# The enigma of biofilms

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*Biofilms represent a self-contained and self-sustained ecosystem resulting from a synergistic response of bacteria to stress imposed on them by their environment. Biofilms are formed when microorganisms attach to a substratum and to one another in a matrix composed of extracellular polymeric substances. Biofilms are resilient and exhibit properties that confer on them the ability to resist and tide over stress conditions. The study of biofilms can go a long way in unravelling secrets of the multicellular life of prokaryotic bacteria. This note introduces the multi-faceted nature of biofilms which has spurred research aimed at better understanding of microbial physiology.*

## What are biofilms?

Bacteria are known to alternate between the free-swimming (planktonic), unicellular form and a sessile, multicellular form, commonly referred to as a biofilm. Antonie Van Leeuwenhoek, a Dutch scientist who first observed bacterial colonies on tooth surfaces using his rudimentary microscope in the 17th century, could be credited with the discovery of biofilms; however, the 'unified theory of bacterial biofilm formation' was first described by Costerton *et al.*[1]. Though our understanding of microbial physiology and genetics stems from studies using laboratory-grown pure cultures of bacteria in nutrient-rich culture media, bacteria predominantly exist as biofilms in natural settings.

Biofilms are defined as 'aggregates of microorganisms in which cells are frequently embedded in a self-produced matrix of extracellular polymeric substances that are adherent to each other and/or a surface'[2]. The extracellular matrix is known to be a complex of water, polysaccharides, proteins, lipids, metabolites, extracellular DNA, signalling factors, waste products, detritus from the immediate surroundings and cell debris. These polymers often interact with each other and with receptors on microbial cells to provide a unique structural composition. The extracellular matrix also confers unique properties to biofilms by providing a stable environment which prevents desiccation and allows for communication between bacterial cells[3]. Biofilms occurring in different environments exhibit diversity in the chemical composition of the extracellular matrix terms as well as in the constituent microbes. A biofilm may contain only a single bacterial species or even a combination of bacterial species, fungi, algae and protozoa. Biofilm activity depends not merely on the type of microbes and their genetic constitution, but also on the nature of the substratum and the soluble environment around it[4].

## Why and how are biofilms formed?

As shown in Figure 1, biofilm formation involves five major phases – (i) initial attachment, (ii) cell–cell adhesion, (iii) proliferation, (iv) growth and maturation and (v) dispersion – that can be further elaborated in nine steps as explained by Simões *et al.*[5]. A biofilm mode of life provides bacteria with a favourable niche for growth, confers on them the ability to survive adverse stress conditions such as low nutrient availability, pH fluctuations, variations in temperature and allows for division of metabolic burden akin to multicellular organisms[6]. These advantages have led to the recalcitrant nature of bacterial biofilms. It is thus hardly surprising that biofilms occur in extreme natural environments ranging from deep-sea hydrothermal vents to the 'desert-like' lake ice cover in Antarctica.

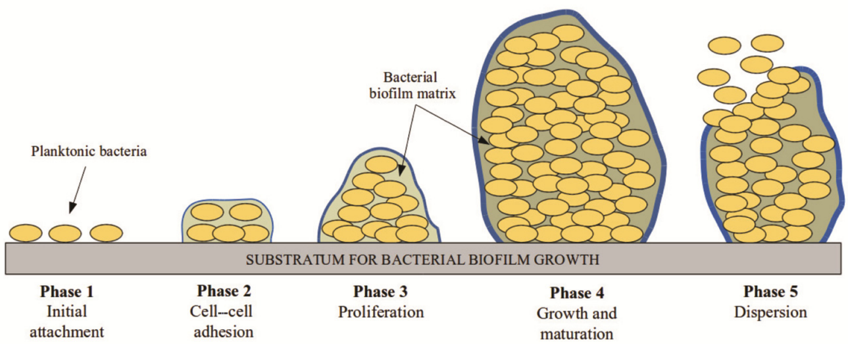


**Figure 1.** Five major phases of biofilm formation.

## Biofilms are a nuisance

The relevance of biofilms often sparks off a debate as to whether they are a boon or a bane. Much has been spoken and written about bacteria as agents of infection and disease. Bacterial biofilms are implicated in diseases such as endocarditis, otitis, prostatis, cystic fibrosis and many others. Although antibiotic therapy continues to be the most common treatment against such diseases, biofilms are highly resistant to antibiotics and are often not removed effectively with mere antibiotic treatment[7]. When antibiotics burst into the health-care scenario in the first half of the 20th century, it was believed that diseases of bacterial origin would be short-lived. However, as scientists explored and still continue to explore various sources of antibiotics to build up the arsenal against bacterial infections, the seemingly innocuous targets have also been rallying to evolve mechanisms for combating them[8]. Planktonic bacteria might be susceptible to specific antibiotics, but biofilm formation is an effective strategy adopted by bacteria to counter the barrage of antibiotics used against them. The minimum inhibitory concentration of antibiotics required for biofilms is much higher compared to planktonic bacteria because the biofilm matrix hinders the antibiotics physically and chemically from reaching their destination[9].

Apart from their role in disease, biofilms are also known to be a hindrance for biomedical implants and water distribution systems, and a menace in the food industry. Their active involvement in biodeterioration of materials has made it all the more critical to find solutions for removal of biofilms. Over a decade ago, in a novel study, biofilm bacteria containing knockout mutations for matrix formation were efficiently lysed[9], indicating the role of the extracellular matrix in the survival of biofilms. As biofilms are known to regulate their physiology by quorum sensing, strategies for producing anti-biofilm compounds involve targeting the key molecules involved in quorum sensing between microbes[10].

## Biofilms are beneficial too

Biofilms are like a glass half-filled with water; some say it is half empty, others find it half full. There is a flip side to biofilms. A number of biotechnological processes exploit them for the production of enzymes, antibiotics, secondary metabolites of interest and in bioconversions; however, the most extensive use of biofilm reactors has been for wastewater treatment. Biofilms are of tremendous importance in trickling filter systems, fluidized bed reactors and specialized systems for nutrient or waste removal from wastewater[11]. The resistance of biofilms to changing environmental conditions renders them effective in the wastewater treatment process. Biofilms provide operational flexibility, occupy lesser space and are cost-effective. They present a specialized, immobilized niche in which their biotic and abiotic components interact with wastewater. Effectiveness and efficiency of biofilms in wastewater treatment can vary based on specific extrinsic and intrinsic factors which affect the biological, chemical and physical components of the biofilm matrix. The ability of microbes to degrade diverse organic substances and to cycle carbon, nitrogen and phosphorus is unparalleled in nature. Biofilms are known to employ different processes like biodegradation, biosorption and biomineralization in the remediation process.

The interest in electroactive biofilms has provided a new impetus towards solving two important present-day challenges – wastewater treatment and clean energy generation. Electroactive biofilms comprise a consortium of microbes that are capable of transferring electrons to a conductive surface. These biofilms serve as a conduit for transferring electrons and thereby play a key role in the performance of microbial electrochemical systems such as microbial fuel cells at the anode and cathode[12]. Although electrochemically active microorganisms are key members, electroactive biofilms may also harbour electrochemically inactive microorganisms which may aid in the breakdown of complex organic substrates by means of fermentation or utilization of alternate electron donors or acceptors[13].

## The future of biofilms

Biofilms are as ubiquitous as they are obscure; their maleficence in nosocomial infections standing against their beneficence in wastewater treatment. As a seemingly impregnable multicellular alter ego of the ostensibly vulnerable unicellular prokaryotes, biofilms have time and again proved to be a conundrum for researchers. Biofilms have provided valuable clues to understanding hitherto unknown facets of microbial physiology and growth. The decade-old statement by Branda *et al.*[3] is relevant even today – '...no single experimental approach has adequately fulfilled all of the needs of the biofilm investigator; rather, each method has complemented the others, and the accumulated knowledge gained from their combined application has provided new insights into the nature of biofilms.' As the quest for strategies to dislodge biofilms competes with those to stabilize their establishment, biofilms are in no uncertain terms pointing to the fact that they will continue to sustain the interest of researchers for a long time to come.


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