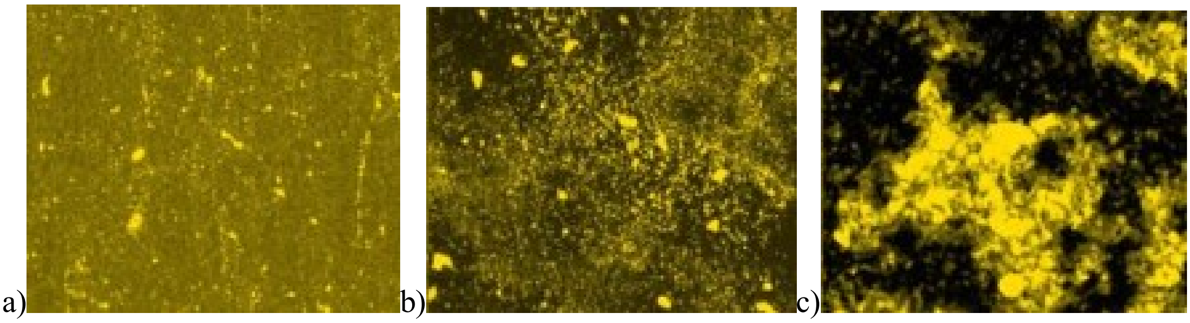

Figure 2 Ten percent a) beef extract, b) cod extract and c) whey solution deposited on stainless steel surfaces demonstrating that different organic material produce different patterns of retention of organic material (yellow) across surfaces. This difference in the pattern of organic material retention will presumably also affect cell retention and the formation of initial biofilm architecture.

a) b)

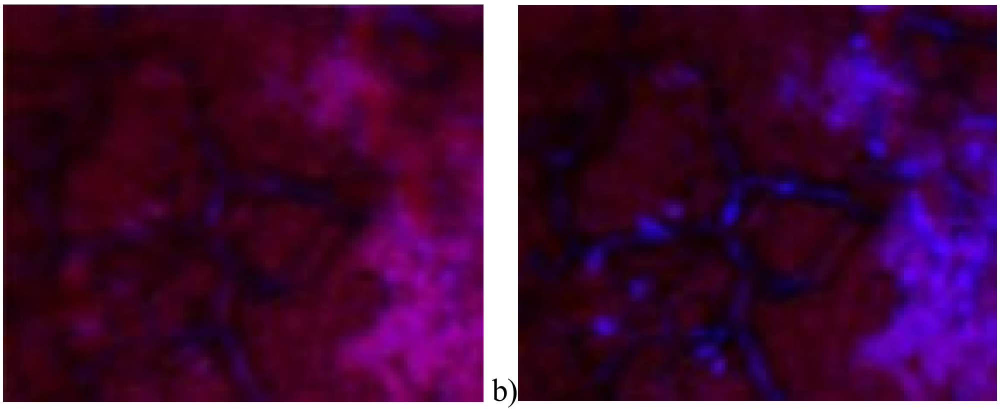

Figure 3 Organic material and cells retained on a surface, resulting in the heterogeneous distribution of a) organic material (red) and b) cells (blue).

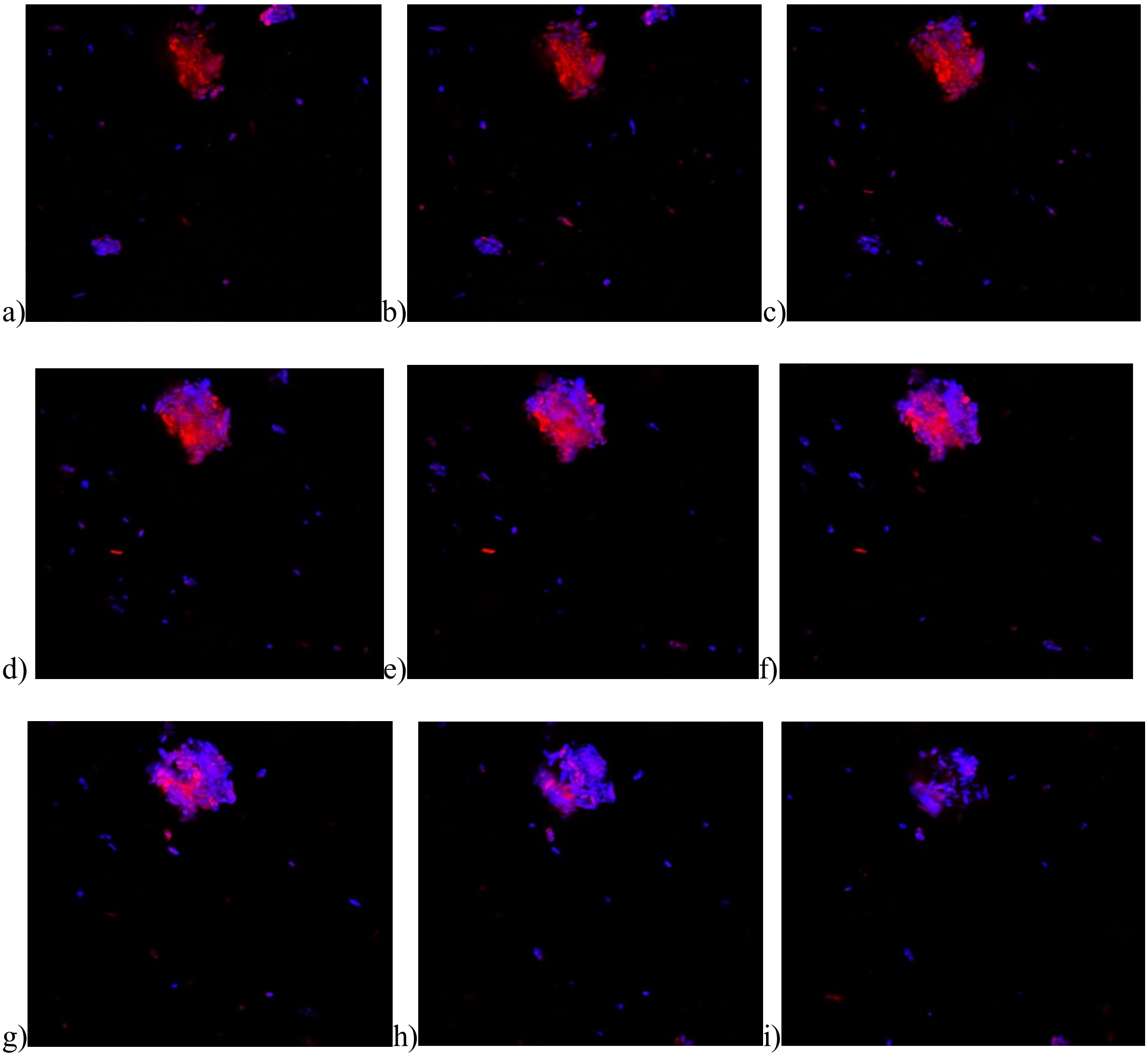

Figure 4 Using confocal microscopy it can be demonstrated that as food material and cells are visualised, the distribution of cells (blue) and organic material (pink) form a heterogeneous matrix which may protect the cells. a-c) Nearest the surface the conditioning film is most prevalent but visualising up from the surface to the top of the food particle (d-f) the bacteria become more obvious. At the top of the food particle (g-i) bacteria predominate.