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# A Review: Role of Bacterial Exopolysaccharides in Biofilm Formation

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## ABSTRACT

**Biofilms are a group of microbial cells that are attached to various abiotic or living surfaces and submerged in an extracellular polymeric substance produced by these microorganisms. Biofilm-producing bacteria are more resistant to antibiotics compared to planktonic cells and that is why nowadays, for the removal of pharmaceuticals from the environment biofilms are used. The presence of various substances in water sources is a major concern these days because it was observed that continuous accumulation of these active compounds in water causes harm to various aquatic organisms. Therefore, removal of these antibiotics from water bodies is compulsory and for this biofilm-producing bacteria are used in various studies. This review aims to determine that compared to planktonic cells, how bacterial biofilms are more effective for bioremediation of antibiotics from the environment.**

***Keywords***- Bioremediation, Accumulation, Antibiotics, Biofilm, Planktonic.

## I. INTRODUCTION

Bioremediation is a biological process in which pollutants like personal care products, heavy metals, hydrocarbons, drugs, etc., are removed using microorganisms under controlled conditions into simple products [1]. Those microorganisms which can degrade such pollutants from the environment are known as bioremediation and they can be fungus, algae, bacteria, etc. Bacteria can produce varied biopolymers (having different chemical properties) because of the accumulation of simple to complex substrates. Certain biopolymers serve the same purpose, whereas others are taxonomic-specific and have various biological functions [2,3]. Depending on their cellular location, biopolymers may be extracellular or intracellular. There are few intracellular biopolymers with limited applications; however, extracellular biopolymers are numerous and can be divided into four categories: inorganic polyanhydrides like polyphosphates, polysaccharides, polyamides, and polyesters [4] and have been generally referred to as extracellular polymeric substances, micro capsular polysaccharides, and slime [5,6].

The biofilms are communities of microbial cells that are connected to solid surfaces. Extracellular components like proteins, lipids, and exopolysaccharides enclose these bacterial cells in a slimy extracellular matrix [7]. In comparison to individual bacterial cells, biofilm-forming bacteria are more resistant to nutrition depletion, pH variations, temperature, and the effects of reactive by-products of oxygen, disinfectants, and antibiotics.

The presence of drugs in water bodies changes behavior or causes the death of many fishes and other marine animals as explained in several studies, for example, Ibuprofen, the third most highly consumed antibiotic, when exposed to a Mediterranean mussel (*Mytilus galloprovincialis*), it was observed that the action of the enzyme catalase, superoxide dismutase, glutathione reductase, phase II glutathione S-transferase and was increased up to the seven days and also the activity of lipid peroxidation level and membrane damage in digestive gland was found increasing in mussel [8,9]. Therefore, removal of such antibiotics from water sources is necessary and for their removal biofilms are used, which can utilize these active compounds and degrade them into other simpler forms.

***Biofilm formation***

An exopolysaccharide plays important role in information of biofilm as it is a highly complex process in which the planktonic cells of bacteria exhibit to sessile growth mode [10]. Bacterial exopolysaccharides form biofilms to protect bacterial cells from unfavorable conditions and the occurrence of nutrient deficiency [11]. There are mainly five stages for biofilms formation i.e., Early development of biofilm formation, Maturation, dispersion, reversible attachment and irreversible attachment as shown in Fig.1.

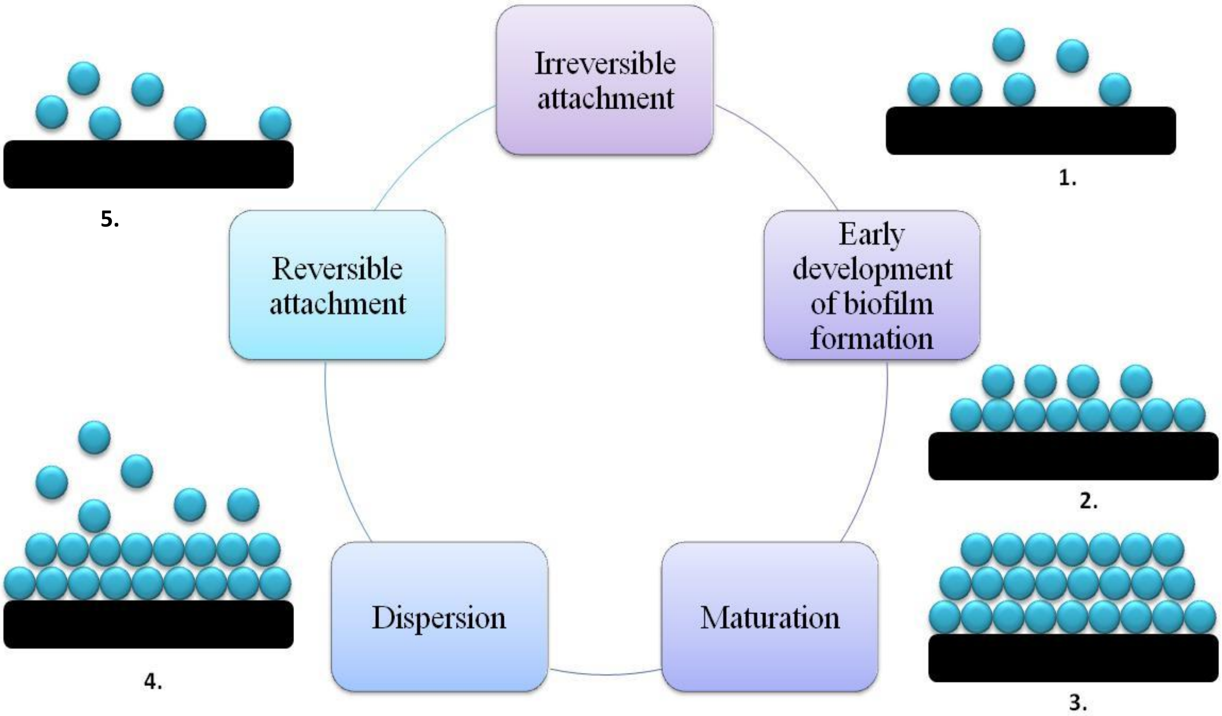

**Figure 1: Stages of Biofilm Formation**

***i. Early development of biofilm formation:*** In this stage, the bacterial cells are capable to adhere to the cell surface or any other microorganism already present on it. Through coated and hydrophilic surface prominently favors the cell's attachment and the formation of biofilms. A solid and liquid interface can provide an ideal environment for attachment [12]. There are some other favorable conditions like the presence of nutrients, proteins, carbohydrates, locomotor structures, and temperature.

***ii. Maturation of Cell:*** In this stage, mature cells of the individual bacterial cells now come nearer to each other and attach to the surface and thus forming microcolonies.

***iii. Cell proliferation and growth:*** In this stage, the biofilm-forming bacterial cells start to continue dividing and as a result of that, they release some chemicals released that part that resembles the form of signals when it crosses to minimum threshold resulting in inactivating the production of the exopolysaccharide matrix [13].

***iv. Dispersal of cell:*** In this stage the bacterial cells grow and adhere to acquire three-dimensional structures and the expression of particular genes, as well as water-filled channels for the transport of nutrients, takes place. These water channels provide not only nutrients as well as remove waste materials also.

***v. Irreversible attachment:*** In this stage, the bacteria stop producing EPS so that it would release from the biofilms and form new cells. The dispersion of bacterial cells from biofilms due to quorum sensing and have some characteristics like antibiotic resistance and some of the cells are able to return to their original planktonic phenotype quickly [14].

***Secretion of EPS***

Polysaccharides are actively released by living cells. In case of bacterial polysaccharides and proteins, there are several biosynthetic routes and separate export mechanisms, like, the translocation of EPS through membranes of bacteria to the surface of a cell or into the surrounding medium. Bacteria may create extracellular DNA during growth, but it's unclear about the DNA that it is secreted actively or released passively when the cell membrane permeability increases. Another route for extracellular polymer substances release is the sudden release of integral cellular components from the cells like LPS (lipopolysaccharides) from gram-negative bacteria on the outer membrane. This might occur via the development of blebs, which are a typical mechanism of secretion in Gram-negative bacteria [15]. Surface blabbing is a method in which cellular macromolecules such as periplasmic substances and the components of the membrane (such as LPS, phospholipids, nucleic acids, enzymes) are released into the extracellular environment as membrane vesicles during normal development. This method may release cellular components because of processes of metabolic turnover. Alternatively, vesicles of membrane containing hydrolytic enzymes (such as peptidoglycan hydrolases) may destroy surrounding biofilm cells ("predatory vesicles"), releasing nutrients for the bacteria that form vesicles in biofilms.

### *Bacteria producing exopolysaccharides*

Microbial exopolysaccharides (EPSs) have recently received a lot of attention owing their health advantages and are now being recognized as high-potential compounds [16]. According to their activities, microbial polysaccharides are divided into three groups: capsular polysaccharides like K30 Antigen, intracellular storage polysaccharides like exopolysaccharides including cellulose, dextran, and xanthan [17]. Exopolysaccharides are produced by both prokaryotes (eubacteria and archaebacteria) and eukaryotes (fungi, algae, and phytoplankton). The enzymes that make exopolysaccharides (EPSs) are found in the cell wall [18]. Dextran, xanthan, curdlan, cellulose, succinoglycan, and colanic acid are examples of bacterial exopolysaccharides. *Acetobacter spp*., *Pseudomonas aeruginosa*, *Lactobacillus helveticus*, *Lactobacillus rhamnosus*, *Xanthomonas spp*., *Shigella spp*., *Escherichia coli*, *Salmonella spp*., *Enterobacter spp*., and other bacteria produce exopolysaccharides [19].

### *The physiological role of Exopolysaccharide in biofilm formation*

Exopolysaccharide plays a key part in the production of biofilm on solid surfaces, which is one of its most well-known physiological functions. Because exopolysaccharides have a glue-like nature, they integrate most of the biofilm-forming processes on solid substrates, whereas DNA and proteins perform a minor role. Also, it has been shown that biofilm-forming bacteria are more resistant to antibiotics and surface-active compounds than planktonic bacteria. The matrix of exopolysaccharides that surrounds the cells serves as a barrier to the reactive biocides [20] and influences the microorganism's virulence factor and pathogenicity [21]. The exopolysaccharide in extremophiles serves as a protective layer for the cells in severe environmental conditions (radiation, pH, salinity, temperature) and therefore protects the microorganisms from external environmental stress [20,22]. As a result, the production of exopolysaccharides is required for such microbes to survive. Exopolysaccharide synthesis aids in anchoring to surface, antibiotic resistance, the creation of biofilm structural scaffolds, and host immune defense mechanisms, according to genetic and biochemical investigations on such exopolysaccharides from various pathogens [23].

EPS coats cells in a gelatinous form, which is thought to protect microorganisms against predation, desiccation, and harmful metal ions [24,25]. As a result, the EPS can also be called “the microbes house” (Cloete and Oosthuizen, 2001). They fill and create a space between cells to shield the cells from the severe external environment (such as pH changes, salt, and water content, and antibiotics), as well as to act as storage for carbon and energy during starvation [26]. The cohesiveness of sludge is also a result of EPS. The organisms are held together by the molecules of EPS. As a consequence of the varying adhesion sites, a network of matrices is formed, and the resultant matrix may act like a gel [27]. Proteins and carbohydrates are the two most common EPS constituents. Humic compounds may also make up a significant portion of the EPS in biological wastewater treatment reactor sludge, accounting for around 20% of the overall total quantity [28,29]. EPS from diverse matrixes has also been shown to include uronic acids, nucleic acids, lipids, and certain inorganic components. EPS are organic macromolecules made up of identical or similar blocks that could be organized as repeating units inside molecules of polymer, as seen in numerous polysaccharides. Low-molecular-weight non-polymeric substituents in EPS may have a significant effect on their physicochemical characteristics and structure. Proteins may be glycosylated using oligosaccharides to make fatty acids, or glycoproteins can be replaced to form lipoproteins. EPS are found on or near the cell surface, regardless of their source. Active secretion includes cell lysis, shedding of cell surface material, and adsorption from the environment are all possible causes of EPS extracellular composition and localization [30].

**Table 1. Bacterial EPS General Composition: humic compounds are mentioned here.**

| EPS | Principal components (precursors, subunits) | Linkage type between subunits | Polymer's backbone structure | Substituents (e.g.) |
|---|---|---|---|---|
| **Protein(polypeptides)** | Amino acids | Peptide bonds | Linear | Fatty acids(lipoproteins), Oligosaccharides (glycoproteins) |
| **Polysaccharides** | Monosaccharides<br>Amino sugars<br>Uronic acids | Glycosidic bonds | Branched, Linear | Inorganic: phosphate, sulphate Organic: N-acetyl, O-acetyl, pyruvyl, succinyl |

| | | | | |
|---|---|---|---|---|
| **Nucleic acids** | Nucleotides | Phosphodiester bonds | Linear | - |
| **Humic substances** | Simple sugars<br>Phenolic compounds<br>Amino acids | Ether bonds,<br>Peptide bonds<br>C-C bonds | Cross-linked | - |
| **(Phospho) lipids** | Phosphate<br>Fatty acids<br>Serine<br>Glycerol<br>Sugars<br>Choline<br>Ethanolamine | Ester bonds | Sidechains | - |

### *Factors affecting biofilms Formation*

Biofilm formation is influenced by several factors such as:

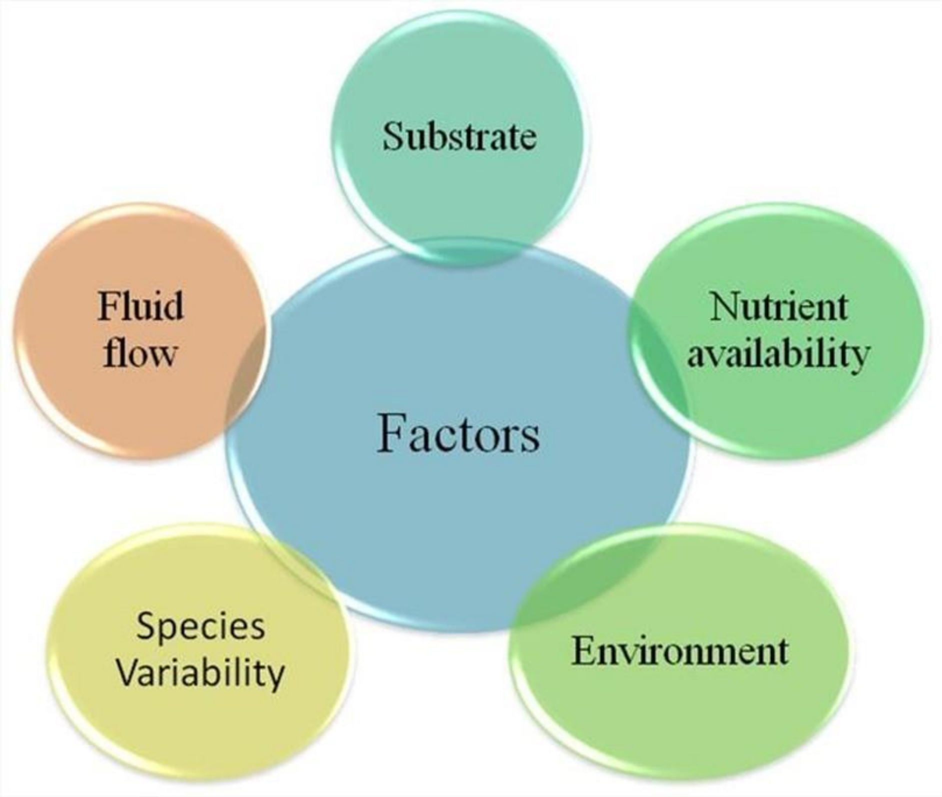

**Figure 2: Factors affecting biofilm growth**

***Fluid Flow:*** It is an important modulator of biofilm; it impacts the exchange of nutrient material as well as the structural integrity of biofilm. There have been several attempts to treat these ailments *in vitro*, including simulating the flow of blood, urine, and saliva as well as evaluating bacterial biofilms and their use in continuous flow cells.

***Substrate:*** The substrate activity plays a crucial part in enhancing the ability of biofilms, demonstrating the significant effect that various substrates may have on the morphology, thickness, and architecture of biofilms.

***Nutrients:*** The development of the biofilm-forming ability generated on silicone-elastomer catheter discs, which are examined by confocal laser microscopy and scanning electron microscopy, depends on the availability of nutrients, which include lipids, serum, and sugars.

***Species Variability:*** The ability to produce biofilms may range greatly between and among different species. The majorly used techniques are electrophoretic karyotyping and pulsed-field gel electrophoresis to demonstrate genotypic variation and slime production.

***Microbial Cohabitants:*** The existence of additional species or different bacterial cohabitants widely affect the activity. For instance, it has been shown that *Streptococcus Gordonii* binds to the common oral fungus *C. tropicalis* and *C. albicans*, but not with two other species. Based on the *Streptococcal* available favourable growing environment, the saliva either encouraged or somewhat hindered the adhesion of *C. Albicans* to *S. gordonii* [31].

***Role of Biofilms producing microorganisms in Bioremediation of pharmaceuticals***

The presence of pharmaceuticals and their active compounds in the aquatic environment has been recognized as one of the most concerning environmental issues nowadays. Every day, large numbers of drugs discharge from domestic or industrial wastewater and ultimately enter municipal wastewater. Where after entering municipal wastewater, these drugs do not remove properly even after treatment and transform into various other toxic intermediates that release out with treated water and enter into other water bodies where these drugs cause harm to aquatic fauna as well flora like feminization of male fish, inhibition of molting in crustaceans [32], changes in liver, kidney, gills [33], and may also cause damage to fish eggs or embryos [34]. So, for the removal of these drugs from wastewater, the bioremediation process is being used. Bioremediation is a less expensive as well an eco-friendly process; in this process microbes especially bacteria, which can transform toxic micropollutants into simple non-toxic compounds are used. The major group of bacteria used for the drug removal is, heterotrophs because they mainly feed on organic carbon sources [35]. In terms of pharmaceuticals removal, it was observed that biofilm bed bioreactor is much better than traditional activated sludge as explained by Zupanc and group, in which they used two types of reactors, i.e., membrane bed biofilm reactor and activated sludge, for the removal of drug diclofenac and they found that membrane bed biofilm reactor was slowly degrading the drug whereas the activated sludge shows no response. This experiment shows that compared to activated sludge, biofilm reactor can give much better results [35,36]. Another experiment on bioremediation of the same drug was done by Tang and his group in which they used intermittently fed biofilms for the removal of the drug diclofenac and they demonstrated that using these biofilms, 50% of diclofenac and atenolol were removed from wastewater treatment plant effluents [37]. These studies clearly explains that biofilms can eliminate the drugs much better than those microorganisms which cannot produce biofilms.

Bacterial biofilms are more resistant to antibiotics compared to their planktonic counterparts and because of this, biofilms are more capable to degrade broad spectrum of antibiotics such as biphenylol, *p*-chloro-m-cresol, chloroprene, 5-fluorouracil, gemfibrozil, ibuprofen, ketoprofen, naproxen, trichloroxen and valproic acid, which was all completely degraded using biofilm as observed by Onesies and Bouwer [38]. The same result was found by Escola Casas and their group, they used staged treatment system for the removal of 26 pharmaceuticals and they demonstrated that 21 out of 26 drugs were completely degraded using biofilms [39]. Other than these active compounds, various other studies were found in which biofilms were used for degradation of drugs like, for the removal of non-steroidal anti-inflammatory drug phenazone and methylaminoantipyrine, natural biofilms were used which were collected from river water. Biofilms were then incubated in both batch and continuous mode, where within 12 hours complete degradation of methylaminoantipyrine and slow degradation of drug phenazone was observed by biofilm reactor operated in batch mode [32,40]. Thus, these studies explained that treatment of pharmaceuticals using biofilms not only significantly reduces the impact of drugs in receiving surface water but also prevents further contamination in the environment [41].

## II. CONCLUSION

Biofilms are a group of single bacterial cells or also composed of the mixed microbial population like bacteria, algae, fungi, and protozoa which are embedded in an extracellular matrix of polysaccharides. Exopolysaccharide is a substantial part of microbial biofilm which helps them to survive against the deleterious effect of harsh environmental conditions. Because of their non-toxic and biodegradable nature, these exopolysaccharides formed by microorganisms during extreme environmental conditions are highly used in various biotechnological processes like bioremediation. Bioremediation is a general process in which microorganisms are used for the removal of toxic compounds from the environment. Toxic compounds like pharmaceuticals in wastewater are a major concern these days because continous accumulation of these drugs in wastewater affecting the survival of aquatic organisms. Still there are some antibiotics in wastewater which are not yet detected because of their low concentration but continuously accumulating and may causing harm to aquatic life. So, detection and removal of these undetected drugs is necessary for the environment and for this biofilm-producing microorganisms can be used to degrade these toxic compounds into other simpler forms that do not further harm the environment.

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