Biofilms: Community Behavior by Bacteria

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Biofilm is a lifestyle exhibited by bacteria. This is an intricate process that involves cell–cell communication which leads to the regulation of certain genes. This affects the laying down of the extracellular matrices which form the substratum for the biofilm. In this review, we have discussed in detail about the mechanism behind biofilm formation and the physiological importance of biofilm lifestyle for the bacteria.

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Background

The purpose of this article is to provide a basic overview on biofilms. Biofilms have been affecting mankind long before we even knew that something such as biofilms exist. The plaque on our teeth, the slime that accumulates on the walls of a fish tank, the growth seen on the hulls of ships; they are all biofilms. A biofilm is basically a highly structured community of bacteria which adheres to each other as well as the other surfaces. Biofilms have been spotted unknowingly for centuries; only recently has research in this field become prominent. Historically, microbiological investigations have focused on the study of planktonic bacteria – bacteria that are unicellular, and their ability to cause diseases. More recently, microbiologists have realized that bacteria exhibit both unicellular as well as multicellular lifestyles, biofilms constituting the multicellular phase.

This article introduces the history of study of biofilms in the first section, and how they began to be studied and researched. The next two sections describe the process of formation of biofilms and why this is preferential to bacteria. Finally, we take a look at the benefits as well as hindrances biofilms bestow on mankind.

History of Biofilm Breakthrough

Most bacteria form multicellular communities called biofilms in

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which cells are protected from environmental hazards. Although observations on these communities were documented as early as 1684 by Anton Von Leuwenhoek, the earliest use of the term 'biofilm' in publications was 1977. It is only now that scientists have begun to understand their true significance.

Biofilms have also been mentioned in fictional works. For example, in the famous book '*Twenty Thousand Leagues under the Sea*' by Jules Verne, in which he used real ship logs, he mentions 'milk seas' (*Box* 1).

Box 1. Excerpt from *Twenty Thousand Leagues Under the Sea*

About seven o'clock in the evening, the Nautilus, half-immersed, was sailing in a sea of milk. At first sight, the ocean seemed lactified. Was it the effect of the lunar rays? No; for the moon, scarcely two days old, was still lying hidden under the horizon in the rays of the sun. The whole sky, though lit by the sidereal rays, seemed black by contrast with the whiteness of the waters.

Conseil could not believe his eyes, and questioned me as to the cause of this strange phenomenon. Happily, I was able to answer him.

"It is called a milk sea," I explained. "A large extent of white wavelets often to be seen on the coasts of Amboyna, and in these parts of the sea."

"But, sir," said Conseil, "can you tell me what causes such an effect? For I suppose the water is not really turned into milk."

"No, my boy; and the whiteness which surprises you is caused only by the presence of myriads of infusoria, a sort of luminous little worm, gelatinous and without colour, of the thickness of a hair, and whose length is not more than seven-thousandths of an inch. These insects adhere to one another sometimes for several leagues."

"Several leagues!" exclaimed Conseil.

"Yes, my boy; and you need not try to compute the number of these infusoria. You will not be able, for, if I am not mistaken, ships have floated on these milk seas for more than forty miles."

Towards midnight the sea suddenly resumed its usual colour; but behind us, even to the limits of the horizon, the sky reflected the whitened waves, and for a long time seemed impregnated with the vague glimmerings of an aurora borealis.

Mariners have reported witnessing 'milky seas' for years. 'Milky sea' is the name given to the nocturnal emergence of a uniform, intense glow that extends in all directions and is sustained for long periods of time [1]. Both dinoflagellates[1] and luminous bacteria were considered possible reasons for these observations. However, dinoflagellates only emit brief sources of light, and sightings were also made in very calm seas, where dinoflagellates could not be expected to be present.

[1] Dinoflagellates are a large group of flagellate protists. They are mostly unicellular, marine and photosynthetic, commonly regarded as algae (division Dinoflagellata).

Bacteria were better candidates, as they emit a continuous but relatively faint glow. What was unbelievable was the postulation that these bacteria were responsible for this phenomenon due to autoinduction or quorum sensing, a system of stimulus and response which is discussed in detail later on. It was shown that planktonic bacteria typically did not emit light in the sea, even though they were luminous when cultured. However, if a colony was grown, concentrations of the autoinducer would increase, causing luminescence.

The discovery of quorum sensing opened up new inquiries and research. If such a large phenomenon like luminous seas could be caused by these microscopic bacteria, then there was certainly something to investigate.

How do Biofilms Blossom?

For a microbe, being in a biofilm is a community effort. Biofilms are characterized by complex three-dimensional structures, with water channels, exopolysaccharides, curli, DNA, pili, flagella, detritus, etc. They have significant properties such as increased resistance to harmful environments, phenotypic versatility and quorum sensing by autoinducers. So, how are these amazing biofilms formed?

Biofilm formation is a five-step process (*Figure* 1).

Reversible attachment: Formation of a biofilm begins when free-living planktonic microorganisms adhere to a surface. When these bacteria near the surface, they experience attractive van der Waals

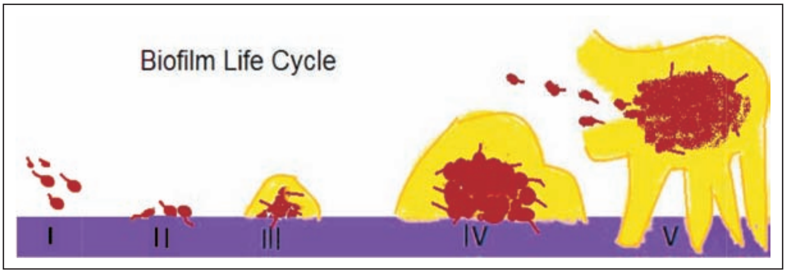


Figure 1. Different stages of lifecycle of bacterial biofilm.

forces as well as a net negative electrostatic repulsion. Any external increase in kinetic energy increases the probability of the bacteria overcoming the repulsion and making physical contact with the surface through their surface receptors such as pili and flagellae, and various adhesins. Some are unable to attach themselves directly to the surface but anchor themselves to the earlier colonists.

Irreversible attachment: The cells are typically enclosed in a polymeric matrix that they produce, resulting in an irreversible attachment to the matrix.

Maturation I and II**:** Once the microcolony is established, its mass and bulk increase slowly through a mixture of cell division and additions.

Dispersal: Biofilms often have a limited lifespan due to exhaustion of nutrients coupled with biological as well as environmental factors such as abrasion and fluid shear due to the surrounding particles. There is ever-growing evidence to support the fact that biofilms initiate their own disassembly [2]. Many labs describe a phenomenon called seeding dispersal, where the interior of the biofilm liquefies as the exterior is broken, permitting release of phenotypically planktonic bacteria, leaving behind an empty colony. Dispersal is usually concomitant with detachment[2].

***Factors Affecting Biofilm Growth and Dispersal*:** Biofilm dispersal is closely linked with environmental triggers, such as variations in availability of nutrients, oxygen depletion and changes in temperature [3]. To disperse, bacteria must break their 'encasings'.

[2] Microbiologists have used the terms 'detachment' and 'dispersal' for stages in biofilm development when masses of biofilm or individual cells may separate from the biofilm and travel to other areas either within the fluid flow or aided by their flagellar motion.

To this end, a number of effectors such as enzymes and surfactants are developed. Bacteriophages also help in degrading the biofilm matrix, eDNA, polysaccharides and proteins, leading to dispersal.

Apart from these, stress, as a result of habitat decay, is a major dispersal initiator. Formation of biofilms consisting of variants through dispersal cells can increase the number of habitats colonized by these cells.

Why do Bacteria Opt for Biofilm Lifestyle?

United we stand, divided we fall. This is a motto humans have used successfully for generations, be it in armies, families or countries. Even in Nature, the same philosophy is equally effective for fish or birds or insects with communal societies. This strategy has been found in the most basic organisms as well. Since the nineteenth century, we know that many single-celled organisms have used this ideology and created highly sophisticated colonies to survive many different environments. Bacteria are speculated to use biofilms as the main mode of growth. Biofilms are considered to be the product of evolution against adverse conditions. Biofilms are comparable with higher organisms in terms of their structural functionality and diversity. Bacteria living in biofilms have significantly different properties from their planktonic counterparts.

The main characteristics that distinguish biofilms are as follows:

Quorum Sensing: Scientists began reporting a unique phenomenon in the 1970s. They were investigating bioluminescence in *Vibrio fischeri*. Microbiologists were curious about the fact that at low population density, the bacteria produced no light. As the bacterial community grew, the cells 'switched on'. The cell's adaptability in being able to turn genes 'off or on' based on cell numbers was named quorum sensing. The investigators concluded that *Vibrio* produced a substance they called autoinducer (AI) which, above a threshold concentration, induced the enzyme luciferase to initiate emission of light. Since its first observation,

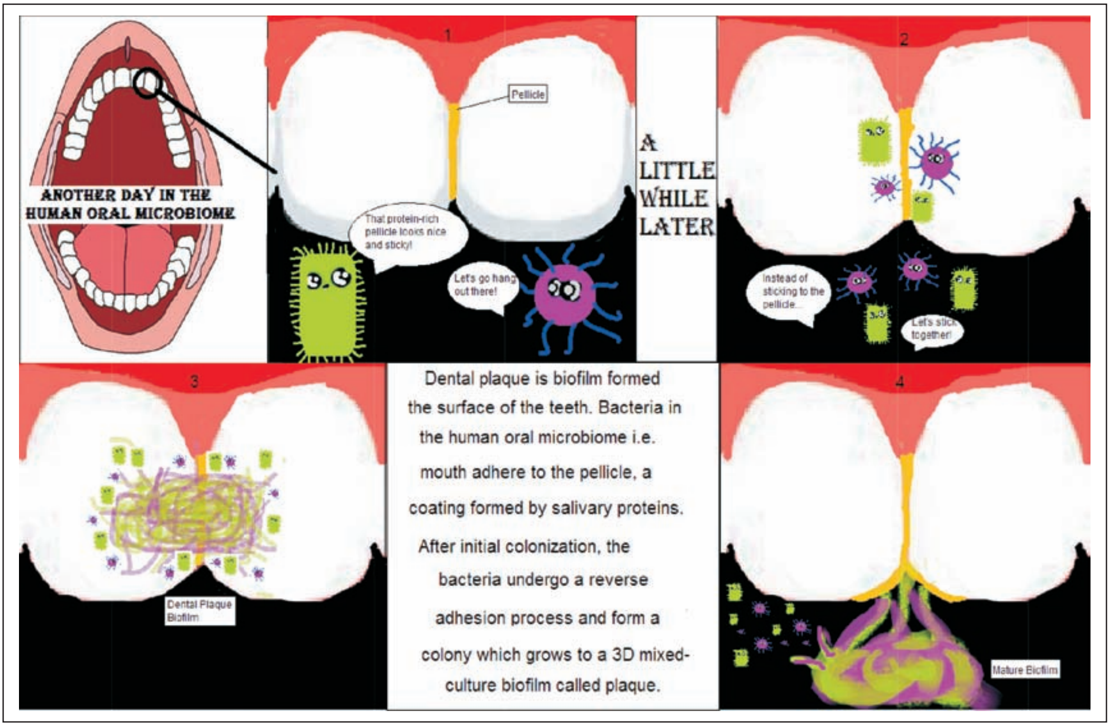


Figure 2. This comic strip shows the formation of a specific biofilm – the dental plaque. The formation of dental plaque biofilms includes a series of steps that begins with the initial colonization of the pellicle and ends with the complex formation of a mature biofilm. Dental plaque biofilms exist on a variety of tooth surfaces including fissures, smooth surfaces and gingival crevices; however they are most likely to be seen in their mature state in more stagnant sites, such as fissures and crevices, as these places provide protection from the forces of removal, such as toothbrush. Dental biofilms 'grow' by two mechanisms: accretion in which additional microorganisms are added successively to the community, increasing its diversity; and cell division of the individual cells forming microcolonial islands which contribute to the biofilms mass. Oral biofilm cells accumulate in a very specific sequence much similar to a typical ecological succession.

autoinduction or quorum sensing has been shown to be widely distributed and is responsible for many important functions. Biofilms are considered evolutionary adaptations mainly because of quorum sensing, which allows the bacteria to flip between different modes of behavior and facilitates cell-to-cell signalling.

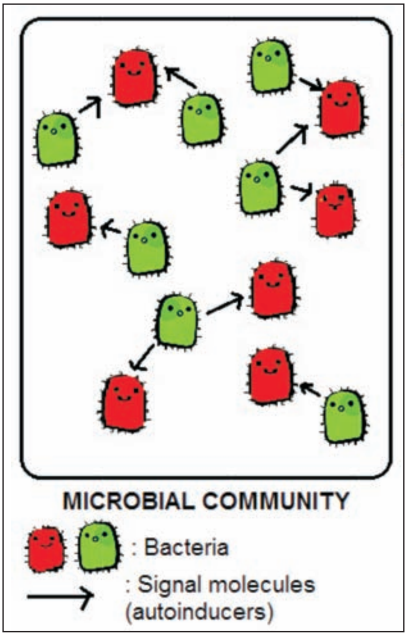
One of the best understood quorum-sensing systems is that of *Pseudomonas aeruginosa*. Quorum sensing is the primary reason for its virulence in plants and animals including humans. It controls biofilm development, swarming motility and the production of its stock of extracellular virulence factors capable of causing extensive tissue damage and invading the bloodstream.

Quorum sensing is generally considered to facilitate gene expression. It is also shown to cross interspecies barriers[3] in that these signal molecules [4] influence the behavior of eukaryotes in both plants and animals and can facilitate biofilm survival by influencing the given environmental nook. Crosstalk has many implications as bacteria almost always exist as mixed species in nature (*Figure* 3). Quorum-sensing research has many potential applications, most of which are involved in interfering with the biofilm signalling system and thus controlling the bacteria. Already, mutants have been found which take advantage of the production of quorum-controlled factors [5].

[3] While species-specific quorum sensing allows recognition of self in a mixed population, bacteria also need mechanisms to detect the presence of other species. Evidence for the existence of these types of complex mechanisms first came from the study of *Vibrio harveyi.* One signal termed AI-2 appears to be universal and facilitates interspecies communication.

Resistance: Biofilm cells offer increased resistance when compared to constituent members. Such biofilm cells can cause a variety of diseases. Microbial biofilms are detrimental and unwanted in hospitals and industries and are tougher to eradicate. The main functions of biofilms are capturing environmental nutrients and providing protection. Greater resistance of biofilm cells to antibacterial agents and sanitizers has been noted. *Salmonella typhimurium* can form biofilms with defence reactions against immune cells of the host.

Figure 3. Sessile cells in a biofilm 'talk' to each other via quorum sensing to build colonies and keep water channels open.



Diffusion: Diffusion is the primary means of molecular transport in biofilms. Quorum sensing is accomplished by acyl homoserine lactones (AHL), which are highly diffusible compounds that move across the plasma membrane and out into the surrounding environment. When the concentration becomes significant, these molecules bind allosterically to an RNA polymerase enzyme via a regulator. Diffusion is also responsible for transporting nutrients. Biofilm growth and physiology depend on nutrients obtained from the environment. Liquid transport channels also expedite mechanisms of transport.

Biofilms: Boon or Bane

Useful Biofilms: Many biofilms play a major role in the ecology of the earth and sustenance in general. One of the best examples of successful, beneficial applications of biofilms is in the treatment of waste water. In some filtration systems, the filter medium presents surfaces for microbes to attach to and eat away the organic material in the water. It has been found that waste water treated by the biofilm method (activated sludge) is very effective.

Biofilms can also be used to 'eat up' petroleum and other oil products. There is also the concept of microbial leaching. For example, low grade ore is mildly acidified to encourage the growth of bacteria which oxidize the ore to release cupric ions that can be recovered. However, one of the most influential and important biofilms to man is the gut flora[4]. The human intestine has an enormous number of bacterial strains. These provide us with some genetic and metabolic attributes that we have not acquired on our own. There are now many studies on the co-evolution of our microbial ensemble. The composition of our gut flora shows natural selection at the microbe as well as host levels [6]. Suboptimal functionality of the gut microbiota reduces host fitness. Many infectious diseases involve the gut, and our 'good' flora help in keeping away 'bad' microorganisms. In developed countries, a firm control over intestinal diseases is maintained, yet allergies and inflammations are extremely common. It is thought that the absence of overt gut flora upsets the balance between the normal colonizing bacteria and the immune system. The normal flora synthesizes and secretes vitamins, prevents colonization of pathogens, stimulates the development of tissues and the production of antibodies.

[4] Gut flora refers to the complex of microorganism species that live in the gastrointestinal tract. It is considered the largest reservoir of human flora. The human intestine carries about 100 trillion microorganisms.

Another potential use of bacteria is in treating cancer. Many bacterial species show a preference to colonize cancerous tissue [7]. One of the hallmarks of solid tumors is that they lie in hypoxic regions, i.e., regions with low oxygen concentration. Certain anaerobic bacteria seek out these environments and colonize the tumor, leaving normal tissue untouched. This colonization can result in retardation in growth of tumor or even complete disappearance.

Harmful Biofilms: Biofilms can be very harmful. Due to their resistance, they are formed in many undesirable locations and cause infections. Biofilms such as those formed by *Salmonella* are undesirable in food processing units [8]. It is known that bacteria in biofilm mode are up to 1000-fold resistant to antibiotics than their planktonic counterparts. These biofilms are a continuous source of contamination to food coming in contact with them. They also pose severe problems in clinics. Often, medical devices such as catheters that are implanted in patients can be contaminated by the growth of biofilms and such growth can cause persistent infection and sepsis[5]. Often the only solution for an infected catheter[6] is complete removal, an inconvenient and painful process. *S. aureus* is a leading cause of hospital-acquired infections. These problems stem from its ability to form biofilms. Research in preventing the formation and eliminating these biofilms is necessary [9]. Other common ICU infections are biofilm formation on the surface of endotracheal tubes, urinary tract infections, etc. Central venous catheters[7] are estimated to account for 90% of all nosocomial[8] bloodstream infections.

Another interesting aspect of biofilm colonization is converting hosts into carriers of a disease. In particular, 3% to 5% of the population infected with *S. enterica serovars* typhi will become chronic carriers. Salmonella is contracted in regions with poor hygiene due to the ingestion of faecal matter containing the pathogens. Some of these individuals develop a sustained infection of the gallbladder and are referred to as carriers. It has been shown that *Salmonella* can form biofilms on gallstones, and survive for long periods [10]. The most famous example of this is 'Typhoid Mary'[9], who was outwardly asymptomatic but infected those around her.

6. Bridling Biofilms

Practitioners across many diverse fields have the same concerns

[5] Sepsis is a potentially fatal condition characterized by a whole-body inflammatory state called systemic inflammatory response syndrome (SIRS) caused by severe infection.

[6] Catheters are thin tubes that can be inserted in the body to perform surgical operations or treat diseases. By adjusting its components, catheters can be tailormade for certain applications.

[7] A central venous catheter (CVC) is a catheter placed in a large vein. It could be in the internal jugular vein (neck), subclavian/ axillary vein (chest) or femoral vein (groin).

[8] A nosocomial infection is a hospital-acquired infection (HAI). It is basically an infection whose development is favored by hospital environment.

[9] Mary Mallon (1869–1938) was the first person in USA to be identified as an asymptomatic carrier of the pathogen associated with typhoid fever. In carrying out her duties as a cook, she infected at least 51 people, three of whom died. She evaded authorities and continued to work and infect people even after being forcibly isolated by health authorities, and is known as 'Typhoid Mary'.

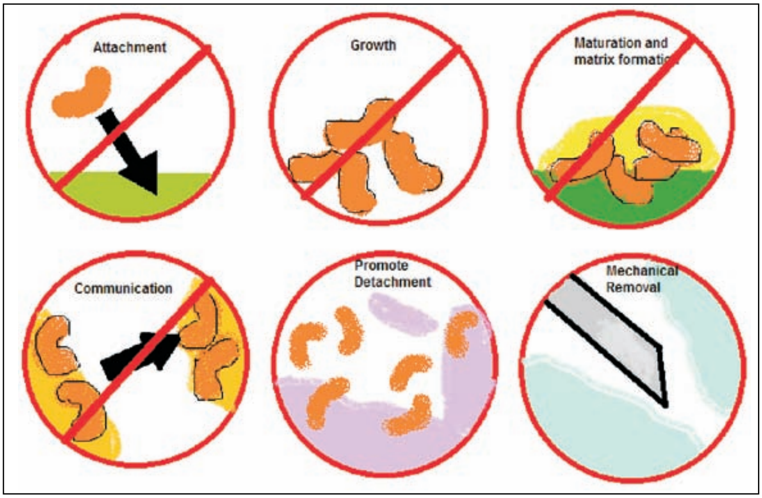


Figure 4. Options for controlling biofilm accumulation.

of detecting biofilm formation both qualitatively and quantitatively, and controlling its growth (*Figure* 4). The question is how to decimate biofilm structures, and the best inspiration is derived from natural mechanisms. It is now being understood that plants and animals have been dealing with biofilms since their inception. For instance, some animals use mucin polymers to inhibit biofilm formation [11].

Brominated furanones are known to disrupt quorum sensing and thus are used to fight biofilms. There is also ongoing research on the use of phages to combat biofilms. Phages are able to break through a biofilm, but they first have to overcome the biofilm's defences towards viral attack. As promising as this idea seems, there are several risks of integration which makes this strategy unlikely.

In order to eliminate biofilms, it is necessary to identify the bacteria and select the best available treatment, tailormade for that strain. The problem is the resistance of biofilms to antibiotics. How is this brought about? Although some believe that antibiotics cannot penetrate a biofilm, it has been shown that this is more often false than true. According to Kim Lewis, "In most cases involving small antimicrobial molecules, the barrier of the polysaccharide matrix will only postpone the death of cells rather than afford protection." However, after penetration, a number of cells called persisters are left behind. Persisters, as their name suggests, are simply cells that are able to survive the first onslaught of antibiotics. If left unchecked, they can gradually reform the biofilm. Therefore, with prolonged dosage of antibiotics, it is possible to eradicate biofilms [12]. Also, it is now known that *P. aeruginosa* displays antibiotic tolerance by expressing specific genes only in the biofilm mode.

Biofilms are a permanent fixture of our lives. They are ubiquitous whether you search for them in the frozen deserts of Antarctica, the depths of the oceans, the interstices of underground rocks, in rain forests or in your very own body. It now seems that many phenomena can be explained using biofilms. Estimates indicate that more than half the earth's biomass is composed of biofilms. Even fossilized biofilms have been observed, which indicate that they are among the Earth's earliest residents.

The concept of bacteria as exclusively unicellular is obsolete, and in its place, we find functionally and structurally advanced communities that have the ability to influence the environment. Thus, it is necessary that we all have an understanding about the roles biofilms play and how they are both useful and harmful. Understanding the cause is the only way to find solutions.

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