

Biofilms and Their Advantages/Disadvantages in Food Industry

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A biofilm is known as a microbial community formed surface-associated cells embedded in the extracellular polymeric matrix. Microorganisms change their behaviours under inadequate conditions by cooperating with each other and it can cause some undesired results, such as biofilm-associated infections which are very common in medicine. Taking part in biofilm matrix found on surfaces following the adherence of various microorganisms to the food processing area is considered as a potential source of food contamination. Biofilm forming microorganisms have different physiological properties in comparison to planktonic cells. Behaviour changes of strains lead to some results such as antimicrobial resistance. “Quorum sensing (microbial communication with each other by spreading their signal molecules)” appears and the bindings in biofilm layer become permanent that not allow penetration into the matrix. The biofilm formation has been correlated with pathogeny in general, however, its presence could provide the desired properties to the microorganism which are especially used in food industry. This chapter overviews the mechanism of biofilm formation and its advantages/disadvantages for the food industry.

Keywords: Biofilm; advantages; disadvantages; food industry

1. Introduction

In food industry biofilm formation has known as a microbial colonization which is embedded into the extracellular polymeric substance (EPS). Many microbial species can live together in biofilm composed of organic polymer matrix containing polysaccharides, proteins, nucleic acids and DNA that lead to be glued microorganisms together. In biofilm layer, several microbial strains might be found in the medium. The synergistic effect has been observed among the microorganisms living together in the biofilm layer. This means biofilm formation either poses a risk of biofouling for food industry or brings an advantage by enhancing the desired properties of microorganisms used as a starter or natural food preservatives. In this circumstance, the desired behaviours of microorganisms could be supported in biofilm matrix and also the prevention from pathogeny or/and spoilage properties of microorganisms might be provided, as well. However, when the biofilm is formed by pathogenic or spoilage microorganisms, the formation may cause cross contamination to the food during the processes [1].

Microorganisms adhere to the different food contact surfaces as aluminum, glass, stainless steel, Teflon and nylon structures. The attachment begins and forms greater on the hydrophilic surfaces than hydrophobic surfaces. Besides, the gene transfer efficiency from one to another strain and existence of functional surface proteins are observed higher for microorganisms in biofilm matrix than for planktonic cells, because microorganisms found in biofilm layer have different physiological properties in comparison to planktonic cells. Behaviour changes of strains lead to some results and the most important result is considered as the antimicrobial resistance of biofilm forming microorganisms. Slow penetration of antimicrobial agents through the biofilm layer, changes of chemical structure in the matrix and existence of persisting cells which are extremely tolerant to the adverse conditions could play an important role in failure the application of antimicrobial agents to biofilm layer. Biofilm formation occurs in three steps which are called early, intermediate and mature levels. In the early level of biofilm formation, antimicrobial agents can be still effective to inhibit microorganisms, however, the biofilm matrix becomes permanent when the maturation and the development of the matrix have been completed, consequently, antimicrobial agents remain too insufficient to affect and degrade the biofilm layer [2].

2. Biofilm formation and development

The term of biofilm have been defined as a heterogeneous, three-dimensional structure with channels allowing liquid and gas flow and the complex architecture is composed of various populations of microorganisms surrounded by an extracellular polymeric matrix that provides the attachment to the biotic and abiotic surfaces such as plastic, glass, skin, mucosa and etc. [2, 3]. Biofilm layer is formed in three steps which are known as attachment to the biotic/abiotic surface, maturation, and release. The active cells which are found out of the biofilm layer are named as sessile cells. The structural properties of the surface is the most important factor for attachment and binding abilities of microbial strains. The roughness, the charge of the surface and etc. affect the firmness properties of the biofilm layer [2].

Extracellular polymeric matrix is a complex mixture of proteins, nucleic acids, and exopolysaccharides. This matrix allows for adhesion to the surfaces, cell-to-cell communication, antimicrobial resistance and organisation of microbial structure [2]. It has been reported that the amyloid fibers are found naturally in biofilm matrix because the amyloid fibers promote to create an extracellular matrix that provides attachment to the surfaces and also biofilm formation.

Amyloid fibers are resistant to proteases and also denaturation by chemically or thermally and they are also organized protein aggregates which are found in association with some diseases such as Alzheimer and prion diseases. Amyloid fibers can be produced by *Escherichia coli* and *Salmonella* spp. as they form the biofilm layer [4].

3. Quorum sensing mechanism and biofilm formation

The interactions between the microbial groups have been observed for many years. Commensalism, mutualism, synergism, antagonism, parasitism, and competition are some of the interactions appeared between the microorganisms.

Some mechanisms of interactions have inhibitory effects on another microbial strain, whereas some mechanisms lead to enhance microbial growth. The concept of quorum sensing as a mechanism is a communication way for microorganisms to regulate population growth found in the medium [5].

Quorum sensing (QS) can be defined as cell to cell interactions which are mediated by diffusible chemical signal molecules called autoinducers (AIs). The signal molecules are produced and released into the medium by microorganisms and the extracellular concentration reaches a threshold level the signal molecules lead the change the behaviours of the microbial population because the transcriptional regulator is activated and the gene expression has been carried out by the regulators. The signal molecules affect the gene expression in the population as a whole. The virulence factors are also included in the changed gene expressions. The gene expressions are changed by the signalling and cooperative behaviours are observed among the microbial species [6,7,8]. The quorum sensing molecules and their expression ways vary according to the microbial diversity. The signal molecules and the molecule synthase genes are different in Gram positive and Gram negative bacteria cells [7]. The regulation and the response to the gene expression are correlated with the cell density which is known as the key factor in quorum sensing. Auto inducers have the same meaning in both G-(+) and G(-) bacteria, however, the phenotypes and genetic expressions are different from each other. In G(-) bacteria the autoinducer system is carried out by homoserine lactones (HSL) synthesizing. HSL are fatty acid derivatives and synthesized by LuxI and LuxR homologue and the complex quorum sensing mechanism is called as LuxI/LuxR systems. In G-(+) bacteria, the auto inducers are amino acids and secreted short peptides unlike the system observed in G(-) bacteria for quorum sensing. The quorum sensing response is observed after the phosphorylation of a response regulator protein [9]. In yeast strains, bicarbonate, acetaldehyde and ammonia are known cell to cell signalling molecules. Besides, farnesol, which is found in the biofilm matrix produced by *Pseudomonas aeruginosa*, is another signal molecule to provide the inhibition of *Candida albicans* [5]. The mechanism of quorum sensing is first discovered in bacterial cells. The fungal quorum sensing mechanism has been studied in recent years. The quorum sensing mechanism is an advantage for the adaptation of microbial communities to the rapid changes in environmental conditions. Farnesol is the most known molecule for communication in between fungi species but on the other hand, aromatic alcohols, tyrosol, dodecanol and γ -butyrolactone are other molecules which have been identified as mediators of QS processes in eukaryotic organisms. Furthermore, filamentous fungi have QS mechanism. The signal molecules found in the filamentous fungi such as *Aspergillus* and *Penicillium* species are secondary metabolites in general. Penicillin is one of a QS molecule as well as the secondary metabolite that regulates growth profile of *Aspergillus nidulans* [10]. The cell to cell communications could be also observed in the different genus of microorganisms such as bacteria-fungi, yeast-fungi interaction [11].

4. Biofilm phenotypes

Microscopic and molecular methods allow the examination of the changes in protein and gene expressions in the microorganisms during the incubation periods. The examination of the relevant protein molecules is performed in general by using matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF-MS). The reporter genes could be determined by using the green fluorescent protein (GFP) fusion reporter constructs. In previous studies, the different stages of biofilm development could be observed with suchlike methods. In a bacterial biofilm development, five different stages were determined during a 12-day incubation period. The initial and developing stages of the biofilm formation could be determined by using the microscopic and spectroscopic equipment [12,13,14].

5. The Advantages of Biofilm Formation in Food Industry

It has been known that the microbial biofilm formation has been associated with the virulence of pathogen microorganism in general. In this regard, the spoilage and/or pathogen microorganisms become more resistant to harsh conditions and also chemicals and drugs. In pathogeny, the biofilm formation has known and accepted as an undesired health risk. On the other hand, biofilm forming can be a desired property in some cases in the food industry. Raw materials have to be processed before they are put up for sale in bazaars and markets. According to the researches, biofilm formation is observed typically in the food industry, such as table olives which have been economical and nutritional sources for especially Mediterranean countries. Olive such a fruit containing oleuropein which has a bitter taste and also the fruit contains high oil content and sugar. The ratio of the components could change depending on the

maturity and variety of the olive. Olive fruits have to be processed after harvesting because the taste and aroma of under ripe fruit are out of demand of consumers. Alkaline oxidation, immersion in the brine are the examples of the treatments for obtaining table olives. During these treatments, olive mesocarp is a basis of nutrient source for microbial strains which induce the fermentation period. Biofilm formation has been observed on the skin of the olive fruit when they especially fermented naturally. The biofilm layer has been formed by various yeast and lactic acid bacteria which are found on the mesocarp inherently. Such bacteria and yeast strains act a role in the fermentation of olive and also they become a dominant flora on the fruit which prevents the olive from microbial spoilage originated by Gram negative bacteria. In this regard, the quality and safety of the table olive and also the taste and flavour of the last product have been determined by biofilm forming microorganisms found on the mesocarp of the fruit. Besides, biofilm forming ability is a desired property of fermented fruit products [2]. Another example of the beneficial effects of the biofilm formation is about the yeast strains used commonly in the food industry. The recent studies have been showed that some yeast species having biotechnological relevance such as *Saccharomyces cerevisiae* might regulate the QS type. In the QS mechanism of the yeast strains, aromatic alcohols are the most observed signal molecules and the regulation of the

QS mechanism can result in modification and improvement of industrial processes that performed by yeast species. The signalling network could be controlled by tryptophol and phenylethylalcohol in *S. cerevisiae* and the molecules are produced by *C. albicans* and *S. cerevisiae* respectively [10].

The microbial interactions have an importance for food industry. Fermentation and brewing and also cheese ripening are some fields microbial interactions have been observed. The wine production has been leaded by mixture of fungi, yeast and bacteria species from ripening of grapes in vineyards to wine bottling. The cell to cell communication has a key role to produce approvable last product of the wine. On the other hand, during the cheese ripening period mixed communities have been found in the fermentation medium as well. According to the previous studies, the microbial communication affects the growth of the microbial species in the medium. The growth mechanism has been observed in cheese ripening period, therefore it has been reported that the growth of some bacterial species, such as *Leucobacter* sp. or *Brevibacterium aurantiacum*, significantly relied on the presence of the yeast *Geotrichum candidum* [11].

In the food industry, especially lactic acid bacteria act a key role in fermented products, therefore the choice of resistant strains among the lactic acid bacteria has a big importance for prevention economic loss and also for promoting the fast growth and fermentation during the period. The resistant strains are of value in the fermentation process. Among the lactic acid bacteria, probiotic species are remarkable in food industry because of the benefits to human health. In this regard, the desired effects of probiotic microorganisms having the ability of biofilm formation are increased resistance to temperature, gastric pH and some mechanical forces and such properties could not be observed in their planktonic counterparts. Although the harmful effects of biofilm formation have been known and pointed out because it has been correlated with pathogeny in general, the importance of the lactic acid bacteria strains such as *Lactobacillus plantarum*, *Lactobacillus rhamnasus*, *Lactobacillus fermentum* and their ability of being in a biofilm matrix has become discoverable due to their technological and industrial significance [15].

In some natural environments, the quality of the water could be affected and improved by the microbial metabolism in biofilm matrix. In the biofilm layer, the toxic compounds are removed or/and degraded by the present microorganisms. Therefore, the size reduction has been performed due to the microbial metabolism in the biofilm matrix. The microbial reactions help the management of waste water. In the food industry, removal of the waste products have been carried out by some plants, however, the significant part of waste could not be removed or reused industrially. The waste products are accepted as pollutant constituents and their removal industrially might be provided by choice the sufficient strains to degrade, minimise and convert the waste products to the non-toxic, biodegradable components [16].

6. The Disadvantages of Biofilm Formation in Food Industry

Both natural and artificial environments, biofilm formation has been accepted as the biofouling agent and the biofouling can cause significant problems in the food and various industries. The biofilm formation is composed of complex series interdependent steps, however, the first step is known as microbial adherence to the biotic/abiotic surface has been recognized as a crucial step. In this circumstance, the adherence and growth of the pathogen and spoilage microorganisms should be prevented at the beginning of the formation of biofilm layer on the surfaces. Whether the responding to the adherence of the microorganisms to the surfaces is delayed, the biofilm layer covers the surfaces. At this step, the high resistance property has been observed against the antimicrobial agents [17]. The first scientific observation of biofilm formation has been determined in infections. In medicine, biofilm forming microorganisms have been a big threat to human health for many years [18,19]. Biofilm forming microorganisms play a crucial role in the infectious diseases such as endocarditis, otitis, urinary tract infection, Legionnaire's disease, cystic fibrosis and etc.

Most of the diseases can be a threat the human life because the infection related microorganisms have a potential to adhere to surfaces and form a biofilm in the several tissues and organs, hospital settings, and devices used for food products, and the food contact surfaces in the food industry. In most cases, the antimicrobial resistance of the infections has been correlated with the biofilm formation [20]. In this circumstance, the biofilm forming capacity has been correlated with the pathogeny or spoilage in medicine and food industry. Biofilm formation has three stage which are

the attachment of the cell to the surface, aggregation as a microcolony and the maturation phase which is the growth of the biofilm in the extracellular polymeric matrix. Biofilm formation is an important mode of the growth and possessing of pathogeny of microorganisms. Besides the formation of biofilm is the main reason of persistence of chronic infections caused by microorganisms. The most important thing in biofilm is the change of microbial behaviours. Some microorganisms become a pathogen in biofilm matrix. The resistance of the strains to the environmental changes, chemicals and also antimicrobials, antibiotics and other components which are used for prevention of the microbial diseases is the commonly known property in the industry [18,19]. Poor sanitation and hygiene in food plants cause food-borne diseases and outbreaks. The foodborne diseases appeared because of cross contaminations, hygiene problem and therefore the biofilm matrix formation on the surfaces of the devices found in the food plants. Especially the diseases have been correlated with *Listeria monocytogenes* and *Salmonella* in the food industry. Such diseases caused by microorganisms become more insistent if they form biofilm matrix. Food spoilage may result in essential economic losses [21]. Furthermore, the prevalence of biofilms produced by the major foodborne pathogens that can be listed as *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni* and etc. threatens human health with consumption of the contaminated food product. In food processing environments, several microbial species can colonise in the food or/and the food contact surfaces. The microorganisms can grow and some of them can form a biofilm layer in time. The biofilm development is a potential risk for consumer health and it has been reported that many outbreaks are associated with the consumption of fresh produce, such as lettuce, onions, spinach, and tomatoes. This could be explained that the surface colonization by one or more biofilm forming pathogen microorganisms [20]. The cross contamination is another risk factor for human health. The microorganisms in sufficient numbers found in the biofilm matrix might lead the infection. In this regard, *S.aureus*, another pathogen bacteria that causes foodborne disease, can be a potential risk for food cross-contamination too. The biofilm formation has been linked with gene expression of the microorganisms, such as for *S.aureus* several genes are involved in the production of staphylococcal biofilm formation. Most of the results of biofilm formation could be determined and understood with the researches however the regulatory mechanism of the biofilm formation has been poorly understood [22].

Microbial adhesion onto the mineral surfaces and the biofilm formation is an important step for an aggregate occurrence. Microorganisms lived in soil and the aggregate stability is correlated with the biofilm formation of the microorganisms found in the soil environment. The microorganism-mineral interactions are not understood completely.

The observation of the interaction between the microorganisms and the particles is carried out commonly by using the atomic force microscope (AFM) that allows the microbial examination [23]. The soil microorganisms have an importance for soil-grown raw food materials such as green vegetables that are used in the salads without heating treatment. In this regard, the biofilm formation poses risk in green vegetables for human health after consumption. The removal of the biofilm layer is difficult by washing the green vegetables. Because the microorganisms can be removed from the surfaces of the vegetables by washing process however once the biofilm formation develops initially, the inhibition and removal of the microorganisms found in the matrix get difficult. The microorganisms have a resistance against the antimicrobial agents and also adherence has been completed to the surface of the raw food, therefore washing under the tap water is inefficient for removal of the biofilm.

7. Microbial-Surface Interactions

The structure and properties of the cell surface regulate the adhesion of microorganisms and biofilm formation. The surface and cell charges are the most critical phenomena for adhesion. It has been known that the microorganism surfaces are charged negatively. The charge of the biotic/abiotic surfaces is important for adhesion of the microorganism. If a microorganism is negatively charged, the positively charged surfaces have been induced to attract to the microorganisms easily. The adhesion can be reversible and irreversible according to the firmness of the biofilm layer. If the adhesion firmer, the fimbriae, pili and capsule have been observed in the microbial cells that they strengthen the biofilm matrix [24].

8. The Measurement Methods of Microbial Adhesion

Microbial contaminations and microbial spoilages result in infections because microorganisms form biofilm layer on the devices used in medical and food industry and the removal of the biofilm layer included several microbial species is more difficult than the removal of planktonic cells. Such microbial infections become morbid and mortal as well as it is expensive during the recovery time of the patients. In many cases the device infections are caused by the adhesion of microorganisms to the tissues during the surgery in medical cases and also the adhesion could cause significant microbial spoilage that results in foodborne diseases. The simulations which allow the observation of the adhesion to the surface and the behaviours of the microbial cells in the biofilm layer and also the initial period of the attachment provide to determine the formation of biofilm. Therefore some prevention and removal methods for biofilm forming might be developed. AFM is used for suchlike purposes to define the microbial behaviours from the adhesion to the surface to the development of the biofilm layer [25]. AFM allows nanoscale observation directly on the surface

morphologies of a microbial aggregates and biofilm layer formed on the particles, minerals, and etc. found on the surfaces. Biofilm occurs after adhesion step and the adhesion is completed once, the energy is required to remove the cell from the surface. The microbial adhesion can be measured by biochemical, physicochemical, macroscopic and microscopic methods [24]. Microbial-mineral aggregates and biofilm formation could be observed by advanced techniques such as confocal laser scanning microscopy with fluorescent labelling, scanning transmission X-ray microscopy, magnetic resonance imaging, electron microscopy are some direct observing systems which are used in the researches of biofilm formation [23].

The microbial population found on the surface could be observed optical, laser scanning confocal and scanning electron microscopy. Besides, these observing methods can be combined with various antibodies, dyes and fluorescence. According to the macroscopic measurement methods, the contact angles could be observed and measured, therefore the surface energies and the potential of the adhesion event might be detected. The interaction force could be detected by atomic force microscope effectively [24]. The strength of biofilm depends on its rheological and viscoelastic properties. It has been known that the structure of the biofilm is changeable according to the members of the microcolony and the surface structure of the material. The cohesive structure of the biofilm layer is a result of the cell-to-cell interactions and the EPS production in the biofilm matrix. Cohesive and adhesive properties of the biofilm matrix affect the irreversible or reversible responses about the attachment of the surfaces [26].

9. Screening Methods of Microbial Biofilm Formation

Biofilm layer formation and its observation could be carried out by different methods. The methods are classified 4 groups which are known as tissue culture plate (TCP) method, tube method (TM), Congo Red Agar (CRA) method and modified CRA (MCRA) method [27,28].

Tissue culture plate method: Fresh culture of the test microorganism was diluted 1:100 into sterile suitable broth medium. Diluted culture was transferred to each well as 100 μ L and is incubated in 96-well microtiter plates at appropriate temperature and period for the microorganism. For control groups, only an individual broth medium should be found in the wells. All procedures are applied to control groups too. After incubation period total cell mass is measured as absorbance at 630 nm in a spectrophotometer. The plate is decanted and the wells are washed by submerging into the distilled water, followed by drying at 37 °C for 30-45 min. Dried plate is stained by addition 125 μ L of a 0.1% crystal violet solution (in deionized water) to each well of the microtiter plate. Stained microtiter plate is kept at room temperature for 20 minutes. Crystal violet has an ability to bind the adherent cells found at the bottom of the wells. After binding, the excess dye is decanted and the biofilm layer and dye complex could be resolubilized in the wells by 95% ethanol. The absorbance of soluble dye and biofilm layer complex is measured at 492 nm. The ratio of absorbance at 492 nm and 630 nm was named as “B” represents the level of biofilm formation [29].

The ratio was measured according to the formula: “ $B = A_{492}/A_{630}$ ”. Test microorganisms were categorized according to the scale values of B. The scale values were considered as [29];

$B < 0.1$ (non-biofilm producer),

$0.1 \leq B < 0.5$ (weak biofilm producer),

$0.5 \leq B < 1$ (moderate biofilm producer),

$B \geq 1.0$ (strong biofilm producer).

Tube method: The test microorganism is activated into the relevant broth medium and the fresh culture is inoculated as 100 μ L into the individual broth medium. After the test tubes are incubated at the proper temperature and period, medium is removed from the tubes and the tubes are washed with sterile phosphate buffer saline (pH: 7.3). The tubes are inverted and kept them at room temperature to dry inner sides of the tubes. The tubes are stained with crystal violet (0.1%), and excess stain is washed with deionized water. The tubes are dried in the inverted position. The scoring for tube method is performed according to the results of the negative control tubes including only individual broth medium.

The detection of biofilm forming ability of the test microorganism is carried out according to the occurrence of a visibly stained film layer at the bottom or the side walls of the tubes. The stained layer observation by naked eye means that the test microorganism could be accepted as biofilm positive strain. The amount of biofilm formed is scored as 0-none, 1-weak, 2-moderate, 3-high [30].

Congo red agar (CRA) method: Exopolysaccharide (EPS) production and therefore the biofilm formation is assessed by cultivating the strains on Congo Red Agar (CRA). EPS and/or biofilm formation is detected by variation in the colour of colonies grown on the CRA medium. The preparation of the medium is carried out by addition Congo red dye in a proper agar medium [22]. It has been reported that, amyloid fibers which can be produced by some microorganisms lead the biofilm formation and also the production of these proteins is correlated with pathogeny.

Congo red dye can bind to the amyloid fiber. In this circumstance the binding is observed with the stained colony formation on the agar medium. The biofilm positive strains can be detected by this method [4,31]. In this method the supplemented sucrose could be added in different concentrations without the addition any carbohydrate source into the medium to determine the influence of the concentration of sucrose on the phenotypic production of EPS and/or biofilm.

The microbial culture is streaked or spread on the CRA and incubated at the proper temperature and for a period. It has been reported that; a six-colour reference scale might be used for a fine classification of colony colours. The six-

colour tones of the scale are as follows: very black; black; and almost black, which were considered as positive results, and Bordeaux; red; and very red; considered as negative results [22,32].

Modified CRA (MCRA) method: In some cases, the slime and EPS production ability of the clinical isolates have been examined by modified CRA method. According to the method, the concentration of Congo red and saccharose is changed compared with Congo red agar method. In this method, alternative agar mediums are also used to determine the slime production. According to a research, the in vitro slime production ability of the strains on Congo red agar is detected by the colonies in black colour but pigmentation decreased with time. However, the constant pigmentation is observed when the strains grown on Modified Congo Red Agar (MCRA). The strong black pigmented colonies remain constant even after 4-6 days. A black pigmentation is accepted as biofilm positive in contrast with red pigmented colonies which are accepted as biofilm negative strains. Permanent intense black pigmentation has been occurred in the colonies with the reduction in the concentration of agar constituents over time. The permanent pigments are formed in the colonies which are included ica A and D genes. The modification of agar content provides the stability of black pigmentation of the colonies. The modification is an advantage to determination of the biofilm formation and also it can reduce the agar medium cost [33].

10. Conclusion

There are many advantages and disadvantages of biofilm formation in the food products. This situation can be changed according to the microbial genus, species and strains. Besides, the stress factors could influence the results and the responses of the microorganisms and therefore their behaviours. The biofilm formation in the food industry could be turned into an advantage in the microbial strains having great importance biotechnologically. The biofilm formation is an important research area for food microbiology field because of their changeable properties and genetic expressions.

Creating the new starter cultures having ability to produce biofilm might be the new strategy in the food industry.

Therefore, the beneficial microorganisms could compete with the pathogen microorganisms. The spoilage in food product could be retarded or prevented. In this regard, the biofilm forming beneficial strains could be used as shelf-life extender.

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