Review on Biofilm and Microbial Adhesion

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Abstract: Biofilms were observed in 1674 by Antonie Van Leuwenhoek in his primitive microscopic observation. Biofilm is defined as a structural community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface. Biofilm may form on living or nonliving surface and can be prevalent in natural, industrial and hospital settings. Biofilm development is considered to progress in five stages (Reversible attachment, irreversible attachment, maturation I, maturation II and dispersion). Biofilm formation is regulated by different genetic and environmental factors. Genetic studies show that bacterial motility, cell membrane proteins, extracellular polysaccharides and signaling molecules play a significant role in biofilm formation. On the other hand, different signals from environment such as nutrients, oxygen, temperature and pH take part in regulation of biofilm formation. Biofilms have negative and positive attributes in home and industries. The mechanism of resistance of biofilm towards antimicrobial therapy is not yet explained but on hypothesis it is due to delayed penetration, altered growth rate and other physiological changes. In elimination of biofilm, combinations of physical and chemical methods are needed. Finally further studies on mechanisms of their resistance towards therapy are recommended.

Key words: Adhesion • Antimicrobial Resistance • Biofilm • Bacteria • Polymeric Substance

INTRODUCTION

Biofilms were observed as early as 1674, when Antonie van Leuwenhoek used his primitive but effective microscope to describe aggregates of “animalcules” that he scraped from human tooth surfaces [1]. Biofilm represents a specific life form of microorganisms which provides not only efficient protection from negative outside influence, but also physically and chemically suitable micro-environment necessary for growth and survival [2].

A biofilm is any group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm extracellular polymeric substance, which is also referred to as slime (Although not everything described as slime is a biofilm), is a polymeric conglomeration generally composed of extracellular DNA, proteins and polysaccharides. Biofilms are produced by microorganisms and consist of a sticky rigid structure of polysaccharides and other organic contaminants [3].

Biofilms may form on living or nonliving surfaces and can be prevalent in natural, industrial and hospital settings [4]. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium. Microbes form a biofilm in response to many factors, which may include cellular recognition of specific or nonspecific attachment sites on a surface, nutritional cues, or in some cases, by exposure of planktonic cells to sub-inhibitory concentrations of antibiotics [5].

The adhesion process of bacteria to the surfaces includes interactions, such as van der Waals, Lewis acid-base, hydrophobic and electrostatic interactions. It has been reported that hydrophobic substrata favor bacterial adhesion and that the hydrophobic effect may be the primary driving force for the adhesion of most pathogens. Bacteria growing in a biofilm on a surface are generally more resistant to many antimicrobial agents than the same bacteria growing in a free-swimming (Planktonic) state. The resistant characteristic of biofilms leads to persistent infections in the human body, as well as to
troublesome biofilms in industrial processes. Biofilms including pathogenic bacteria growing inside the human body, e.g. in lungs or on implant surfaces [6] or in drinking-water distribution systems can threaten human health. In industrial processes biofilms cause malfunction of equipment, lower the efficiency of heat exchangers and lower the end-product quality or safety in food industry [7].

Biofilms are also associated with a number of medical diagnoses, including dental caries, gastric ulcers, implanted medical devices (Vascular catheters, urinary catheters and artificial joints), keratitis, kidney stones, meningitis, osteomyelitis, otitis media, pneumonia, sinusitis, tonsillitis, gallstones and chronic wound infections [8]. Dental plaque, S. pneumoniae and aggregate together as easily as motile bacteria [6].

Biofilm Development:

Biofilm development is considered the ability of microorganisms encased in biofilm communities to resist antimicrobial agents and biocides, but prevention and controlling biofilm formation by applying both physical and chemical methods at the earlier stage of the biofilms development can be possible [9].

The objectives of this seminar paper were therefore:

- To review on the definition, formation and development of biofilm.
- To provide an overview on the impact of biofilms on human economy and public health.
- To show the appropriate and cost effective methods of biofilm prevention.

Literature Review

Definition of Biofilm:

Microorganisms gather in masses, cling to various surfaces and capture available moisture and nutrients. The formation of these layers called biofilms is actually a universal Phenomenon. Biofilms are often cooperation associated among several microbial groups, such as bacteria, Fungi, algae and protozoa, as well as plants and animals [10].

Biofilms are defined as a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface. The basic ingredients of a biofilm are microbes, glycocalyx and surface. If one of these components is removed from the mix, a biofilm does not develop [11].

Biofilm Formation:

Biofilms can exist on all types of surfaces, such as plastic, metal, glass, soil particles, wood, medical implant materials, tissues and food products. Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. These first colonists adhere to the surface initially through weak, reversible adhesion via Vander Waals forces. If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili. Hydrophobicity also plays an important role in determining the ability of bacteria to form biofilms, as those with increased hydrophobicity have reduced repulsion between the extracellular matrix and the bacterium. Some species are not able to attach to a surface on their own but are sometimes able to anchor themselves to the matrix or directly to earlier colonists. Non motile bacteria cannot recognize the surface or aggregate together as easily as motile bacteria [6].

Biofilm Development:

Biofilm development is considered to progress in five stages: Reversible Attachment, Irreversible Attachment, Maturation I, Maturation II and Dispersal. Initial event in biofilm development is the interaction between planktonic bacteria and substrate surface. This phase is called reversible adsorption because some bacteria attach to the substrate surface only for a brief period and then detach from it. In reversible attachment, planktonic bacteria adhere to a surface. At this stage, gene expression has not been altered, so the bacteria can easily return to planktonic living. This phase lasts a few minutes [11].

In the second phase, irreversible attachment, bacteria adhere firmly to substrate surface and lose their mobility. Bacterial cells attach to each other and to the substrate surface and thus formation of bacterial micro colonies begins. This phase lasts two hours. Protein analysis of a first two phases in biofilm formation determined that there were significant differences in regulation of the large number of proteins, which showed that there is physiological difference between reversibly and irreversibly attached cells [7].

Maturation I is the third phase in biofilm formation. In this phase, matrixes of extracellular polysaccharide Substances (EPS) are produced. Micro colonies increase and become multi-layered and their thickness is up to 10µm. This phase lasts three days. In the next phase, maturation II, bacterial micro colonies grow to their maximum size and their thickness about 100 µm. This phase lasts six days. Studies of protein expression have shown a significant difference between maturation I and maturation II phases. It is assumed that changes in protein structure are directly correlated to phenotype adaptations of bacterial cells. Comparison of cells in
maturation II phase and planktonic cells has shown a significant difference in protein structure, which proves that there is a great physiological difference between biofilm bacteria and planktonic bacteria [12].

The last phase in biofilm development is dispersion. In this phase, microcolony structure changes since the bacterial cells situated in their central part regain their mobility and detach from the previously formed structure. Micro-colonies are therefore not mushroom-shaped or rod-like any longer, but adopt shell-like structure having an inner empty cavity and the wall consisting of immobile bacteria. The process dispersion probably takes place to allow bacterial cells better access to nutrients. During this phase, water channels form between micro-colonies. It lasts nine to twelve days [2].

**Factors Influencing Biofilm Formation and Development:** Biofilm formation is regulated by different genetic and environmental factors.

Genetic studies have shown that bacterial mobility, cell membrane proteins, extracellular polysaccharides and signaling molecules play significant roles in biofilm formation. Bacterial mobility is enabled by two types of growth on the cell surface, flagella and fimbriae. Flagella are long, spiral growths that enable bacteria to float in liquid medium and fimbriae are short, straight growths that enable limited, twitching movements of bacteria on substrate surface. Bacterial mobility enabled by flagella is necessary for establishing the connection between the bacteria and the surface, while the mobility enabled by fimbriae is necessary for the formation of microcolonies [2].

Initial interaction being established, stable connection between bacteria and substrate surface is maintained by specific cell membrane proteins, adhesions. If adhesion activity is inhibited, there is no biofilm formation, which was proved by studies carried out on *E. coli* and *Vibrio cholerae* [13]. Extracellular polysaccharide matrix (EPS) has a significant role in biofilm formation. Molecular genetic studies on *P. aeruginosa* showed that activation of genes necessary for extracellular polysaccharide synthesis took place after establishing stable connection between bacteria and substrate surface. Interactive communication via signaling molecules enables bacteria to organize into a community so that the biofilm functions as a multicellular organism [14]. Different signals from environment, such as availability of certain nutrients, presence of oxygen, temperature and pH, take part in regulation of a biofilm formation [15].

**Effect of PH:** Environmental pH is also important for biofilm formation, which was shown by studies carried out on *V. cholerae*. Optimal pH for multiplication of *V. cholerae* is 8.2 and if pH value is less than 7, that is if the solution is acid, the ability of these bacteria to form a biofilm is reduced due to the fact that bacteria cells lose their mobility. Unlike *V. cholerae*, bacteria *S. epidermidis* and *E. coli* do not need alkaline environment to multiply so that they can form a biofilm on urethral catheters where urine pH is acidic [2].

Large variations in external pH can overwhelm such mechanisms and have anbiocidal effect on the microorganisms. Bacteria respond to changes in internal and external pH by adjusting the activity and synthesis of proteins associated with many different cellular processes [16]. Studies have shown that a gradual increase in acidity increases the chances of cell survival in comparison to a sudden increase by rapid addition of HCl. This suggests that bacteria contain mechanisms in place which allow the
bacterial population to adapt to small environmental changes in pH. However, there are cellular processes which do not adapt to pH fluctuations so easily. One such process is the excretion of exopolymeric substances (Polysaccharides). Optimum pH for polysaccharide production depends on the individual species, but it is around pH 7 for most bacteria [17].

**Effect of Temperature:** The optimum temperature for a microorganism is associated with an increase in nutrient intake resulting in a rapid formation of biofilm. Nutrient metabolism is indirectly associated and dependent on the presence of enzymes. So it may be fair to say that the formation of a biofilm is dependent on the presence and reaction rates of enzymes, which control the development of many physiological and biochemical systems of bacteria. Optimum temperature results in the healthy growth of bacterial populations. Conversely, temperatures away from the optimum reduce bacterial growth efficiency. This is due to a reduction in inorganic enzyme reaction rates [18].

**Nutrient Effects on Biofilm Formation:** Bacteria monitor and respond to the types and amounts of nutrients in their environments. The nutritional status of the environment greatly affects the response of a bacterium to form a multilayer biofilm or to remain in suspension. The nutrient environment is most likely so influential in this response due to the energetic costs of joining and leaving the biofilm. It seems that biofilm formation fulfills different needs for different bacterial species depending on nutrient environments [8].

**Effect of Enzymes on Biofilms:** To gain more understanding on the chemistry of attachment the sensitivity of *Deinococcus geothermalis* biofilms towards enzymes that hydrolyze macromolecules expected to represent components of its biofilm matrix was investigated. Treatment with pronase a broad-spectrum protease from *Streptomyces griseus* for 2.5 hrs detached *D. geothermalis* biofilms from the surfaces of glass and polystyrene and polypropylene. When the buffer solutions were placed on TSA agar after this 2.5 hrs treatment, a higher number of cells grew out of the enzyme-buffer solution than of the buffer with no enzyme (The negative reference). Light microscopy showed that in the enzyme-buffer solution there were single cells rather than cell clusters. We interpreted these findings to mean that protease treatment released intact living cells from the biofilm matrix and that proteins are involved in the cell-to-cell attachment of *D. geothermalis* in biofilms [19].

**Effect of Oxygen:** Biofilm formation in *E. coli* is regulated by the presence of oxygen. In case of insufficient oxygen supply biofilm does not form, since bacteria cannot adhere to substrate surface [2].

**Process of Bacterial Adhesion:** Adhesion is the process by which microbes gain a more stable foothold at the portal of entry, often involves a specific interaction between the molecules on the microbial surface and the receptors on the host cell. The process of bacterial attachment to an available surface (Living or abiotic) and the subsequent development of a biofilm can be described in fairly simple or incredibly elaborate terms depending on the level of detail required or sought. Obviously, the process is dictated by a number of variables, including the species of bacteria, surface composition, environmental factors and essential gene products [10].

**Mechanisms of Bacterial Adhesion and Development:** Biofilm growth is governed by a number of physical, chemical and biological processes. Attachment of a cell to a substrate is termed as adhesion and cell-to-cell attachment is termed cohesion. It is the mechanisms behind these forms of attachment, which ultimately determine the adhesive and cohesive properties a biofilm will exhibit. The accumulation of microorganisms on a collecting surface described as a process of three stages: (1) adsorption, or the accumulation of an organism on a collector surface i.e. substrate (deposition); (2) attachment, or the consolidation of the interface between an organism and collector; (3) colonization, or growth and division of organisms on the collector’s surface [1].

**The Effect of Growth Media and Temperature on Microbial Adhesion:** Bacterial attachment to inert surfaces is influenced by the properties of both substratum and bacterial cell, such as charge, hydrophobicity, surface roughness, the presence of fimbriae, flagella and production of exopolysaccharides (EPS). The properties of the bacterial cells are affected by the environmental conditions (Temperature, pH or composition of the culture medium); hence, alterations in these conditions can affect the bacterial adhesion [6].
The adhesion assays of the five bacteria *Listeria monocytogenes* ATCC 19112, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Micrococcus luteus* ATCC 4698 and *Serratiamarcescens* ATCC 8100 under different conditions are displayed in Fig. 2. *L. monocytogenes* showed the highest adherence when cultured in TSYEA. When peptone agar was the culture medium, *L. monocytogenes* presented the lowest adhesion values; these results are in agreement with the data that showed that *L. monocytogenes* cells were better biofilm producers in rich nutrient media, whereas the decrease in concentration of nutritive compounds reduced their growth [20].

For *S. aureus*, *M. luteus* and *P. aeruginosa*, the adhesion was also higher in TSYEA, the richest medium studied. The growth medium that resulted in the lowest adhesion of *M. luteus* and *P. aeruginosa* was peptone agar while for *S. aureus* was the lactose agar. On the contrary, *S. marcescens* presented lower attachment to polystyrene surface when grown in TSYEA and higher in lactose agar [21].

The adhesive assay was performed with bacteria cultured in TSYEA medium. The bacterial suspensions in saline solution were transferred to the polystyrene surface and samples were withdrawn every hour during 6 hours, at 25°C. Regarding the effect of temperature shifts, a pattern behavior was observed only in TSYEA, where the adhesion decreased with the decrease of temperature; adhesion was higher at 35°C and lower at 4°C for most of the bacterial strains, except for *P. aeruginosa*, that presented an opposite behavior, showing higher adhesion at 4°C in all media studied. This temperature shift could induce a stress in the strains that could affect the adhesion [21].

**Biofilms at Home and in Industry:** There are a number of ways in which we use bacterial biofilms to our advantage, including water purification systems, biochemical compound production and toxic waste disposal. Biofilms have immense potential in bioremediation of hazardous waste sites, biofiltering municipal and industrial water and wastewater and forming biobarriers to protect soil and groundwater from contamination [22]. Biofilms are profoundly important forces in the development of terrestrial and aquatic environments. They dwell permanently in bedrocks and the Earth’s sediments, where they play an essential role in recycling elements, leaching minerals and soil formation. Biofilms associated with plant roots promote the mutual exchange of nutrients between microbes and roots [10].

However, biofilms are also common nuisances in industry and in healthcare, causing clogging in water pipelines, chronic disease in patients and food safety hazards. Biofilm are associated with a number of medical diagnoses, including dental caries, gastric ulcers, implanted medical devices (Vascular catheters, urinary catheters and artificial joints), keratitis, kidney stones, meningitis, osteomyelitis, otitis media, pneumonia, sinusitis, tonsillitis, Gallstones and chronic wound infections [23].

Fig. 2: Time course of bacterial adhesion to polystyrene
Source: Zeraik and Nitschke [21].
Biofilms in the Industry: Biofilms represent great benefits in biotechnology industries because of their self-immobilization with high concentration of biomass within EPS that provide the high resistance to toxic compounds, long term activity which all facilitate continuous process with the high stability [24].

Biofilm formation can be found in all types of microbes which can lead to serious hygiene problems, economical losses due to the food spoilage and equipment impairment. The biofilm is probably forms by single species or mixed species of microbes. If the biofilm formed by spoilage or pathogenic microorganisms in the food industry, it will create serious problems which can cause the cross contamination to the food. Microorganisms in biofilms are also able to catalyze chemical and biological reactions causing metal corrosion, reduce heat transfer efficiency of heat exchangers and pipelines. Biofilm formation in the pipe reduces the liquid flow rate, heat transmission efficiency and pipe corrosion in terms of acid production from the bacterial consortium in the biofilm [25].

Biofilm commonly contaminate industrial pipelines, food contact surfaces, floors when the inappropriate sanitizing has been applied in the industrial cleaning up since the biofilm can develop on various kinds of surface materials in the food industry biofilms cause serious engineering problems such as impeding the flow of heat across a surface, increases in fluid frictional resistance of surfaces and increases in the corrosion rate of surfaces leading to energy and production losses. Pathogenic micro flora grown on food surfaces and in processing environments can cross-contaminate and cause post-
processing contamination. If the microorganisms from food-contact surfaces are not completely removed, they can lead to mature biofilm formation and so increase the biotransfer potential. Examples of the food sectors that pay particular attention to the possibility of cross-contamination are the milk industry and the slaughter industry [26].

**Biofilms in the Home:** Humans have considerable use of microbial biofilms, primarily in the area of habitat remediation. Water treatment plants, waste water treatment plants and septic systems associated with private homes, remove pathogens and reduce the amount of organic matter in the water or waste water through the interaction with biofilms [27].

**Biofilms and Infectious Diseases:** Infectious processes in which biofilms have been implicated include common problems such as urinary tract infections, catheter infections, middle-ear-infections, formation of dental plaque, gingivitiscoating contact lenses and less common but more lethal processes such as endocarditis, infections in cystic fibrosis and infections of permanent indwelling devices such as joint prostheses and heart valves [28]. More recently it has been noted that bacterial biofilms may impair cutaneous wound healing and reduce topical antibacterial efficiency in healing or treating infected skin wounds [29].

**Dental Plaque:** Dental plaque is an oral biofilm that adheres to the teeth and consists of many species of both fungal and bacterial cells (Such as *Streptococcus mutans* and *Candida albicans*), salivary polymers and microbial extracellular products. The accumulation of microorganisms subjects the teeth and gingival tissues to high concentrations of bacterial metabolites which results in dental disease. The biofilm on the surface of teeth is frequently subjected to oxidative stress and acid stress [30].

**Streptococcus pneumoniae:** *Streptococcus pneumoniae* is the main cause of community-acquired pneumonia and meningitis in children and the elderly and of septicemia in HIV-infected persons. When *S. pneumoniae* grows in biofilms, genes are specifically expressed that respond to oxidative stress and induce competence [31]. Formation of a biofilm depends on competence stimulating peptide (CSP). CSP also functions as a quorum-sensing peptide. It not only induces biofilm formation, but also increases virulence in pneumonia and meningitis. It has been proposed that competence development and biofilm formation is an adaptation of *S. pneumoniae* to survive the defenses of the host [32].

**Legionellosis:** *Legionella* bacteria are known to grow under certain conditions in biofilms, in which they are protected against disinfectants. Workers in cooling towers, persons working in air conditioned rooms and people taking a shower exposed to *Legionella* by inhalation when the systems are not well designed, constructed, or maintained [33].

**Biofilm Resistance to Antimicrobial Agents:** It is difficult to eradicate bacterial biofilm which is therefore the cause of numerous chronic infections. Bacteria in a mature biofilm are more resistant to antimicrobials (Biocides and antibiotics) than freely swimming cells. Different mechanisms have been proposed to account for this increased resistance that is most likely multifactorial. The bacteria within the biofilm are 10–1000 times more resistant to antibiotics than planktonic cells, but the resistance mechanism is still unexplained. So far three hypotheses have been formulated in attempt to explain biofilm resistance to antibiotics [2].

The nature of biofilm structure and the physiological attributes of biofilm organisms confer an inherent resistance to antimicrobial agents, whether these antimicrobial agents are antibiotics, disinfectants, or germicides. Mechanisms responsible for resistance may be one or more of the following: (i) delayed penetration of the antimicrobial agent through the biofilm matrix, (ii) altered growth rate of biofilm organisms and (iii) other physiological changes due to the biofilm mode of growth [6].

**Delayed Penetration of the Antimicrobial Agent:** Antimicrobial molecules must diffuse through the biofilm matrix in order to inactivate the encased cells. The extracellularpolymeric substances constituting this matrix present a diffusion barrier for these molecules by influencing either the rate of transport of the molecule to the biofilm interior or the reaction of the Antimicrobial material with the matrix material [34].

**Altered Growth Rate of Biofilm Organisms:** Another proposed mechanism for biofilm resistance to antimicrobial agents is that biofilm-associated cells grow significantly more slowly than planktonic cells and, as a result; take up antimicrobial agents more slowly.
The slowest growing *Escherichia coli* cells (in biofilms) are the most resistant to cetrimide [35]. At growth rates higher than 0.3 per h, biofilm and planktonic cells were equally susceptible. Another study showed that *S. epidermidis* biofilm growth rates strongly influenced susceptibility: the faster the rate of cell growth, the more rapid the rate of inactivation by ciprofloxacin [36].

**Other Physiological Changes Due to Biofilm Mode of Growth:** Gram-negative bacteria respond to nutrient limitation and other environmental stresses by synthesizing sigma factors. In *E. coli*, those sigma factors that are under the control of the rpoΣ regulon regulate the transcription of genes whose products mitigate the effects of stress. The rpoSE *coli* biofilms had higher densities and a higher number of viable organisms. Since rpoS is activated during slow growth of this organism, it appears that conditions that elicit the slowing of bacterial growth, such as nutrient limitation or build-up of toxic metabolites, favor the formation of biofilms [37]. Nutrient limitation and increases in toxic metabolite concentrations might be particularly acute within the depths of established biofilms. Agar-entrapped *E. coli* cells are more resistant to an amino glycoside as oxygen tensions were decreased. This is due to lowered uptake of the antibiotic by the oxygen-starved cells [38].

**Biofilm Control Strategy:** Since biofilms create problems in various food industrial sectors such as brewing, dairy processing, fresh produce, poultry processing and red meat processing, thus many biofilm control strategies have been risen up. Biofilm mode of life leads to increased resistance to antimicrobial compounds which has made the elimination from food processing facility becomes more challenge. In order to provide the effective control of undesirable biofilm, the understanding of the type of microbial biofilm need to be known before performing the sanitation process. The formation of biofilm can be prior avoided by choosing the correct materials and performing the appropriate cleaning methods at the first place. Also, the equipment design should not contain any fault exist sanitation like dead spaces, crevices, corners, cracks, gaskets, valves and joints which are vulnerable area for biofilm accumulation [39].

In the elimination of biofilm, the combinations of physical and chemical methods need to be applied in the cleaning up process. The physical methods that have been applied include super high magnetic fields, mechanical grinding, ultrasound treatment, high pulsed electrical fields, brushing with high pressure with steam is one of the effective methods [40].

Chemicals such as chlorine, lauricidin, hydrogen peroxide, chlorinated alkaline detergent, acetic acid and iodine have been widely used for the industrial clean up [41]. However, the disinfectant resistances are found to be directly proportional to the thickness of 3-dimensional structure of biofilm and the resistance is lost as soon as the biofilm structure is disrupted. The inappropriate concentration of the disinfectants or ineffective cleaning is also found to develop more resistance of the biofilm against the cleaning agents [20].

Generally, disinfectants do not penetrate the biofilm matrix. Therefore, cleaning is the first step and the most important step to improve the sanitation of the processing equipment. Biosurfactant produced from the microbes was also found to impair biofilm forming abilities. Biosurfactant produced by *Lactococcus lactis* impaired biofilm formation on silicone rubber. Surfactant from *Bacillus subtilis* was found to disrupt biofilm without affecting cell growth and prevent biofilm formation of *Salmonella enteric* and *E. coli* [42].

The physical treatment prior the chemical treatment has been found to be the most effective since the detachment of the biofilm from the physical treatment make it more sensitive to the disinfectants or antimicrobial molecules like nisin, reuterin and pediocin have been reported on their abilities to control biofilm formation by *L. monocytogenes* [43].

Recently, biological treatment like the utilization of enzyme has been emerged as an alternative cleaning method as green chemicals. However, the use of biological control is not a cost effective method in comparison to the chemical used. As chemical disinfectants have been widely used to eliminate biofilms, the properties of the chemical have been concerned based on effectiveness, safety, easily apply, easily rinsed off from surfaces, leaving no toxic residues that can affect the health properties and sensory values of the final products. In the past, efficiencies of biological and chemical disinfectants were previously tested on planktonic (Free cell) rather than biofilm mode of growth. Biofilms have been reported to be 100-1000 times resistant to disinfectants [44].

The main strategy to prevent biofilm is to clean and disinfect regularly before bacteria attach firmly to surfaces. The cleaning in the short time interval would be highly recommended as the most effective method to eliminate the biofilm since the elimination would be performed at the earlier stage of the biofilm development in which the EPS is less and disinfectant is accessible to kill the microbes underneath the biofilm. Other attempts are to identify materials that do not promote or even
suppress biofilm formation. The coating, painting walls, ceiling and floor with antimicrobial agents have been applied. The impregnation of surface material with biocides or antimicrobials also plays an important role in minimizing the bacterial colonization or modifying the surface physicochemical properties [45]. Coating surfaces with silver is also found to inhibit biofilm formations [46]. Non-ionic and anionic surfactants were evaluated to prevent the bacterial adhesion on stainless steel and glass surfaces which gave more than 90% inhibition of adhesion [47].

CONCLUSIONS

Biofilm is a well-organized, cooperating community of microorganisms whose cells are attached to each other and to the surface of the host individual. Biofilms can exist on all types of surfaces, such as plastic, metal, glass, soil particles, wood, medical implant materials, tissues and food products. Biofilm development and microbial adhesion can be influenced by genetic and environmental factors. Biofilms have negative and positive attributes in home and industries. Bacteria in a mature biofilm are more resistant to antimicrobials (Biocides and antibiotics) than freely swimming cells. The main strategy to prevent biofilm is to clean and disinfect regularly before bacteria attach firmly to surfaces.

Based on the aforementioned conclusions, the following recommendations are forwarded;

- Professionals and workers should proceed their work on laboratory in a hygienic condition.
- Regular cleaning and disinfecting materials and vehicles are necessary for effective elimination of biofilm.
- Further research on mechanism for their resistance towards antimicrobial therapy is suggested.

REFERENCES


