Biofilms

Biofilms are the aggregation of microbial cells, which are associated with the surface in almost an irreversible manner, i.e. cannot be removed by gently rinsing. They are attached with a biotic or abiotic surface integrated into the matrix that they have produced.

Biofilm formation steps

Step1. Attachment: Conditioning layer is formed which have a loose collection of carbohydrates and proteins which gets unite with minerals in water. It attracts the microbial cells to get attached with the surface.

Step2. Irreversible attachment: As soon as conditioning layer formed, electrical charge accumulates on the surface which attracts the bacteria having opposite charge that result in irreversible attachment of microbial cells. The charges are sufficiently weak that microorganisms could be easily removed by the mild cleanser and sanitizers.

Step3. Proliferation: In this phase, bacteria get attached to the surface as well as with each other by secreting EPS (an extracellular polymeric substance) that entraps the cells within a glue-like matrix.

Step4. Maturation: The biofilm environment consists of the nutrient-rich layer which supports the rapid growth of microorganisms. Complex diffusion channels are present in a mature biofilm to transport nutrients, oxygen and other components required for bacterial growth and removes waste products and dead cells

Step5. Dispersion: It is the process of dispersal of biofilm in which actively growing cells gradually sheds daughter cells. Because as long as fresh nutrients are kept providing, biofilm continues to grow and when they get nutrient deprived, they return to their planktonic mode by detaching themselves from the surface. This process probably happens to allow bacterial cells to get sufficient nutrients. There is also a possibility of the detachment process to be species-specific as *Pseudomonas fluorescence* recolonizes surface after approx. 5 hours, *Vibrio harveyi* after 2 hours and *Vibrio parahaemolyticus* after 4 hours.

3. The composition of biofilm

Biofilm is primarily composed of bacterial micro-colonies which are non randomly distributed in a shaped matrix or glycocalyx. Mostly, these micro-colonies are rod-like or mushroom-shaped or they can have one or more types of bacteria. Based on bacteria type, the composition of micro-colonies contains 10–25% (by volume) of microbial cells and 79–90% (by volume) of the matrix. Extensive bacterial growth assists in the rapid formation of visible layers of microbes accompanied by excretion of EPS (extracellular polymeric substance) in an abundant amount. At bottom of most of the biofilms, a dense layer of microorganism is bound together in polysaccharide matrix with other organic and inorganic components. The successive layer is highly irregular and loose and may extend into surrounding medium**.**

3.1 Water channels

These are present in between the micro-colonies which act as the simple circulatory system for distributing nutrients and receiving harmful metabolites.

3.2 EPS

Exopolysaccharide which is produced by the bacteria, are the major component of a biofilm. It constitutes about 50–90% of the total organic matter in a biofilm. It is mainly composed of polysaccharides, some of which may neutral or anionic in case of Gram-negative bacteria or cationic as in case of Gram-positive bacteria. The anionic property of polysaccharide is confirmed by the presence of uronic acids. EPS also contains proteins, DNA, RNA as well as some lipids and humic substances.

Factors affecting biofilm formation

A number of factors such as substratum effects, hydrodynamics and various properties of cell surface play an important role in microbial attachment.

1 Substratum effects

As the surface roughness increases microbial colonization increases because as the roughness increases, surface area increases and shear forces get diminished. And considering extent and rate of attachment, it has been seen that microorganisms get attached to more rapidly to hydrophobic and nonpolar surfaces as Teflon and other plastics rather than to glass and other materials having hydrophilic properties.

2 Conditioning films forming on the substratum

When a material surface gets exposed to any aqueous medium, it gets immediately coated with polymers from that surface or become conditioned. The coating or film is found to be organic in nature formed within minutes of exposure. The nature of these films is found to be quite different for surfaces exposed in the human host. As an example, “acquired pellicle,” a proteinaceous conditioning film, develops on tooth enamel surface. A pellicle is composed of glycoprotein, lysozymes, phosphoproteins, albumin, lipids and gingival crevice fluid. Oral cavity bacteria get adhered within hours of exposure to this pellicle conditioned surface.

3. Characteristics of the aqueous medium

Characteristics of the aqueous medium such as temperature, pH, nutrient level and ionic strength possibly play an important role in attachment of microbes with the substratum. As an example, it has been found that the attachment of *Pseudomonas fluorescens* to glass surface is affected by an increase in the concentration of several cations (sodium, calcium, lanthanum, ferric iron).

4. Environmental factors

4.1 Availability of certain nutrients

It has been shown by studies on *Listeria monocytogenes* that an optimum level of phosphate is very important for biofilm formation and gets stimulated by the presence of carbohydrates mannose and trehalose.

4.2 Presence of oxygen

Presence of oxygen regulates Biofilm formation in *Escherichia coli*. In the absence of sufficient oxygen supply biofilm does not form as bacteria could not adhere to the substrate surface.

4.3 Environmental pH

Environmental pH effects were observed by studying on *Vibrio cholerae*. Optimal pH for multiplication of *V. cholerae* is 8.2 and below pH 7 i.e., in acidic environment the bacteria lose their ability to form biofilm as they lose mobility.

On the other hand, bacteria like *E. coli* do not need an alkaline environment for multiplying hence they easily form a biofilm on urethral catheters where urine pH is acidic.

4.4 Temperature

When temperature was kept high, most of the organisms did not form biofilm as the bacteria wasn’t able to adhere itself to the substrate surface

Diseases due to biofilm

Besides infecting the industrial pipelines, waste water channels, oral cavity, ventilators, catheters, and medical implants, they are a major cause of human diseases. Infections and diseases in humans are mostly due to development of biofilm on or within indwelling implants or devices such as contact lenses, bio prosthetic and mechanical heart valves, pacemakers, stunts, IUCD’S etc.

Gram-positive microorganisms	**Site of infections and diseases**
Acidogenic gram-positive cocci (e.g. *Streptococcus*)	Dental caries
Gram-positive cocci (e.g. *Staphylococci*)	Musculoskeletal infections
S. epidermidis, E. faecalis	Urinary catheter cystitis
S. epidermidis, S. aureus, Corynebacterium species, *Micrococcus* species, *Enterococcus* species, *Candida albicans*, Group B *Streptococci*	IUDs

Viridans Streptococci, Enterococci	Mechanical heart valves
Hemolytic Streptococci, Enterococci	Orthopedic devices
Gram-negative microorganisms	**The site of infections and diseases**
P. aeruginosa and Burkholderia cepacia	Cystic fibrosis pneumonia
Klebsiella pneumoniae, Proteus mirabilis	Urinary catheter cystitis
K. pneumoniae, P. aeruginosa	Central venous catheter
Proteus mirabilis, Bacteroides species, *P. aeruginosa, E. coli*	Orthopedic devices

Applications of Biofilms

Plant Protection Agents

Biofilm formation triggers a number of beneficial effects such as biocontrol and symbiosis. In plants, bacterial biofilms can be formed on the surfaces of leaves, roots, and stems. Biofilm-forming rhizobacteria can act as biocontrol agents due to their successful colonization of plants surfaces). Such rhizobacteria belong to *Bacillus, Pseudomonas sp*. Beneficial bacteria could also be used as biofertilizers to promote plant growth through nitrogen fixation, mineral nutrient uptake, phytohormone production, and disease suppression as well as protection from both biotic and abiotic stresses.

For example, *Bacillus subtilis* is a prominent rhizobacterium which is used as an efficient biocontrol and growth promotion agent to protect plants from bacterial and fungal pathogens due to the formation of robust biofilms and the production of several antagonistic metabolites. These metabolites mainly include lipopeptides (such as surfactin, iturin, and fengycins), bacteriocins and siderophores. The colonization by *B. subtilis* in plant roots is associated with surfactin production and biofilm formation, and the surfactin confers protection of plants from pathogens.

Bioremediation

Bioremediation is a process that employs living organisms or their derivatives for treatment of hazardous substances from the environment (soil, water, and air) into lesser or harmless compounds. It is thought to be a better option than conventional physical and chemical remediation measures with regard to cost and environmental safety. Moreover, biofilm-mediated remediation methods exhibit higher efficiency in transforming toxic wastes because of improved bioavailability of the pollutants to degrading organisms and enhanced adaptability of degrading microorganisms to different toxic compounds. Microbial bioremediation can be at the site of contamination (*in situ*) or off the place of contamination (*ex situ*). It can be achieved through the incorporation of limiting nutrients and electrons (biostimulation) or by the addition of microbes at the polluted sites (bioaugmentation) to promote the transformation process.

Biofilm mediated remediation can harbor diverse species of both aerobic and anaerobic bacteria that often use the degradation of pollutants as an energy source.

During aerobic degradation, bacteria can use oxygen as final electron acceptor to breakdown toxic contaminants into innocuous products, mainly carbon dioxide, and water. In anaerobic conditions, electron acceptors such as nitrate and sulfate can play the role of oxygen to transform contaminants into less toxic or harmless substances and the byproduct may depend on the electron acceptor.

Wastewater Treatment

Wastewater is composed of a broad range of organic and inorganic contaminants originating from storm water, agriculture, industry, domestic, and commercial sewage. Bacterial communities have been employed to neutralize and degrade organic and inorganic compounds in wastewater through the use of biofilm-based wastewater treatment technology. The basic nutrients present in wastewater are mostly nitrogen and phosphorous. Hence, among the bacterial species used in wastewater treatment are often denitrifying species or those capable of neutralizing phosphorous.

Biologically active carbon (BAC) process, one of the water treatment biotechnologies, uses granular activated carbon (GAC) as a water filtration media to physically remove water-borne disease causing microorganisms, organic matter and in organic substances. After the GAC media particles became exhausted, the rough porous surfaces of this GAC are amenable to colonization of bacteria and formation of bacterial biofilms, which degrade phosphorous and nitrogen-containing compounds, organic carbon as well as other entrapped contaminants in the influent water

Biofilms can also be used in bioelectrochemical systems. BESs are bioreactors that utilize microorganisms as catalysts to convert the energy present in organic wastes into electrical energy. BESs can facilitate wastewater treatment, bioremediation as well as production of power, fuels and chemicals

Prevention and Control of Corrosion

Both chemical and biological factors can accelerate the rate of corrosion. Obviously, the activities of microbes on surfaces of metallic materials can either inhibit or promote corrosion. Recently there has been increased interest in the use of beneficial bacterial biofilms to prevent corrosion because of their effectiveness, cost effective and nature friendly behavior. The potential strategies may involve: (i) removal of corrosive substances such as oxygen by aerobic bacteria through respiration; (ii) inactivation of corrosive inducing bacteria like sulfate reducing bacteria by inhibitory antimicrobial compounds secreted within biofilms; (iii) production of protective coats such as γ-polyglutamate by biofilms; and (iv) biofilms formation serving as a diffusion barrier to hinder dissolution of metals. A gramicidin-S-producing *Bacillus brevis* biofilm has been reported to curtail the rate of corrosion in mild steel by suppressing the growth of sulfate-reducing bacterium *Desulfosporosinus orientis* and the iron-oxidizing bacterium *Leptothrix discophora*.). Anticorrosive approach via beneficial biofilms has been successfully reported for stainless steel, carbon steel, copper, and aluminium. The use of bacterial biofilms for prevention and control of corrosion is a relatively new direction and deserves special attention.