A Review on Biofilm Formation with Health and Industrial Impacts

Getinet Ayalew

Department of Animal and Range Science, Wolaita Sodo University, Dawro-Tarcha Campus, Tarcha, Ethiopia, B.O.B. 138.
quine2003@gmail.com

Abstract: Biofilms are assemblages of microbial cells formed by one or more species (bacteria, fungi, algae, and protozoa) that are irreversibly associated with a surface and enclosed in a matrix of primary polysaccharide materials that allow the growth and survival in sessile environments. Biofilm is formed when microbes adhere to surfaces in aqueous environments and (EPS) that can anchor the cells in all kinds of material such as metals, plastics, soil particles, medical implant materials, living tissues, industrial or potable waste-system piping or natural aquatic systems. Species of microbes forming Biofilm mainly characterized by a high degree of interaction between different types of organisms and by more or less immobilized form of life. Biofilm development is considered to progress in five stages (reversible attachment, irreversible attachment, maturation I, maturation II and dispersion). This is regulated by different genetic and environmental factors. Genetic studies show that bacterial motility, cell membrane proteins, extracellular polysaccharides and signaling molecules play 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.
[Getinet A. **A Review on Biofilm Formation with Health and Industrial Impacts.** *Biomedicine and Nursing* 2019;5(4): 1-13]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). http://www.nbmedicine.org. 1. doi:10.7537/marsbnj050419.01.

Key words: Biofilm, Bacteria, microbe, Adhesion, Polymeric substance, Antimicrobial resistance

1. Introduction

Biofilm is a community of microorganisms attached to substrate surface and submerged into extracellular slimy matrix (Chandra *et al*., 2001). Bacterial biofilm, as a sessile life form, ensures existence of bacterial life forms and it is a dominant phenotype in the nature over the free floating, planktonic form. Biofilm has positive effects in biotechnology, but it is extremely harmful in industry and in medicine. Biofilm causes numerous chronic infections, such as chronic osteomyelitis, chronic cystitis, chronic prostatitis, chronic otitis media (Roland, 2002), chronic pneumonia in patients with cystic fibrosis. In addition, biofilm also causes various infections of biomaterial used in medicine, such as infections associated with the use of intravascular and urethral catheters, infections of orthopedic devices, contact lenses, prosthetic heart valves, vocal cord prosthesis (Warren, 1997). 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 (Garrett *et al*., 2008). 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 (Maric and Vranes, 2007). 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, 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 (Rao *et al*., 2005). Biofilms may form on living or nonliving surfaces and can be prevalent in natural, industrial and hospital settings (Lear *et al*., 2012). 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 (Karatan and Watnick, 2009). 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 (Donlan and Rodney, 2002), or in drinking-water distribution systems can threaten human health. In industrial processes biofilms cause malfunction of equipments, lower the efficiency of heat exchangers, and lower the end-product quality or safety in food industry (Flemming, 2002). 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 (Price, 2012). 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 methods at the earlier stage of the biofilms development can be possible (Okuno, 1993). There the following points are the objectives of this review:

- To provide compiled information on development of biofilms.
- To provide an overview on the impact of biofilms.
- To show the appropriate methods of biofilm prevention.
- To Provide confined information to determine EPS compositions.

2. Biofilm and Microbes

2.1. Features of Biofilm

Biofilm was 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 (Costerton *et al*., 1999). 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 (Cowan and Talaro, 2006).

2.2. Structure of Biofilm

Basic structural units of a biofilm are microcolonies, separate communities of bacterial cells embedded into EPS matrix. These microcolonies are in most cases mushroom- shaped or rodlike and they can consist of one or more types of bacteria (Burns *et al.,* 1986*)*. Depending on bacteria type, microcolonies consist of 10–25% of cells and 79–90% of EPS matrix. EPS matrix protects biofilm cells from various negative environmental conditions, such as UV radiation, abrupt changes in pH values, draining. Between microcolonies, there are channels through which water flows. These water channels function in a biofilm as a simple circulatory system distributing nutrients to microcolonies and receiving harmful metabolites *(*Drring *et al*., 1986*)*. Biofilms contain mixed populations of bacteria, fungi, protozoa and if conditions allow, they can host even higher organisms in the food chain such as nematodes and larvae. All bacteria within a biofilm live together and depend on other microorganisms for energy, carbon and other nutrients (Prakash *et al.,* 2003). The extracellular matrix contributes to the mechanical stability of the biofilms enabling them to withstand shear forces. Biofilm formation occurs in response to a variety of environmental triggers including high cell density, nutrient deprivation and physical environmental stress (Li *et al.,* 2003). Biofilms are common form of microbial ecosystems associated with surfaces and they are found in an extremely varied environment, from pure water systems to stream beds. The EPS matrix is important both in the formation and structure of the biofilm and also protects the cells by preventing the access of the antimicrobial and xenobiotics to the cells in the biofilm and confers protection against environmental stresses such as UV radiation, pH shift, osmotic shock and desiccation (de Carvalho, 2007).

2.3. Typical Features of Biofilm

A distinguishing feature of biofilms from that of other colonizing infections is the presence of aggregated microcolonies of cells that are attached to a surfac (Dorn *et al.,* 2000) Importantly, biofilm formation as a protective mechanism could have profound implications for the host, because the microorganisms that are growing in these matrix-enclosed aggregates are more resistant to antibiotics and host defences. The biofilm model proposes that microbial cells growing in biofilms are clustered. It fundamentally challenges the assumption that infectious agents are evenly distributed and therefore equally susceptible to the host immune response or antibiotic therapy. It might further account for several problematic clinical challenges, such as symptomatic,

but unculturable, inflammation, antibiotic resistance, recurrence or persistence, and metastasis or the spread of infectious emboli. However, a problem with assessing the contribution of biofilms in human disease is the lack of defined criteria with which to characterize biofilm-induced pathogenesis. According to Parsek and Singh propose four criteria for defining a biofilm aetiology of an infection: the pathogenic bacteria are surface associated or adherent to a substratum; direct examination reveals bacteria in clusters, encased in a matrix of bacterial or host constituents; the infection is localized; and the infection is resistant to antibiotic therapy despite the antibiotic sensitivity of the constituent planktonic organisms (Dorn *et al*., 2000). The infections discussed in this review were chosen because they illustrate consistencies between biofilm growth in the environment and published literature investigating clinical infections. Owing to a great increase in the number of medical biofilm papers, however, space does not allow a comprehensive review of the medically relevant biofilms and the readers are referred to several reviews (McCoy and Costerton. 1982). Device-related infections were the first clinical infections to be identified as having a biofilm aetiology and show that biofilm formation can be facilitated by the host inflammatory response because host inflammatory molecules facilitate adhesion to the surface of the device. Bacterial endocarditis shows how microorganisms on the skin or in the oral cavity that transiently enter the bloodstream can colonize abnormal or implanted valves, or altered endothelial surfaces in the heart. Surface attachment within vegetations occurs as a result of interactions between microbial cells and host products (Dorn *et al*., 2000).

3. Biofilm Formation and Development

3.1. 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 reduce drepulsion 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 (Donlan and Rodney, 2002).

3.1.1. Microbial Taxonomic Diversity

Many different bacteria form biofilms, including gram-positive (e.g. *Bacillus* spp, *Listeria monocytogenes*, *Staphylococcus* spp, and lactic acid bacteria, including *Lactobacillus plantarum* and *Lactococcus lactis*) and gram-negative species (e.g. *Escherichia coli*, or *Pseudomonas aeruginosa*). Cyanobacteria also form biofilms in aquatic environments. Biofilms are formed by bacteria that colonize plants, e.g. *Pseudomonas putida*, *Pseudomonas fluorescens*, and related pseudomonads which are common plant-associated bacteria found on leaves, roots, and in the soil, and the majority of their natural isolates form biofilms. Several nitrogen-fixing symbionts of legumes such as *Rhizobium leguminosarum* and *Sinorhizobium meliloti* form biofilms on legume roots and other inert surfaces *(Danhorn and Fuqua, 2007).* Along with bacteria, biofilms are also generated by a range of eukaryotic organisms, including fungi e.g. Cryptococcus laurentii and microalgae. Among microalgae, one of the main progenitors of biofilms is diatoms, which colonise both fresh and marine environments worldwide (Aslam *et al*., 2012).

3.1.2. 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 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 (Cowan and Talaro, 2006).

3.1.3. Mechanisms of Bacterial Adhesion

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 a collector, often involving the formation of polymer bridges between the organism and collector; (3) colonization or growth and

division of organisms on the collector's surface (Garrett *et al.,* 2008).

3.1.4. Factors for 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 (Donlan and Rodney, 2002). The adhesion assays of the five bacteria *Listeria monocytogene* ATCC 19112, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Micrococcus luteus* ATCC 4698 and *Serratiamarcescens* ATCC 8100 under different conditions are displayed in *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 were 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 (Hood and Zotolla, 1995). For *S. aureus, M. luteus* and *P. aeruginosa,* the adhesion was also higher in TSYEA, the richest medium studied. The growth medium that result the lowest adhesion of *M. luteus* and *P.aeruginosa* was peptone agar while for *S. aureus* it was the lactose agar. On the contrary, *S.marcescens* presented lower attachment to polystyrene surface when grown in TSYEA and higher in lactose agar (Zeraik and Nitschke, 2012). 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 ºC. Source: (Zeraik and Nitschke, 2012). 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 35ºC and lower at 4ºC for most of the bacterial strains, except for *P. aeruginosa,* that presented an opposite behavior, showing higher adhesion at4ºC in all media studied. This temperature shifts could induce a stress in the strains that could affect the adhesion (Zeraik and Nitschke, 2012).

3.1.5. Floating Biofilms

As the criteria for the biofilm mode of growth are very broad, the environments suitable for microorganisms to colonize and establish biofilms are practically limitless. Biofilms may occur attached to a surface, suspended in fluid as flocs or exist as pellicles at air–liquid interfaces, also referred to as floating biofilms. In general, floating biofilms are 30–300 mm thick and common in both anthropogenic and natural aquatic environments (Jennings *et al*., 2003). Such films contain numerous microorganisms, some of which are harmful to humans like *E. coli*, *P. fluorescens*, *V. cholerae* and *Salmonella* spp. Disturbance of floating biofilms by mechanical or natural means may lead to the production of aerosols that allow transmission of biofilm-associated pathogens over considerable distances, until they are inhaled by susceptible persons (Jennings *et al*., 2003).

3.2. Biofilm Development

Biofilm development is considered to progress in five stages: Reversible Attachment, Irreversible Attachment, Maturation I, Maturation II and Dispersal. 1) ***Reversible Attachment****:* the 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 (Costerton *et al*., 1999). ***2) Irreversible attachment***: in this phase 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 (Flemming, 2002). 3) ***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 (Danese *et al.,* 2001). 4) ***Maturation II*** *phase*: bacterial micro colonies grow to their maximum size and their thickness is 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 significant difference in protein structure, which proves that there is great physiological difference between biofilm bacteria and planktonic bacteria (Danese *et al.,* 2001). 5) ***Dispersion Phase***: *is* the last phase in biofilm development. In this phase, micro colony 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 (Maric and Vranes, 2007). At some point, the biofilm reaches a critical mass and a dynamic equilibrium is reached at which the outermost layers of growth begin to generate planktonic organisms. These organisms are free to escape the biofilm and colonise other surfaces. Cells nearest the surface become inactive or die due to a lack of nutrients, decrease in pH, pO2 or an accumulation of toxic metabolic byproducts. The primary development, maturation and breakdown of a biofilm might be regulated at the level of population density dependent gene expression controlled by cell-to cell signaling molecules such as acylated homoserine lactones. Once fully matured, a logical cooperation and metabolic efficiency provides a form of functional communal coordination that mimics primitive eukaryotic tissues (Costerton *et al*., 1995).

3.3. Cell-To-Cell Communication in Biofilm

Cells are densely packed in biofilms, enabling the high accumulation of signaling molecules as well as metabolites and secretion products. The relationship between biofilm formation and cell-to-cell communication (Davies *et al.,* 1998). Cell-to-cell communication plays a role in the organization and differentiation of the cell in biofilms depending on conditions. Bacteria communicate using various chemical signal compounds.

Quorum sensing (QS): it is one type of communication used to recognize the population density of the same species is QS, which plays an important role in bacterial cell-to-cell communication. Microorganisms can use quorum sensing to coordinate their communal behavior such as biofilm formation, motility and production of EPS. Bacteria produce signal compounds, which accumulate with increasing cell density. After the concentration of the signal reaches a threshold level, it is recognized by receptor located in the cytoplasm or cell membrane and activates gene expression involved in signal production. This feed-forward auto regulation loop of QS genes promotes synchronization of the cell community in terms of the QS response. However, when grown at high cell density under optimal growth conditions, pathogenic bacteria collectively produce toxins. Gram-negative bacteria produce acyl-homoserine lactone and derivatives of S-adenosyl methionine (SAM) such as AI-2. Gram-positive bacteria use peptides called autoinducing peptides (AIPs) as signaling molecules, and actinomycete produces A-factor (Bassler and Losick, 2006).

3.4. Detachment & Dispersal of Cells

As the biofilm gets older, cells detach and disperse and colonise a new niche. This detachment can be due to various factors including, fluid dynamics and shear effects of the bulk fluid. Some bacteria are shed from the colony and some stop producing EPS and are released into the surrounding environment. Biofilm cells may be dispersed either by shedding of daughter cells from actively growing cells or detachment as a result of nutrient levels. The released microorganisms may be transported to new locations and restart the biofilm process (Prakash *et al.,* 2003). As the thickness of the EPS increases, anaerobic conditions develop within the biofilm. Because of the film thickness and the activity of anaerobic species, the film detaches and sloughs off from the surface of the substrate. Polysaccharides enzymes specific for EPS degradation for different organisms may be produced during different phases of biofilm growth and contribute to detachment. It has been suggested that the escape of *P. aeruginosa* cells from the biofilm matrix involved the action of an enzyme that digests alginate (Prakash *et al.,* 2003).

3.4.1. Biofilm Dispersal Strategies

Detachment can by external perturbations, such as increased fluid shear, by internal biofilm processes, such as endogenous enzymatic degradation, or by the release of EPS or surface-binding proteins. Detachment is normally viewed from the perspective of control (biofilm removal strategies), or the contamination of food and water production facilities or medical and dental devices (Hagberg *et al.,* 1986). Three distinct biofilm dispersal strategies can be identified: ‘swarming/seeding dispersal’, in which individual cells are released from a microcolony into the bulk fluid or the surrounding substratum; ‘clumping dispersal’, in which aggregates of cells are shed as clumps or emboli; and ‘surface dispersal’, in which biofilm structures move across surfaces (Hagberg *et al.,* 1986).

3.4.1.1. Swarming dispersal

After initial biofilm growth, the microcolonies differentiate to form an outer ‘wall’ of stationary bacteria, while the inner region of the microcolony ‘liquefies’, which allows motile cells (of planktonic phenotype) to ‘swim’ out of the microcolony, leaving a hollow mound. Liquification has been attributed to lysis of a subpopulation due to prophage-mediated cell death. The lysing population can be regarded as a third phenotype, whereas the remaining swarming cells might be a surviving, apoptosis-negative, ‘persister’ phenotype. Hollow microcolonies have been seen in *Staphylococcus epidermidis* growing on agar plates (P. Stewart, personal communication), and transmission

electron micrographs (TEM) indicate that the hollowing occurs through localized lysis. Bacteriophages have also been shown to reduce the viscosity of purified *P. aeruginosa*. The authors of this study concluded that this increased the transport of bacteriophage through the biofilm to enhance infection. However, it is possible that this phenomenon is also important in swarming/seeding dispersal. A similar phenomenon has been reported in other species, including the non-motile dental pathogen *Actinobacillus actinomycetemcomitans* (Kudo *et al.,* 1987).

3.4.1.2. Clumping dispersal

Whole aggregates are continually shed from the biofilm. The aggregates consist of biofilm cells that are surrounded by EPS and which might be more similar physiologically to the attached biofilm than to planktonic cells. The tendency to shed clumps containing hundreds of cells by *S. aureus*, a non-motile human pathogen, contrasts with the detachment pattern for *P. aeruginosa* biofilms, in which the loss of single cells and small clumps predominates (P.S. and S. Wilson, unpublished observations). Moreover, the antibiotic resistance of detached *S. aureus* clumps is similar to the resistance that is associated with attached biofilms (Kudo *et al.,* 1987).

3.4.1.3. Surface dispersal

Another strategy for biofilm dispersa is movement across surfaces. Although it is known that in some species single cells can actively move across surfaces through gliding or twitching motility, there is evidence that whole biofilms can also move across surfaces through shear-mediated transport. Migratory ripple structures travelling at velocities of up to 1 mm hour–1 have been reported in laboratory studies on *P. aeruginosa* and mixed-species biofilms, and similar structures have also been seen in natural biofilms. Rippling transport might have consequences in medicine. Ripple structures have been reported in biofilms in endotracheal tubes and it has been hypothesized that the flow of biofilms down the tubes is related to dissemination into the lungs and subsequent cases of ventilator-associated pneumonia (Lam *et al*., 1983).

3.5. Factors Affecting Biofilm 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 enabled by flagella is necessary for establishing the connection between the bacteria and the surface, while the mobilityen abled by fimbriae is necessary for the formation of micro colonies (Maric and Vranes, 2007). 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 *Vireocholera* (Watnick and Kolter, 1999). 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 (Pratt and Kolter, 1998). Different signals from environment, such as availability of certain nutrients, presence of oxygen, temperature and pH, take part in regulation of a biofilm formation (Davies and Gesse, 1995).

3.5.1. Nutrients

Biofilms can form under diverse nutrient concentrations, ranging from high to almost non-detectable. They are, however, more abundant, densely packed and thicker in environments with high nutrient levels (Prakash *et al.,* 2003). High nutrient concentrations promote the transition of bacterial cells from the planktonic to biofilm state while depletion of these nutrients has shown to cause detachment of biofilm cells from surfaces. In an open reticulating system, there are abundant nutrients derived from water particularly in cooling towers (Melo *et al.,* 1997).

3.5.2. Temperature

For many bacteria found in cooling water systems, the optimum temperature for maximum growth is about 40C (Melo *et al.,* 1997). At this temperature small changes in temperature are likely to produce substantial changes in biofilm growth because microbial activity is very sensitive to temperature. For instance, studies have shown that biofilm thickness of *Escherichia coli* increased by 80% by raising the temperature from 30^{O}C to 35^{O}C (Melo *et al.,* 1997).

3.5.3. Surface condition

The surface could be a dead or living tissue or any inert surface. The attachment of microorganisms to surfaces is a complex process with many variables affecting the outcome. Attachment will occur most readily on surfaces that are rougher, more hydrophobic and coated by surface conditioning films. Furthermore, growth requires complex developmental pathways that are regulated in response to

environmental and bacterial derived signals. Studies based on the effect of substratum were made and results found showed that the extent of microbial colonization appears to increase as the surface roughness increases (Prakash *et al.,* 2003). It has been demonstrated that the surface condition (e.g. whether rough or smooth) affects the ability of bacteria to adhere to a surface. A material surface exposed in an aqueous medium will inevitably become conditioned or coated by polymers from that medium, and the resulting chemical modification will affect the rate and extent of microbial attachment. Studies based on the films were made on surfaces exposed in seawater and results found showed that films were organic in nature and they formed within minutes of exposure and continued to grow for several hours (Prakash *et al.,* 2003).

3.5.4. Velocity, Turbulence and Hydrodynamics

The area from the surface where no turbulent flow is experienced is known as the boundary layer. Within this area, the flow velocity has been shown to be insufficient for biofilm removal. The area outside this layer is characterized by high levels of turbulent flow and has an influence on the attachment of cells to the surface. An increase in water flow velocity resulted in an increased bacterial number in biofilms. This is attributable to better mass transfer of growth limiting nutrients at the higher flow velocity of water (Lehtola *et al.,* 2006).

3.5.5. Enzymatic Effect

To gain more understanding on the chemistry of attachment we investigated the sensitivity of *Deinococcus geothermalis* biofilms towards enzymes that hydrolyze macromolecules expected to represent components of its biofilm matrix. Treatment with pronase a broad-spectrum protease from *Streptomyces griseus* for 2.5hrs 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 (Kolari, 2003).

3.5.6. 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 (Maric and Vranes, 2007).

4. Impact of Biofilms in Medicine & Industry

4.1. Positive Impact of Biofilm

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 waste water, and forming biobarriers to protect soil and ground water from contamination (Cunningham *et al.*, 2011). 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 (Cowan and Talaro, 2006).

4.1.1 Role of 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 (Sigth *et al*., 1991).

4.1.2. Role of. 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 (Verran and Jones, 2000).

4.1.3. Enzyme Activity in Sludge Flocs

The activated sludge process has long been employed to treat a wide variety of waste water (Yan *et al.,* 2008). It has been reported that a number of enzymes such as aminopeptidase, galactosidase, glucosidase, lipase and phosphatase and protease have been extracted from sludge. These enzymes found in sludge may originate from the effluent sewage, from the sludge itself or even as actively secreted extracellular enzymes (de Beer *et al.,* 1996; Watson et *al.,* 2004; Burgess *et al.,* 2008). Proteolytic, lipolytic and cellulolytic enzymes synthesized within bacterial cells are secreted into the extracellular environment and hydrolyse the absorbed macromolecules into small units that can be transported across the cell membrane and then metabolized (Watson *et al.,* 2004; Li and Yang, 2007). Protease, _- amylase and _ glucosidase play important role in the biological waste water treatment. In the bulk solution of activated sludge, the amount of extracellular enzymes is immobilized in flocs. How the extracellular enzymes distribute in sludge flocs determines the contact probability of enzymes with proteins or polysaccharides, hence

affecting the process treatment efficiency (Yu *et al.,* 2007).

4.2. Undesired Impact of Bofilm

However, biofilms are also common nuisances in industry and in health care, 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 (James *et al*., 2008).

4.2.1. Biofilms in the Industry

Biofilm formation can be found in all type 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 (Wong, 1998). 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 (Petrak *et al*., 1999).

4.2.2. Biofilms with Medicine 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, gingivitis coating 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 (Lewis, 2001). 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 (Davis *et al*., 2008).

4.2.2.1. Dental plaque

Dental plaque is an oral biofilm that adheres to the teeth and consists of many species of 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 subject to oxidative stress and acid stress (Marquis, 1995).

4.2.2.2. Streptococcus pneumonia

Streptococcus Pneumonia is the main cause of community-acquired pneumonia and meningitis in children and the elderly, and of septicemia in HIV-infected persons. When *S. pneumonia* grows in biofilms, genes are specifically expressed that respond to oxidative stress and induce competence (Oggioni *et al*., 2006). 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. pneumonia* to survive the defenses of the host (Michod *et al*., 2008).

4.2.2.3. 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 are exposed to *Legionella* by inhalation when the systems are not well designed, constructed, or maintained (Murga *et al*., 2001).

5. 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 multi factorial. The bacteria within the biofilm are 10–1000 times more resistant to antibiotics than planktonic cells, but their resistance mechanism is still unexplained. So far three hypotheses have been formulated in attempt to explain biofilm resistance to antibiotics (Maric and Vranes, 2007). 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 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 (Donlan and Rodney, 2002).

5.1. Delayed Penetration of Antimicrobial Agent

Antimicrobial molecules must diffuse through the biofilm matrixin order to inactivate the encased cells. The extracellular polymeric 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 (Suci *et al*., 1994).

5.2. Altered Growth Rate of Microorganisms

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 (Evans *et al*., 1990). 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 (DuGuid *et al*., 1990).

5.3. Physiological Changes Due to Biofilm

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 *rpoS* regulon regulate the transcription of genes whose products mitigate the effects of stress. The *rpoS. 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 (Adams and McLean, 1999). 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 (Tresse *et al*., 1995).

5.4. Enzyme Mediated Resistance

The resistance of biofilms resistance to antimicrobial agents can be due to enzymes transforming the bactericide to a non-toxic form. The phenomenon is usually investigated from the biodegradation point of view, i.e. the biodegradation of toxic pollutants (Gu, 2007). A host of aromatic, phenolic and other compounds, toxic to many bacteria can be degraded by certain bacteria (Cloete, 2003). Enzyme-mediated resistance mechanism includes heavy metal resistance and formaldehyde resistance. Mercury, antimony, nickel, cadmium, arsenate, cobalt, zinc, lead, tellurite, copper, chromate and silver are some of the compounds where biofilms are found to be resistance to due to enzymatic activity (Cloete, 2003; Bhaskar and Bhosle, 2005). Detoxification is usually by enzymatic reduction of the cation to the metal, whereas some heavy metal resistance genes are carried on plasmids, whilst others are chromosomal. The resistant phenotype is usually inducible by the presence of the heavy metal. Some heavy metals induce resistance to a broader spectrum of heavy metals. Arsenate, arsenite and antimony, for example, induce resistance to each other in *E. coli* (Cloete, 2003).

6. Control and Preventive Strategy

6.1. Control Measure of Biofilm Development

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 (Todhanakasem, 2013). In the elimination of biofilm, the combinations of physical and chemical methods need to be applied in the cleaning up process.

Physical methods: that have been applied include super high magnetic fields, mechanical grinding, ultrasound treatment, high pulsed electrical fields, brushing with high pressure is one of the effective methods (Qian, 1997).

Chemical Methods: uses chemicals such as chlorine, lauricidin, hydrogen peroxide, chlorinated alkaline detergent, acetic acid and iodine have been widely used for the industrial clean up (Carpentier and Cerf, 1993). 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 resistant of the biofilm against the cleaning agents (Hood and Zottolla, 1995). 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. Surfactin from *Bacillus subtilis* was found to disrupt biofilm without affecting cell growth and prevent biofilm formation of *Salmonella enteric* and *E. coli* (Rodrigues *et al*., 2004). 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 anti microbial molecules like nisin, reuterin and pediocin have been reported on their abilities to control biofilm formation by *L. monocytogenes* (Dufour *et al*., 2004).

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 (Gilbert *et al*., 2002). Five enzymes in the biofilm removal reactor (BRR) and among those enzymes was a combination of one protease and two carbohydrates, namely alpha - amylase and beta – glucanase and the enzymatic mixture was found to be effective in digesting slime layers produced by cultures of pure and mixed strains of bacteria Wiatr (1991).

6.2. Preventive Measures of Unwanted Biofilm Development

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 (Rosmaninho *et al*., 2007). Coating surfaces with silver also found to inhibit biofilm formations (Klueh *et al*., 2000). 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 (Meylheuc *et al.,* 2006).

7. Conclusion and Recommendation

Biofilm represents a well-organized, cooperating community of 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. Biofilms are characterized by surface attachment, structural heterogeneity; genetic diversity; complex community interactions and an extracellular matrix of polymeric substances. Biofilms deposit and adhere to all surfaces that are immersed in aqueous environments. A number of parameters including reactor type, substrate composition, substrate loading rate, hydraulic retention time, hydrodynamic shear force, culture temperature etc have been indicated to facilitate the production of biofilm EPS. EPS is highly hydrated and consists of a wide variety of materials including polysaccharides, proteins, nucleic acid, uronic acid and humic substances. Infections of catheters and other biomaterials used in medicine, makes the research on biofilm extremely important for medicine. It is estimated that 65% of all bacterial infections are caused by biofilm. Contemporary interdisciplinary research, based on genetic analyses, microscopic observations and studies of gene expression, has resulted in advanced knowledge of molecular and genetic basis of biofilm development and survival. It has also contributed to an increasing number of strategies for biofilm prevention and control. thus decreasing the potency of the biocides. The tolerance of biofilms to antimicrobials combined with their complex architecture and dynamic nature makes them quite difficult to measure, monitor and remove thus reduces the effectiveness of treatment strategies. Enzymes have been proven to be effective in the degradation of the biofilm EPS. For effective prevention and control of the negative of Biofilm it is worthwhile to apply the following fundamental measures as recommendation**:**

- Stimulate biofilm dispersion by breaking down polymers in extracellular matrix
- Develop new treatments for biofilm destruction
- Carryout further research on mechanisms that lead to increase

Acknowledgements

I Author acknowledge Department of Animal and Range Science.

Corresponding Author:
Dr. Getinet Ayalew
Department of Animal and Range Science
Wolaita Sodo University
Tarcha, Ethiopia, P.O. Box. 138.
Telephone: +251926096499
quine2003@gmail.com

References

1. Adams J. and McLean R. (1999): Impact of *rpoS* deletion on *Escherichia coli* biofilms. *Appl. Environ. Microbiol,* 65:4285–4287.
2. Bassler, B. L, Losick R. Bacterially speaking. Cell. 2006;125: 237–246.
3. Carpentier B. and Cerf O. (1993): Review Biofilms and their consequences, with particular reference to hygiene in the food industry. *J Appl Bacterial,*75: 499-511.
4. Carpentier, B. and Cerf, O. (1993) Biofilms and their consequencies with particular reference to hygiene in the food industry. *J. Appl. Bacteriol*. 75, 499- 511.
5. Chandra J, Kuhn D M, Mukherjee P K, Hoyer L L, Mccormick T, Ghannoumma 2001 Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. *J Bacteriol 183*: 5385–94.
6. Cloete, T.E. Westaard, D. and van Vuuren, S.J. (2003) Dynamics response of biofilm to pipe surface and fluid velocity. *J. Water. Sci. Technol*. 47, 57-59.
7. Costerton J., Stewart P., and Greenberg E. (1999): Review. Bacterial biofilms: *a common cause of persistent infections,*284: 1318-1322.
8. Costerton, J.W. and Lappin-Scott, H.M. (1995) Introduction to microbial biofilms, p. 1-11 in H.M. Lappin-Scott and J.W. Costerton Ed. Microbial biofilms, 1st Ed. Cambridge University Press. New York, N.Y.
9. Cowan M. and Talaro K. (2006): Microbiology A Systems Approach. *Avenue of the Americas New York*: McGraw-Hill Campanies, First Edition.
10. Cunningham A., Lennox J., and Ross R. (2011): *In* Biofilm: The Hyper textbook, 43.*http://www.hypertextbookshop.com/biofilmbook/v004/r003/> Copyright © Eds*/today.
11. Danese P., Pratt L., and Kolter R. (2001): Biofilm formation as a developmental process. *Method Enzymol*,336: 19-26.
12. Davies D.G. and Geese G. (1995): Regulation of the alginate biosynthesis gene in *Pseudomonas aeruginosa* during biofilm development in continuous culture. *Appl Environ Microbiol,*61: 860–7.
13. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science. 1998;280:295–298.
14. Davis S., Ricotti C., Cazzaniga A., Welsh E., Eaglstein W., and Mertz P. (2008): "Microscopic and physiologic evidence for biofilm-associated wound colonization in vivo". *Wound Repair and Regeneration,*16(1): 23–9.
15. de Beer, D. Flaharty, V.O. Thaveesri, J. Lensand, P. and Verstraete, W. (1996) Distrubustion of extracellular polysaccharides and flotation of anaerobic sludge. *J. Appl. Microbiol. Biotechnol.* 46, 197-201.
16. de Carvalho C.C.C.R. (2007) Biofilms: Recent developments on an old battle. *Recent Patents. Biotechnol.* 1, 49-57.
17. Donlan G. and Rodney M. (2002): Biofilms: Microbial Life on Surfaces. Emerging Infectious Diseases. 8(9): Pp. 881-890, Drinking water. *Annu Rev Microbiol,*54: 81-127.
18. Dorn BR, Burks JN, Seifert KN, Progulske-Fox A. Invasion of endothelial and epithelial cells by strains of Porphyromonas gingivalis. FEMS Microbiol Lett. 2000;187: 139-144.
19. Drring, G., Goldstein, W., Botzenhart, K., Kharazmi, A., H~iby, N., et al. 1986. Elastase from polymorphonuclear leukocytes~a regulatory enzyme in immune complex disease. *Clin. Exp. lmmunol.* 64:597-605.
20. Dufour M., Robin S., and Bremer P. (2004): Development of a laboratory scale clean-in-place system to test the effectiveness of natural antimicrobials against dairy biofilms. *J. Food Prot*,67(7):1438-1443.
21. DuGuid I., Evans E., Brown M., and Gilbert P. (1990): Growth rate- dependent killing by ciprofloxacin of biofilm-derived *Staphylococcus epidermidis;* evidence for cell-cycle dependency. *J. Antimicrob. Chemother,*30:791–802.
22. Evans D., Allison D., Brown M., and Gilbert P. (1990): Effect of growth-rate on resistance of gram-negative biofilms to cetrimide. *J. Antimicrob.Chemother,*26:473–478.
23. Flemming H. (2002): Mini-review. Biofouling in water systems – cases, causes and countermeasures. *Appl Microbial Biotechnol,*59: 629-640.
24. Garrett T., Bhakoo M., and Zhibing Z. (2008): Bacterial adhesion and biofilms on surfaces Progress in Natural Science,18: 1049–1056.
25. Gilbert P., Allison D., and McBain A. (2002): Biofilms *in vitro* and *in vivo*: do singular mechanisms imply cross-resistance? *J. Appl. Microbial*. 92Suppl: 98S-110S.

26. Hagberg, L., Lam, J., Svanborg-Ed6n, C., Co,'; terton, J. W. 1986. Interaction of a pye Jonephritogenic *Escherichia coli* strain with the tissue componentos f the mouse urinary tract. *J. Urol.* 136:165-72 53.
27. Hood S. and Zottola E. (1995): Biofilm in food processing. *Food Control*.6:9-18.
28. James G., Swogger E., Wolcott de Lancey R., Pulcini E., Secor P., Sestrich J., Costerton J., and Stewart P. (2008): Biofilms in chronic wounds. *Wound Repair Regen*,16: 37-44.
29. Jennings, S.S., Moran, A.P., and Carroll, C.V. (2003) Bioaerosols and biofilms. In *Biofilms in Medicine, Industry and Environmental Biotechnology*. Lens, P., Moran, A.P., Mahony, T., Stoodley, P., and O'Flaherty, V. (eds). London, UK: IWA Publishing, pp. 160–178.
30. Karatan E. and Watnick P. (2009): "Signals, regulatory networks, and materials that build and break bacterial Biofilm". *Microbiology and Molecular Biology Reviews,*73 (2): 310–47.
31. Klueh U., Wagner V., Kelly S., Johnson A., and Bryers J. (2000): Efficacy of silver coated fabric to prevent bacterial colonization and subsequent device-based biofilm formation. *J. Bio. Med. Mater. Res*,53(6):621-631.
32. Kolari M. (2003): Attachment Mechanisms and properties of bacterial biofilm on non living surfaces, pp 43, Helsinki, Finland.
33. Kudo, H., Cheng, K.-J., Costerton, J. W. 1987. Interactions between *Treponema bryantii* and cellulolytic bacteria in the *in vitro* degradation of straw cellulose. *Can. J. Microbiol.* 33:244-48.
34. Lam, J. S., Mutharia, L. M., Hancock, R. E. W., HOiby, N., Lam, K., et al. 1983. Immunogenicity of *Pseudomonasaeruginosa* outer membranea ntigens examined by crossed immunoelectrophoresis. *Infect. lmrnun.* 42:88-98.
35. Lear G. and Lewis K. (2012): *Microbial Biofilms: Current Research and Applications*. Caister Academic press. ISBN978-1-904455-96-97.
36. Lehtola, M.J. Laxander M. Miettinen, I.T. Hirvonen, T.V. and Martikainen, P. (2006) The effects of changing water flow velocity on the formation of biofils and water quality in pilot distribution system consisting of copper or polyethelene pipes. *Wat. Res*. 40, 2151-2160.
37. Lewis K. (2001): "Riddle of biofilm resistance". *Antimicrobial Agents and chemotherapy,* 45(4):999-1007.
38. Maric S. and Vranes J. (2007): Characteristics of Microbial Biofilms. Department of Biology, 'Josip Juraj Strossmayer' Osijek University Medical School Josipa Huttlera 4 31000 Osijek, Croatia. *Period boil,* 109(2):1-5.
39. Marquis R. (1995): "Oxygen metabolism, oxidative stress and acid-base physiology of dental plaque biofilms". *J. Ind. Microbiol,*15 (3): 198–207.
40. McCoy, W. F., Costerton, J. W. 1982. Growtho f *Spaerothilus natans* in a tubular reactor system. *Appl. Environ. Microbiol.* 43:1490-94.
41. Melo, L.F and Bott, T.R. (1997) Biofouling in water systems. *J. Exp. Therm. Fluid Sci*. 14, 375-381.
42. Meylheuc T., Renault M., and Bellon M. (2006). Adsorption of a biosurfactant on surfaces to enhance the disinfection of surfaces contaminated with *Listeria monocytogenes*. *Int. J. Food Microbiol,*109(1-2):71-78.
43. Michod R., Bernstein H., and Nedelcu A. (2008): "Adaptive value of sex in microbial pathogens". *Infect Genet. Evol,*8(3):267-85.
44. Murga R., Forster T., Brown E., Pruckler J., Fields B., and Donlan R. (2001): "Role of biofilms in the survival of *Legionella pneumophila* in a model potable water system". *Microbiology,*147 (11): 3121–6.
45. Oggioni R., Trappetti C., Kadioglu A., Cassone M., Iannelli F., Ricci S., Andrew P., Pozzi G. (2006): "Switch from planktonic to sessile life: a major event in pneumococcal pathogenesis". *Mol. Microbiol,*61 (5): 1196–210.
46. Okuno K., Tsuchiya K., Ano T., and Shoda M. (1993): Effect of super high magnetic field on the growth of *Escherichia coli* under various medium compositions and temperatures. *J. Ferment. Bioeng,*75:103-106.
47. Petrak T., Kalodera Z., and Novakovic P. (1999): Bacteriological comparison of parallel and counter flow water chilling of poultry meat. *Meat Sci,* 53:269–71.
48. Prakash, B. Veeregowda, B.M and Krishnappa, G. (2003) Biofilms: A survival strategy of bacteria. *J. Cur. Sci*. 85, 9-10.
49. Pratt L.A. and Kolter R. (1998): Genetic analysis of *escherichia coli* biofilm formation-roles of flagella, motility, chemo taxis and type Ipili. *Mol Microbiol,30*: 285–93.
50. Price R.E. (2012): The Effect of various Amino Acids as Nitrogen source on Biofilm Formation of *Aeromonas* species. MSc Thesis, Texas Tech University.
51. Qian Z., Sagers R.D., and Pitt W. (1997): The effect of ultrasonic frequency upon enhanced killing of *Pseudomonas aeruginosa* biofilms. *Ann. Biomed. Eng*,25:69-76.

52. Rao V., Ghei R., and Yildiz C. (2005): Biofilm Research-Implications to Biosafety and Public Health, 10(2):83-90.
53. Rodrigues L., Vander Mei H., Teixeira J., and Oliveira R. (2004): Biosurfactant from *Lactococcus lactis* inhibits microbial adhesion on silicone rubber." *Appl. Microbiology. Biotechnol*, 66(3):306-311.
54. Roland P S 2002 Chronic suppurative otitis media: a clinical overview. *Ear Nose Throat J 81*: 8–10.
55. Rosmaninho R., Nylander S., Paulsson O., Muller S., and Melo L. (2007): Modified stainless steel surfaces targeted to reduce fouling evaluation of fouling by milk components. *J. Food Eng*,80:1176-1187.
56. Singh T., Purohit S., and Parihar P. (1991): Soil Microbiology. Third Edit. Student Edition Behind Nasarani Cinema Chopasani Road, Jodhpur 342002, India, Pp.123.
57. Stepanovic S., Cirkovic I., and Mijac V. (2003): Influence of the incubation temperature, atmosphere and dynamic conditions on biofilm formation by Salmonella spp. *Food Microbial,* 20:339–43.
58. Suci P., Mittelman M., Yu F., and Geesey G. (1994): Investigation of ciprofloxacin penetration into *Pseudomonas aeruginosa* biofilms. *Antimicrobial Agents Chemother,*38:2125–2133.
59. Todhanakasem T. (2013): Microbial Biofilm in the Industry, *African Journal of Microbiology Research*. 7(17): 1-5, Assumption University, Bangkok, Thailand.
60. Tresse O., Jouenne T., and Junter G. (1995): The role of oxygen limitation in the resistance of agar-entrapped, sessile-like *Escherichia coli* to amino glycoside and lactam antibiotics. *J. Antimicrob*. *Chemother,*36: 521–526.
61. Verran J., Jones M. (2000): Industrial biofouling. New York: John Wiley and Sons Ltd.
62. Watnick P., Kolter R. (1999): Steps in the development of *Vibreo cholera* or biofilm. *Mol Microbiology,34*: 586–95.
63. Watson, S.D. Akhurst, T. Whiteley, C.G. Rose, P.D. and Pletschke, B.I. (2004) Primary sludge floc degradation is accelerated under biosulphidogenic conditions. *Enzy. Microb. Technol*. 34, 595-602.
64. Wiatr, C.L. (1991) Application of multiple enzymes blend to control industrial slime on equipments surfaces. United States Patent, Patent No. 5,071,765.
65. Wong A. (1998): Biofilms in food processing environments. *J. Dairy Sci,81(10):*2765-2770.
66. Yan, S. Miyanaga, K. Xing, X.H. and Tanji, Y. (2008) Succession of bacterial community and enzymatic activities of activated sludge by heat treatment for reduction of excess sludge. *J. Bioch. Eng*. 39, 598-603.
67. Yu, G.H. He, P.J. and Shao, L.M. (2007) Enzyme activity in activated sludge flocs. *Appl. Microbiol. Biotechnol*. 77, 605-612.
68. Zeraik A. and Nitschke M. (2012): Influence of Growth media and Temperature on Bacterial Adhesion to Polystyrene Surfaces,55 (4): 569-576, Brazil.

10/7/2019