# Bacterial Biofilm

**Ahmed Kareem Ramadhan,**

Medical Department, Akre Technical Institute, Duhok Polytechnic University, Duhok, Iraq, ahmed.kareem@dpu.edu.krd



## ABSTRACT

Bacterial biofilms are communities consist of single or multi-species bacteria that cooperate and interact with each other in different ways and with the surrounding environment, and perform complex processes, including the intercellular communication to affect the metabolic activities of each other by production of materials, trapping of nutrients, horizontal transmission of genetic material, in order to support each other biologically and physically to resist harsh environmental threats. The acquired characteristics by the bacteria after biofilm formation, including avoiding host immunity defenses, antibiotic therapies even with high doses, and biocides, make them a major health problem, as they are an important cause of medical-device related infection, like in case of prosthetic valves replacement, eye contact lenses and catheters. Recent studies are focusing on further discovering the mechanisms biofilms use to resist treatments to can find alternative effective ways to end their health risks.

**KEY WORDS:** biofilm structure; resisting antibiotics; nosocomial infections; water treatment; waste water.

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## INTRODUCTION

Prokaryotes are almost everywhere in the nature, as they are able to adapt to different conditions, adhere to various surfaces and create communities. As soon as they create a community, bacterial cells make cooperation, interact and compete each other, additional to performing complex processes. The bacteria present as a biofilm, which could be only one species or more than one. They are wide spread and can be isolated from the nature, clinical and industrial sources. For example, dental plaque, which is well studied, carry about 700 different species embedded in EPS (Extra Cellular polymeric secretions) (Chadha, 2014) Biofilms, which are a group of bacterial cells surrounded by a matrix of extracellular polymeric chemicals adhered to a suitable surface. Presence of bacterial cells in a biofilm give them the ability to overcome harsh conditions and also the biofilms formation has a significant role in pathogenesis process in humans and animals. In addition to its role in protection of bacteria from desiccation, predation, and effect of antibacterial chemicals, and also grant survival positions for both opportunistic and beneficial pathogenic bacteria. (Bogino *et al.*, 2013)

This article provides some details about bacterial biofilms as a protective structure from unfavorable environmental conditions and antimicrobial materials, a brief history about biofilms, their characteristics and their formation process, and also antimicrobial resistance mechanisms, their role in nosocomial infections, some novel ways in to avoid biofilms formation and finally some information about beneficial biofilms.

## HISTORY

The formation of biofilm appears in fossils record of about 3.25 billion years ago and this is common in a diverse range of microorganisms in both the Archaea and Bacteria lineages, including living fossils in the most deeply dividing branches of the phylogenetic tree. It is a clue that formation of biofilm is an original and integral part of life cycle of the prokaryotes, and is an important factor to help organisms to survivel in diverse environments. (Stoodley., 2004). Anthony van Leeuwenhoek, the Dutch scientist, before more than three centuries, used a simple microscope to inspect materials he took from his teeth. He found moving objects which were not observable by the naked eye. He named these moving objects animalcules, as he thought that they were very small living animals. In his report to the British Royal Society, he mentioned that he believes these animalcules on the teeth plaque of one man outnumber the population of the United Kingdom. This event made Leeuwenhoek the first who discovered and wrote about biofilm. After about 200 years, an American bacteriologist, Arthur Henrici found an algae growth in his aquarium in the laboratory. He put some slides of microscope in the aquarium, believing to observe algae growth on the microscopic slides. The surprising thing was that in addition to the growth of algae, a very thin and uniform layer of bacteria of different forms, some of strange shape. Henrici wrote in 1933 about his observation by saying that bacteria in the water are not floating freely, but the growth on submerged surfaces could be benthos not only plankton. After few years, two scientists Heller and Heukelekian, were conducting a study about the aerobic and anaerobic technology of sewage solids decomposition, they observed that Surfaces help bacteria to progress in substrates otherwise it is very diluted for enhancing its growth. Development happens as slime of bacteria or growth of colonies adhered to surfaces. During 10 years, the pioneer microbial ecologist Claude ZoBell contributed significantly to microbiology of biofilm field. He discovered during the collection of seawater in sterile glass bottles, that the number of bacteria found on the glass surface in the seawater, were more than the sessile bacteria growth. (Dufour, Leung and Lévesque, 2012)

**Characteristics of Bacterial Biofilms**

The first described evidence for the existence of Bacterial biofilms was in 1970s, in that era several strains of staphylococci with coagulase negative were discovered, which were described as they produce mucoid materials on shunts of cerebrospinal fluid. Observation of some rounded cells by using of scanning electron microscopy (SEM) on peace maker leads, catheters of peritoneal dialysis and intravascular catheters, which were later found as *Staphylococcus epidermidis*, which were forming cluster of cells surrounded by amorphous substance which was at the beginning names as 'slime' , which was a material identified with their presence with other microorganisms like S. aureus. More studies conducted which used molecular methods to aid in founding the locus which is control the creation of biofilm in Gram positive cocci (which is known as ica locus).(Arciola *et al.*, 2012) (Büttner, Mack and Rohde, 2015)

The structure of the slimy substance composed of extra cellular substance matrix carrying bacterial microcolonies which develop in well-organized communities that have functional heterogeneity. Biofilms can be formed by opportunistic or beneficial pathogens, and relying of many factors including the strain, species and also environmental conditions, the compositions could be different. Which has a significant role in pathogenesis of pathogenic bacteria which infectious diseases in both animals and plants. The bacteria within biofilm community get advantages of this situation including resisting unfavorable and harsh environmental conditions and protection against predation, dehydration, phagocytosis, antibiotics or disinfectants exposure, and also other constituents of innate and acquired immunity and host's inflammatory system.

The biofilm consist of several different materials which have different chemical structures, including proteins, teichoic acids exopolysaccharides, and extracellular DNA containing channels which have role in circulation of nutrient. The mentioned structure shows biofilm as well organized structure, in which differentiated cells are recognized in the matrix having their own pattern of expression of gene and conducting complex metabolic activities, which is the cause of its comparison with tissues of more complex higher organisms. The biofilm characteristics will rely on the tissue present and the condition surrounding the structure. There is a possibility of their formation on animal tissues , like mouth, heart, and also surfaces of the plant, rather than dead tissue, like dead bone, medical instruments and equipment, and inert surfaces.biofilm formation in tissues of an animal could stimulate the humoral immunity of the host, leading to production of antibodies and molecules responsible for defense which have no ability to go through the formed exopolysaccharide structure. Which is exactly the same process which occur with antibiotics and other chemotherapeutics, the situation where biofilm and the cells present inside it are protected, while the cells which are not present in the biofilm are sensitive to antibiotics. The mentioned components of bacteria have crucial role in maturation, establishment, and dispersal of biofilms. Bacteria have the ability to form biofilms in various environments where the bacterial cells are able to adhere to a biological or inert surface, as a response to environmental signals that enhance or progress the process.(Arciola *et al.*, 2012) (Bogino *et al.*, 2013) (Costerton, Stewart and Greenberg, 1999).

## Biofilm formation

Biofilm development has four stages which have several specific functional needs during this process, such as highly specialized factors expression which are characterized by various profiles related to their biological functions. The stages include (1) bacterial cells attachment, (2) accumulation and aggregation of multiple layers formed by bacterial cells, (3) maturation of the biofilm, (4) bacterial cells detachment as single or groups into planktonic condition to start a new biofilm structure in another position. During the first stage, the bacteria start to attach to the suitable surface and depend on hydrophobicity character of cells and the biomaterial surface, the adherence is reversible to inert or natural surface, the colonization occur some forces like hydrophobic and electrostatic forces which are physiochemical forces. Several studies A number of studies conducted on the process of infection from medical devices, they found out that there is a protein expressed by bacteria which interact with the extracellular matrix materials of the host, which form a strong connection. Specific molecules of autolysin have role in this stage, such as AtlA and AtlE, were descovered in staphylococci, which are a Gram-positive bacteria doing adhesive and enzymatic functions which degraded the cell wall of bacteria and were connected to polystyrene which is unmodified. In plants tissues, this stage have specific characteristics relying on the surface, creating significant specificity for their own host in several cases, while others work on different types of surfaces. The next stage is known by that the bacterial cells aggregate and accumulate in multiple layers. It is a very active process in which biofilm is formed step by step as bacterial cells surface molecules recognize the molecules in the matrix which are responsible for adhesion process, enhancing the connection between them and the production of a matrix. There are other factors which help adhesion and act as glue, like the adhesion formed by polysaccharide between bacterial cells. Which its production is controlled by an intercellular adhesion (icaADBC) locus, which was firstly recognized and studied in a bacteria called *Staphylococcus epidermidis*. Additional to that, there are another structures and molecules needed for aggregation of bacterial cells into micro colonies, including flagella and pilli, which have a critical role in movement of bacterial cells inside matrix. The third stage of biofilm formation is maturation; this step is specific to each bacterial species. In this stage, the microcolonies embedded in the matrix develop to a complete structure of biofilm, which is basically composed of bacterial cells enclosed by exopolysaccharide which acquire a strong structure that protect them against antimicrobial agents. During this stage, some signal molecules are required for maturation and are synthesized by sessile cells which play a significant role in the process of expression of certain genes which are necessary for development of biofilm. The mentioned molecules are synthesized individually by bacterial cells and when their concentration is high enough to be sensed, certain genes are expressed. This process is called quorum sensing (QS) and involves various signal molecules, including acylatedhomoserine lactones (AHLs) among proteo-bacteria; γ-butyrolactones in *Streptomyces species*, and oligopeptides.(Arciola *et al.*, 2012) (Bogino *et al.*, 2013) (Costerton, Stewart and Greenberg, 1999) (Beoletto *et al.*, 2016) (Chadha, 2014). Vibrio cholera, for example, may use various pathways to initiate attachment relying on the surface that the bacterial cell attaches. For instance, the researches in vivo have realizd that Tcp pilus is necessary for colonization of intestinal tract. (Watnick and Kolter, 1999)



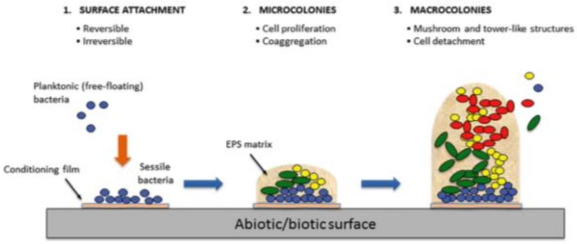


**Figure** 1: Biofilm development stages

## Biofilm Matrix

The main component of matrix in biofilm is water, which could reach about 97%. The synthesis of extracellular polymeric substance is connected to genes which are up-regulated in bacterial biofilms. The extracellular polymeric secretions could be different in their structural composition, physical and chemical characteristics. The mature biofilm phenotype relies on the environment conditions in which it progress in. The researchers discovered that the changes environmental conditions may lead to phenotyping changes in the process of formation of biofilm. Extracellular polymeric secretions are known for their role in the protection of microorganisms from harsh environmental conditions. For example, the EPS role in helping bacterial cells to survive environmental threat was because of that it protect it from dehydration in bacteria which are mucoid, for example E. coli opposite to the ones that does not produce mucoid. The extracellular polymeric secretions has role in bacterial adaptation to different environmental condtions including the stressful and life threatening situations. The slow bacterial growth in biofilm is observed to promote EPS production for a better adaptation.

The mutated bacteria which do not have the ability to produce the EPS usually cannot produce biofilms. For example, E. coli strain that are unable to form normal biofilm , has problems in the production of colonic acid. The colonic acid is one of the main EPS produced by this organism. In spite of that, in population with more than one species, the species that synthesize EPS may give the stability to mutant species which do not have the ability to produce EPS. (Costerton, Stewart and Greenberg, 1999) (Davey and O'toole, 2000)(Sutherland, 2001)

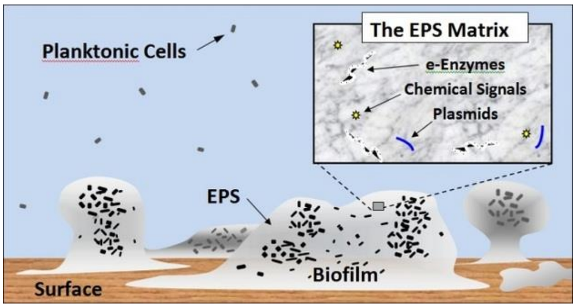


Figure 2: the biofilm consist of a group of bacterial cells enclosed or embedded in a matrix which is composed of extracellular polymeric secretions (EPS), which covers the cells and protect them from environmental threat. The EPS matrix typically contain polysaccharides, lipids, proteins, and extracellular DNA. Extracellular enzymes located within the EPS matrix. Additional to small DNA pieces encoding some specific genes, named plasmids. The biofilm structure protects both Extracellular enzymes and plasmids from degradation. Finally, bacterial cells synthesize signal molecules for a mechanism of communication between the cells known as quorum sensing. The height of a biofilm could be from few to hundreds of micrometers on the surface it attaches, but are provided with several inherent adaptations which are absent in planktonic bacterial cells ( 2013 Nature Education).

## Antibiotic resistance

Biofilms have a significant role in emerging and evolution of bacterial antibiotic resistance. Horizontal genetic material transferring between bacterial cells in the biofilm enhances evolution and genetic diversity of natural bacterial communities. The studies conducted on gene transferring among bacteria in natural environments have given special focus after emerging of multidrug-resistant bacteria and the concerns about the risk of post-antibiotic era. The extracellular polymeric secretions matrix avoid access of specific antimicrobial substances controlling distribution of certain materials from the surrounding environment into the internal structures of biofilm. The chemical structure of classes of antibiotics have role in the process, the antibiotics which are hydrophilic and positively charged, for example aminoglycosides are more preventable than other antibiotics. Antibacterial drugs when used in a dose less than the MIC may lead to formation of biofilm in different bacterial species. This aspect of the process have a major importance due to that the bacterial cells located inside the biofilm are protected from the desirable concentration of the antibiotics, which will lead to enhacement of biofilm formation instead of inhibiting it. Additional to that, is dosing regimen, when bacterial cells are exposed to a lower concentration than Minimum Inhibitory Concentration of antibiotics a dosing regimen's beginning and end. The overuse and misuse of antibiotics in veterinary, aquaculture and agriculture fields may lead to more exposure of microorganism to suc-MIC of antibiotics. (Chadha., 2014)

Various mechanisms are discussed to explain this low impact of antibiotics on bacterial cells in biofilms, proposing many factors which can lead to this resistance against antibiotics. Three major mechanisms of resistance are thought to be:

1. Exopolysaccharide matrix: This substance have role in delaying the entrance of antibiotics or allow limited penetration into internal structures of biofilm because antibiotics are adsorbed into the exopolysaccharide structure of matrix and lead to delay their entry; for instance, aminoglycosides, which are positively charged antibiotic, link to biofilm polymers, which are negatively charged, leading to slower biofilm penetration.

2. Chemical microenvironment: when the biofilm reachs the maturation phase, the number of bacterial cells will grows, inducing the production of the σ factor, RpoS, which is shown only in the log phase of bacterial replication. The increasing of cell density cause the activation of Quorum Sensing systems and the biofilm's environmental conditions produce or choose cells which shows phenotypically resistance to high antimicrobials concentrations. The cell population presence in high density will lead to production of gradients of oxygen and nutrients availability. The available oxygen is utilized by cells which are near the outer layers of biofilm, generating zones which are deprived from oxygen in the deeper layers of the biofilm. Aminoglycosides will be less effective because this group of antibiotics does not work properly in such anaerobic conditions. Also the accumulation of acidic byproducts will generate pH gradients, which will have a direct impact on the mechanism of action of antibiotics.

As a consequence of the deprivation from oxygen and the changing of pH, bacteria cease replication and enter a stasis condition, which will help them to survive. A well-known example for this process is penicillin, which will not affect efficiently because it only kills the bacteria in growing phase because its mechanism of action is targeting the process of bacterial cell wall synthesis. Another result of the slow bacterial growth rate is a general response to this unfavorable environmental condition situation by creating changes to prevent heat and cold shocks and alters in pH and several chemical components. Another consequence, there is an osmotic stress response which produce alteration in porins which in turn decrease the permeability of the bacterial cell envelope to antibiotics.



3. Phenotypic/persister variants: When bacterial cells adhere to a surface during biofilm development process, some alterations in gene expression occur, producing the specific phenotype of biofilm which is responsible for resistance.

This phenotypic characteristic acquired by a subpopulation of bacterial cells resistant to extreme concentrations of antibiotics induced or chosen by the surrounding environmental conditions available in this biofilm matrix. (Mah and O'Toole, 2001).

**Biofilms in infectious diseases and Nosocomial Infections**

Biofilm is one of the major risk factors in the hospitals by contamination of the medical implants by adherence to abiotic surface, including prosthetic valves, eye contact lenses and catheters. Which could be life threatening a high rate of nosocomial infections are related to biofilm formation by pathogenic organisms on medical devices.(Ghannoum *et al.*, 2015). The presence of biofilms on prosthetic valves will lead to endocarditis in patients who undergone heart valve transplantation surgery. The formation of biofilm on catheters is also mentioned in many studies as a main source of UTI. They also formed on eye contact lenses which cause keratitis. Biofilm has a significant role in patients with cystic fibrosis (CF) who have *Pseudomonas aeruginosa* causing infection. (Chadha, 2014). About 95% of UTIs are related to urinary catheters, mechanical ventilation contaminated cause about 80% of pneumonia cases, and about 87% of circulatory system infections are thought to be caused by intravascular devices. The most common hospital acquired infections are Staphylococci and enterococci, which are commensal and are normal flora of skin and upper respiratory tract, uorogenital tract and lower GIT.(Ghannoum *et al.*, 2015).

## The battle against biofilm infections

The bacterial biofilm formation is one of the major obstacles that prevent eradication of pathogenic bacteria using antibiotic therapy, which are resistant to several hundred folded times minimum doses of antibiotics required for killing the bacteria present outside a biofilm, and the expected dose for killing infections caused by bacteria in biofilm exceed the highest dose of antibiotics that could be delivered. Additional to that, the uncontrolled use of antibiotics in various fields, rather than medical field, is playing a critical role in the emerging of new antibiotic resistant bacteria. As a result of that, more efforts are required to understand the mechanisms which have impact on the progress of antibiotic tolerance and to develop novel therapeutic mechanisms and strategies to eradicate biofilm-caused infections. (Dufour *et al.,* 2012).

## Beneficial biofilms in water and wastewater treatment

In addition to medical importance, there are beneficial uses of biofilm in environmental engineering system as the main character of treatment processes. For instance, they are used for elimination of organic materials from drinking water, by a process named biofiltration. And they are used also in biological nutrients removal in treatment of wastewater as shown if figure( ). In both mechanisms, bacteria are used due to their ability to fast break down and changing complex organic materials into simple structures which have less negative effects on the environment. Biofilm forming bacteria are extremely more effective than planktonic bacterial cells in byproducts treatment systems; the non moving bacterial cells with in the biofilms resist and will not be removed away, as a result of that it will act continuously in treatment of large water volumes. This engineering advantage also provides biofilm bacteria with a continuous source of food (i.e., carbon and other nutrients found in the untreated water), which allows the cells to thrive under an otherwise nutrient-poor condition. (Ikuma *et al.*, 2013)

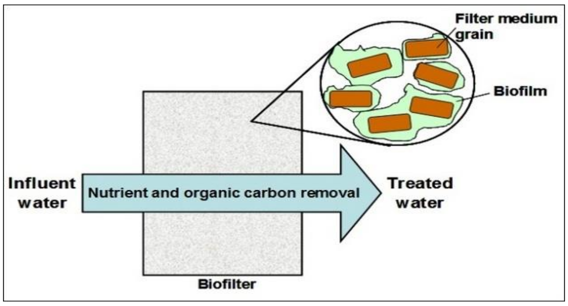


**Figure 3:** Biofiltration as a treatment technology in water and wastewater purification. The arrow indicates the direction of water flow. Biofilters contain filter medium grains (e.g., sand, granular activated carbon) that are covered with biofilms. The biofilm activities break down nutrients (e.g., nitrogen and phosphorous-containing compounds) and organic carbon as well as capture other unwanted contaminants in the influent water. Water coming out of a biofilter typically undergoes disinfection prior to entering drinking water distribution pipes or being released to the environment as wastewater effluent. (2013 Nature Education)

## Conclusion:

Most of microbiological studies were conducted on free swimming bacteria which were cultivated in the laboratory, but recently, the scientists realized that more than 99% of bacteria live in small communities which require focusing on them as they act differently when they are in groups, with higher resistance harsh environmental conditions including antibiotics, desiccation, deprivation of food, by using different mechanisms and perform complex processes, including the intercellular communication to alter the metabolic activities of each other by synthesizing materials, trapping of nutrients, horizontal gene transmission, in order to enhance each other biologically and physically to resist unfavorable environmental conditions. which make them life-threatening. In fact, antibiotics are not developed enough to affect biofilms, even with the available information about biofilms, there is no effective clinical antimicrobial agent for their eradication, yet. But utilization of substances coated with immobilized antibacterial agents, especially cationic antimicrobial peptides, appears to be very innovative and promising. Nanotechnologies and nanomaterials in medical studies have originated novel therapeutic horizons and are fast progressing.

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