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Review on Biofilm and Microbial Adhesion

Alebachew Tilahun, Sibehat Haddis, Ayichew Teshale and Tsehaye Hadush

School of Veterinary Medicine, Wolaita Sodo University, Ethiopia

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 (Reversibleattachment, 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 fromnegative outside influence, but also physically and chemically suitable micro-environment necessary for growthand survival [2].

A biofilmis 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, Lewisacid-base, hydrophobic and electrostaticinteractions. It has been reported that hydrophobicsubstrata favor bacterial adhesion and that the hydrophobic effectmay be the primary driving force for the adhesionof 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

Corresponding Author: Alebachew Tilahun, School of Veterinary Medicine, WolaitaSodo University, Ethiopia.

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 legionellosis are also occurred as a result of bacteria in the biofilm. Eradication of biofilms is highly difficult due to 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 methodsat the earlier stage of the biofilms development can be possible [9].

The objectives of this seminar paper weretherefore:

- 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 associatedamong several microbial groups, such as bacteria, Fungi, algae and protozoa, as well as plants and animals[10].

Biofilmisdefined as a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface.The basic ingredientsof 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 offreefloatingmicroorganisms to a surface. These first colonistsadhere to the surface initially through weak, reversible adhesionvia Vander Waals forces. If the colonists are notimmediately separated from the surface, they can anchor themselves morepermanently using cell adhesion structuressuch as pili.Hydrophobicityalso plays an importantrole in determining the ability of bacteria to formbiofilms, as those with increased hydrophobicity have reducedrepulsion between theextracellular 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: ReversibleAttachment, Irreversible Attachment, Maturation I, Maturation II and Dispersal. Initial event in biofilm development is 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 inregulation of the large number of proteins, which showedthat 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 increaseand become multi-layered and their thickness is up to 10µm. This phase lasts three days.In the next phase, maturation II, bacterial micro coloniesgrow to their maximum size and their thicknessis about 100 µm. This phase lasts six days. Studies of protein expression have shown a significantdifference between maturation I and maturation II phases. It is assumed that changes in protein structure aredirectly correlated to phenotype adaptations of bacterialcells. Comparison of cells in

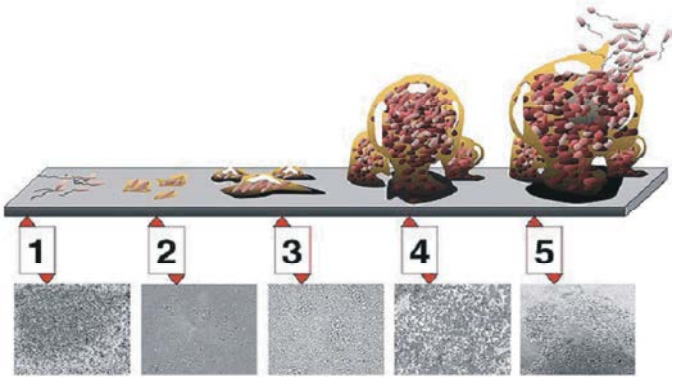

Fig. 1: Five Stages of Biofilm Development: (1) Initial attachment, (2) Irreversible attachment, (3) Maturation I, (4) Maturation IIand(5) Dispersion.Source:Maricand Vranes [2]

maturation II phase and planktoniccells has shown significant difference in proteinstructure, which proves that there is great physiologicaldifference between biofilm bacteria and planktonic bacteria [12].

The last phase in biofilm development is dispersion. In this phase, micro colony structure changes since thebacterial cells situated in their central part regain theirmobility and detach from the previously formed structure.Micro-colonies are therefore not mushroom-shapedor rod- like any longer, but adopt shell-like structure havingan inner empty cavity and the wall consisting of immobile bacteria. The process dispersion probably takesplace 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 protein growths 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 enabledby flagella is necessary for establishing the connectionbetween the bacteria and the surface, while the mobilityenabled by fimbriae is necessary for the formation ofmicrocolonies [2].

Initial interaction being established, stable connection between bacteria and substrate surface is maintainedby 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 availabilityof 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. cholere*. 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 variationsin external pH can overwhelm such mechanisms and have abiocidal effect on the microorganisms.Bacteria respond to changes in internal and external pH by adjusting the activity and synthesis of proteins associatedwith many different cellular processes [16]. Studieshave shown that a gradual increase in acidity increasesthe chances of cell survival in comparison to a suddenincrease by rapid addition of HCl. This suggests thatbacteria contain mechanisms in place which allow the

bacterialpopulation to adapt to small environmental changesin PH. However, there are cellular processes which do notadapt to pH fluctuations so easily. One such process is theexcretion of exopolymeric substances (Polysaccharides). Optimum pH for polysaccharide production depends onthe individual species, but it is around pH 7 for most bacteria [17].

Effect of Temperature: The optimum temperature for a microorganism is associatedwith an increase in nutrient intake resulting in arapid formation of biofilm. Nutrient metabolism isdirectly associated and dependent on the presence ofenzymes. So it may be fair to say that the formation of abiofilm is dependent on the presence and reaction ratesof enzymes, which control the development of many physiologicaland biochemical systems of bacteria. Optimum temperaturesresult in the healthy growth of bacterial populations.Conversely, temperatures away from the optimum reducebacterial growth efficiency. This is due to a reduction inbacterial enzyme reaction rates [18].

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

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

Effect of Oxygen: Biofilm formation in *E. coli* is regulated by the presence of oxygen. In case of insufficientoxygen supply biofilm does not form, since bacteriacannot 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 Bacterialadhesion and Development: Biofilm growth is governed by a number of physical, chemical and biological processes. Attachment of a cellto a substrate is termed as adhesion and cell-to-cell attachmentis termed cohesion. It is the mechanisms behind theseforms of attachment, which ultimately determine the adhesive and cohesive properties a biofilm will exhibit. Theaccumulation of microorganismson a collecting surface described as a process of three stages: (1) adsorption, or the accumulation of an organism on a collectorsurface i.e. substrate (deposition); (2) attachment, or the consolidation of the interface between an organismand a collector, often involving the formation of polymerbridges between the organism and collector; (3) colonization, or growth and division of organisms on the collector'ssurface [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].

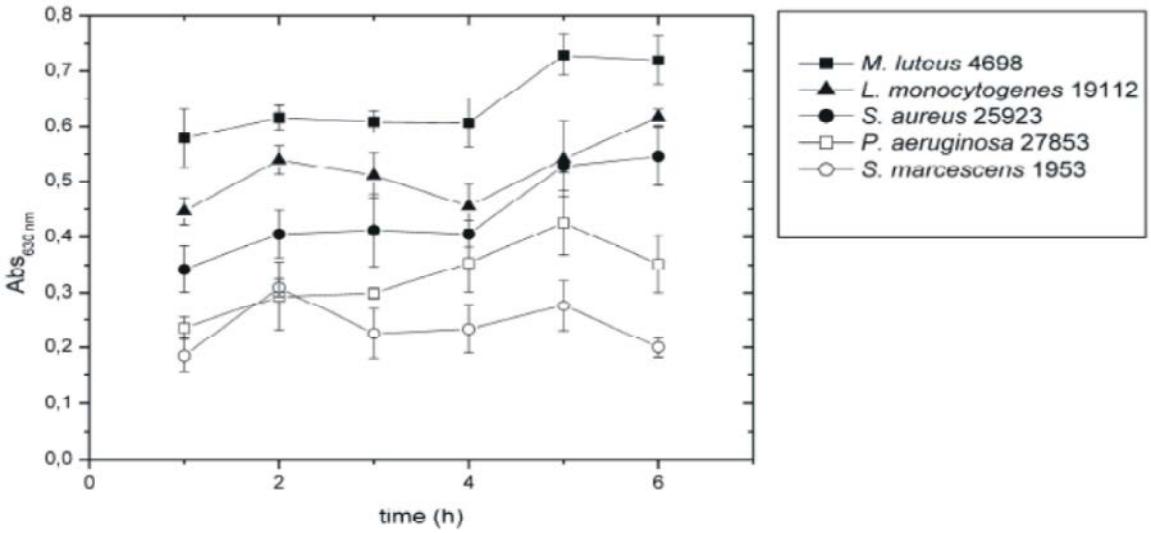

Fig. 2: Time course of bacterial adhesion to polystyrene
Source: Zeraik and Nitschke [21].

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 underdifferent conditions are displayed in Fig. 2. *L. monocytogenes* showed the highest adherencewhen cultured in TSYEA. When peptone agar wasthe culture medium, *L. monocytogenes* presentedthe lowest adhesion values; these results areinagreement with the data that showed that *L.monocytogenes* cells were better biofilm producers in rich nutrient media, whereas the decrease inconcentration of nutritive compounds reduced their growth [20].

For *S. aureus, M. luteus*and *P. aeruginosa,* theadhesion was also higher in TSYEA, the richestmedium studied. The growth medium that resultthe lowest adhesion of *M. luteus*and *P.aeruginosa* was peptone agar while for *S. aureus*itwas the lactose agar. On the contrary, *S. marcescens* presented lower attachment topolystyrene surface when grown in TSYEA andhigher 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 anopposite behavior, showing higher adhesion at 4°C in all media studied. This temperature shiftscould 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 wastedisposal. Biofilms have immense potential in bioremediation of hazardous waste sites, biofiltering municipal and industrial water and wastewater and forming biobarriers toprotect 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 inwater pipelines, chronic disease in patients and food safety hazards. Biofilm are associatedwith a number of medical diagnoses, including dental caries, gastric ulcers, implantedmedical devices (Vascular catheters, urinary catheters and artificial joints), keratitis, kidney stones, meningitis, osteomyelitis, otitis media, pneumonia, sinusitis, tonsillitis, Gallstones and chronic wound infections [23].

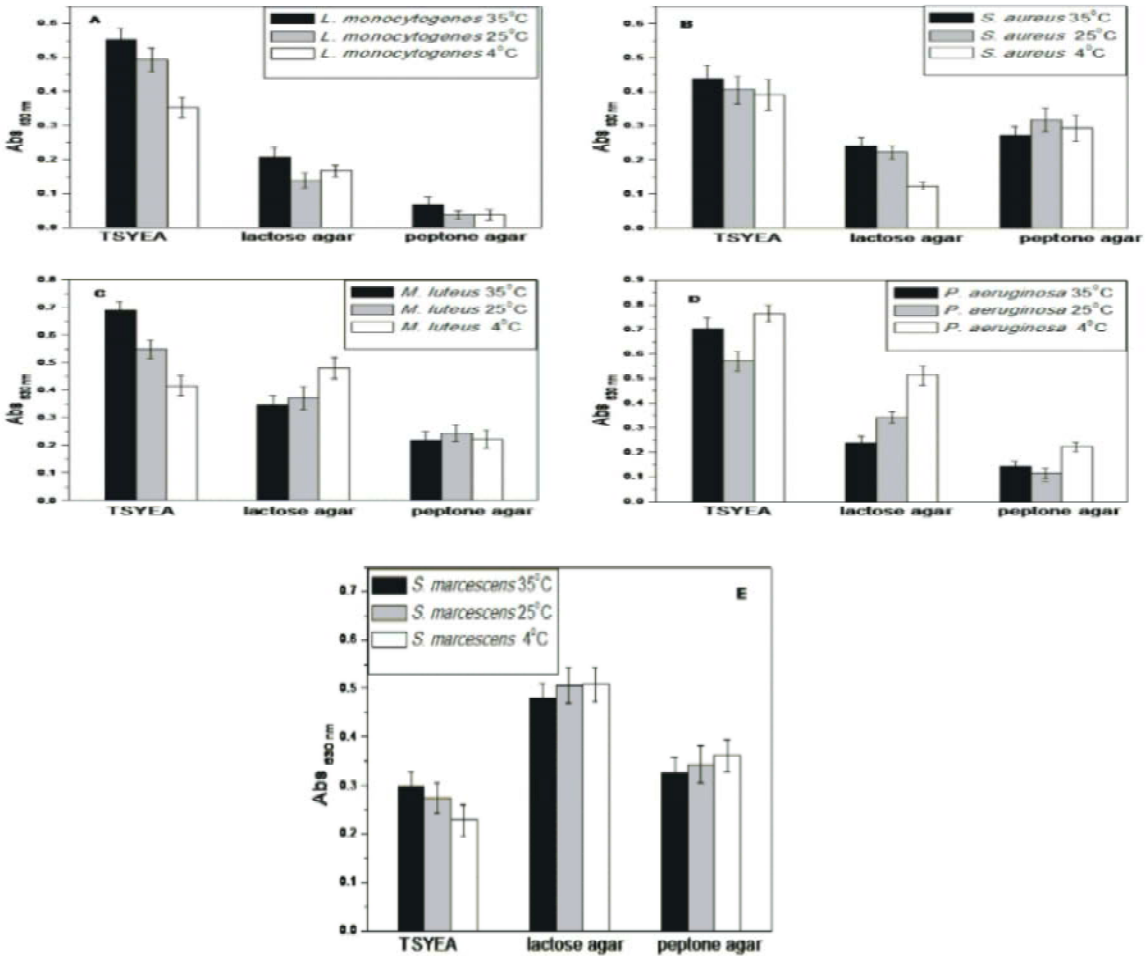

Fig. 3: Adhesion assays of bacterial suspensions of A) *Listeria monocytogenes*B) *Staphylococcus aureus*D) *Pseudomonas aeruginosa*C) *Micrococcus luteus*and E) *Serratiamarcescens*after cultivation on different growth media and adhesion temperatures.Source: Zeraikand Nitschke [21]

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 materialsin the food industry biofilms cause serious engineeringproblems such as impeding the flow of heat across a surface, increases in fluid frictional resistance of surfaces andincreases in the corrosion rate of surfaces leading to energyand production losses. Pathogenic micro flora grownon food surfaces and in processing environments cancross-contaminate and cause post-

processing contamination. If the microorganisms from food-contact surfacesare not completely removed, they can lead to mature biofilm formation and so increase the biotransfer potential.Examples of the food sectors that pay particular attentionto the possibility of cross-contamination are the milkindustryand 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 asurinary tract infections, catheter infections, middle-earinfections, formation of dental plaque, gingivitiscoating contact lenses and less common but morelethal processes such as endocarditis, infections in cysticfibrosis and infections of permanent indwelling devicessuch as joint prostheses and heart valves [28]. More recentlyit has been noted that bacterial biofilms may impaircutaneous wound healing and reduce topical antibacterialefficiency in healing or treating infected skin wounds [29].

Dental Plaque: Dental plaque is an oral biofilm that adheres to theteeth and consists of many species of both fungal andbacterial cells (Such as *Streptococcus mutans* and *Candidaalbicans*), salivary polymers and microbial extracellularproducts. The accumulation of microorganismssubjects the teeth and gingival tissues to high concentrationsof bacterial metabolites which results in dentaldisease. The biofilm on the surface of teeth is frequently subjectto oxidative stressand acid stress [30].

Streptococcuspneumoniae: *Streptococcus pneumoniaeis* the main cause of community-acquiredpneumonia and meningitis in children and the elderly and of septicemia in HIV-infected persons. When*S. pneumonia* grows in biofilms, genes are specificallyexpressed that respond to oxidative stress and induce competence [31]. Formation of a biofilm depends on competencestimulating peptide (CSP). CSP also functionsas a quorum-sensing peptide. It not only induces biofilmformation, but also increases virulence in pneumonia andmeningitis. It has been proposed that competence development andbiofilm formation is an adaptation of *S. pneumonia*tosurvive the defenses of the host[32].

Legionellosis: *Legionella* bacteria are known to grow under certain conditionsin biofilms, in which they are protected againstdisinfectants. Workers in cooling towers, persons workingin air conditioned rooms and people taking a showerare exposed to *Legionella* by inhalation when the systemsare 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. Thebacteria within the biofilm are 10–1000 times more resistant to antibiotics than planktonic cells, but theirresistance mechanism is still unexplained. So far three hypotheses have been formulated in attempt to explainbiofilm resistance to antibiotics [2].

The nature ofbiofilm 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 forresistance may be one or more of the following: (i) delayedPenetration of the antimicrobial agent through the biofilmmatrix, (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 matrixin 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 transportof the molecule to the biofilm interior or the reaction of theAntimicrobial material with the matrix material [34].

Altered Growth Rate of Biofilm Organisms: Another proposed mechanism for biofilm resistance to antimicrobialagents is that biofilm-associated cells grow significantly more slowly than planktonic cells and, as a result; take up antimicrobialagents more slowly.

The slowest growing*Escherichia coli* cells (In biofilms) arethe most resistant to cetrimide [35]. At growth rates higher than 0.3 per h, biofilm and planktoniccells 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 ofinactivation by ciprofloxacin [36].

Other Physiological Changes Due to Biofilm Mode of Growth: Gram-negative bacteria respond to nutrient limitation andother environmental stresses by synthesizing sigma factors. In *E. coli,* those sigma factors that are under the control of the *rpoS*regulon regulate the transcription of genes whose products mitigatethe effects of stress. The *rpoSE. coli* biofilms had higher densities and a higher number of viable organisms. Since *rpoS*is activated duringslow growth of this organism, it appears that conditions that elicitthe slowing of bacterial growth, such as nutrient limitation orbuild-up of toxic metabolites, favor the formation of biofilms [37]. Nutrient limitation and increases in toxic metabolite concentrationsmight be particularly acute within the depths of established biofilms. Agar-entrapped *E. coli* cellsare more resistant to an amino glycoside as oxygen tensions were decreased. This is due to lowereduptake 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 thecleaning 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 stream 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 resistanceof 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.

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