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MICROBIAL BIOFILMS: BENEFICIAL AND DETRIMENTAL IMPACTS

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ABSTRACT

Biofilms are matrix-enclosed microbial accretions that bind to biological or non-biological surfaces, such as stream rocks, as well as to surfaces of plants (roots) or animals (epitheliums). Accretions are often enclosed in the outer polymer layer (EPS) that the microorganism or the colonized host's defensive mechanisms may create. Biofilms are a safe mode of growth that lets cells endure in hostile surroundings and also disperses new niches to colonise. Biofilm development also occurs in a vastly diverse range of microorganisms. The biofilm formation cycle embodies a structurally complex and dynamic system that shares the characteristics of both multicellular primitive organisms and complex ecosystems. Although biofilms confer multifarious advantages to their members, such as adhesion/cohesion capabilities, mechanical properties, nutritional sources, metabolite exchange mechanism, cellular communication, defence and drug resistance (e.g. antimicrobials, antiseptics, and disinfectants), they cause other problems in the hospital environment, food industries, aquatic environments which are described herein this review article.

Keywords: Microbial biofilms; EPS; biofilm-forming microorganisms; quorum sensing

INTRODUCTION

Microorganisms were classified as planktonic, freely suspended cells for much of Microbiology's history, and characterized in nutritionally rich culture media based on their growth characteristics. The rediscovery of a microbiological phenomenon (first described by **Anton van Leeuwenhoek,1683**) involving uniform binding and growth of microorganisms on exposed surfaces, and approximately 180 years after, **Louis Pasteur (1864)** reported bacterial aggregates in wine (**Hoiby, 2014**). Although the term biofilm had been previously used in microbiological and environmental reports, it was in 1985 that **J.W. Costerton** introduced this term to the field of medical microbiology. **Hoiby (2017)** led to studies revealing surface-associated microorganisms (biofilms) exhibiting phenotype with distinct gene transcription and growth rate.
The biofilm inhabiting microbes have been documented for opting specific mechanisms for their initial attachment to surfaces accompanied by the development, growth, and finally detachment of a community edifice (**Donlan, 2000; Muhammad *et al.*, 2020**). Biofilms are produced by a myriad of microorganisms including pathogens and are usually observed on solid substrates that are immersed in or exposed to an aqueous solution, although they may form on liquid surfaces as floating mats and also on the leaves' surface, especially in high humidity climates. Besides being produced on objects living or non- living, biofilms prevalence can also be detected in food, human, medical (hospital) and industrial environments (**Zottola and Sasahara, 1994; Atlas *et al.*, 1995; Williams *et al.*, 1995; Donlan, 2002**). Biofilms exhibit multitude morphologies, depending on the bacteria and the factors under which they are formed (**Rabin *et al.*, 2015**). Biofilms have a momentous role in microbial infection's persistence as they provide these organisms with a means of protecting themselves from antimicrobials. Biofilms comprise an extracellular polymeric material (EPS) matrix composed of EPS, nucleic acid, and proteins (**Flemming *et al.*, 2007; Lopez *et al.*, 2010**). The nature of EPS is hydrophilic (more or less) but hydrophobic EPS also exists; cellulose produced by diverse kinds of microorganisms represents the classical example (Ferdinand *et al.*, 2016). Quorum sensing (cell-to-cell communication) has been demonstrated to have a substantial impact on biofilm formation. Quorum sensing allows bacteria to display an integrated response that benefits the population with the aid of different types of auto-inducers (**Smith *et al.*, 2004**).

DEFINING BIOFILMS

Several definitions of biofilms are available in the literature. For instance, **Jamal *et al.* (2015) defined** biofilm as the concoction of microbes in which their cells bind together on surfaces (living or non-living) within their extracellularly formed polymeric matrix. **According to Hall-Stoodley *et al.* (2012),** the microbial biofilm can be defined as an aggregation of monospecies or polyspecies microbial cells in a **polymer matrix** that is itself secreted **by microbial cells.** However, biofilms are those surface-attached populations of microorganisms, which play a central task in bacterial infections' persistence according to **Rabin *et al.*, (2015)**. As per **Costerton (1995)**, biofilm is a matrix of bacterial species bound together and/or surfaces or interfaces. As defined by **Garrett (2008)**, biofilms are the communities of sessile microbial cells adhered to an EPS matrix surface or interface.

HISTORICAL BACKGROUND

The observation of aggregated microorganisms surrounded by a self-produced matrix adhering to surfaces or in tissues or secretions is as old as Microbiology, with the phenomenon being described by both Leeuwenhoek and Pasteur. Biofilms had already showed (80–90 years ago) imperative in Scientific and Environmental Microbiology on submerged surfaces biofouling e.g. ships. However, the concept and importance of biofilm infections in medicine is < 40 years old and was started by the observations of **Jendresen and Glantz, (1981)** about acquired dental pellicles and **Hoiby's (2014)** observations on *Pseudomonas aeruginosa* cell's heaps from unremittingly compromised tissues of lungs and sputum. In 1985, **Costerton** introduced the word biofilm into medicine (**Nickel *et al.*, 1985**). It became clear in the following decades that biofilm infections are common in medicine, and thereafter, 'biofilm' gradually became accepted I most adequate word for the description of sessile growth *in- vivo* (**Costerton, 1989**).
A Dutch researcher, **Antony van Leeuwenhoek (1632-1723)** of Delft, the Netherlands, observed and described biofilms using his primitive microscope on matter from his own mouth 1683-1708, where he saw aggregated microbes in "the crust of the teeth "and from" particles scraped by the tongue (**Dobell, 1960**). **Louis Pasteur (1822-1895)** observed and described bacterial aggregates as a cause of wine acidification (**Pasteur *et al.*, 1864; Hoiby, 2014**). Growing biofilm forming micro-organisms was neither fascinating nor unknown to medical microbiologists for the next century. From 1933 to 1935, the term 'film', referring to bacterial adhesion, aggregation, and surface multiplication, was used to distinguish adherent (sessile) bacteria from free-swimming 'planktonic' organisms in Marine

Microbiology (**Henrici 1933; Zobell and Allen, 1935**), but earlier in 1923, E.C. Angst stated in his report submitted to the US Navy Department in 1922 that bacteria produced slime to a large extent on the ship's bottom when biofouling occurred on the surfaces (**Zobell and Allen, 1935**). Direct microscopy was used by Henrici to study biocontamination in freshwater and he observed that "it is quite obvious that bacteria were not just free-floating creatures for most of the time, but they could grow attached upon submerges surfaces" (**Henrici *et al.*, 1933**).While, the biofilms formed on the trickling filters used in wastewater treatment processes were examined through scanning and transmission electron microscopy, **Jones *et al.* (1969)** demonstrated that biofilms comprised numerous organisms morphotypes and matrix material connecting and encasing cells inside these biofilms were polysaccharides. Later in 1973, in studying microbial biofilms in industrial water systems, **Characklis (1973)** noticed they were also extremely resistant to chlorine, like disinfectants. **Costerton *et al.* (1978)** put forward a biofilm theory, relied on remarks inferred from both dental plaques as well as sessile ecosystems in mountain streams that clarified the mechanisms by which microorganisms bind to materials (living and non-living) and the advantages this ecological niche has accrued. Since that time, biofilm studies have paralleled one another in industrial and ecological settings and in Environments, more relevant to public health.

The numbers of biofilm publications were three in 1981, which gradually rose in 1996 to 170 when **Costerton** hosted the first biofilm conference of the American Society for Microbiology (ASM) in Utah, United States.

The yearly publications' number in context of biofilm research continued to increase progressively and finally touched 3251in 2013, with a collective number of 22, 887 (**Hoiby, 2014**). Later, most of the research had focused on methods like scanning electron microscopy (SEM) or traditional microbiological cultural techniques used to reveal the characteristic features of biofilms. Two major thrusts over the last decade have significantly changed our understanding of biofilms using confocal laser scanning microscopy to analyse the ultrastructure of biofilms, and examining the genes playing a role in cell adherence and biofilms formation.

COMPOSITION OF BIOFILMS

Biofilms are primarily the microbial communities wherein microbial cells bind together on surfaces (living or non-living) inside a matrix of extracellular polymeric material formed by these cells themselves and consist of DNA, proteins, RNA, polysaccharides and water. The detailed composition of biofilms as depicted in Figure1 reveals that water is the major part of biofilm which handles the flow of nutrients inside the biofilm matrix.

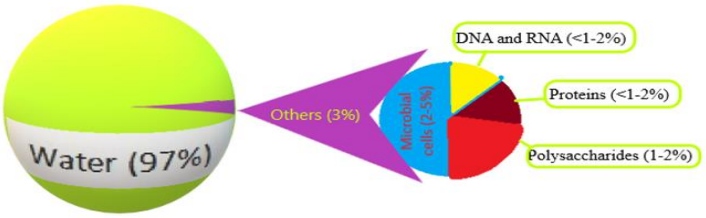

Figure 1 Composition of microbial biofilms **(Lu and Collins, 2007; Jamal *et al.*, 2015)**

Biofilm's architecture has mainly two main components (i) the water channel for the transport of nutrients and (ii) an area of tightly packed cells without prominent pores. In biofilms, the microbial cells are structured in a way with substantially different physiological and physical properties. Bacterial biofilms usually go beyond antibiotic and human immune control. Biofilm-producing microorganisms have augmented their potential to lug and neutralize antimicrobial agents and result in prolonged treatment.

Bacteria involved in biofilm formation switch to those genes that activate the expression of stress genes that turn because of certain changes, such as pH, temperature, osmolarity, cell density, and cell mass, into resistant phenotypes (**Fux *et al.*, 2005**). The biofilm water channels are primitive multicellular species when compared to the circulation system (**Gilbert *et al.*, 1997**). Different components of biofilms like DNA, proteins, RNA, polysaccharides and water indicate the integrity of the biofilm and render it resistant to different environmental factors (**Kumar *et al.*, 2008**).

BIOFILM FORMING MICROORGANISMS

Biofilms are formed by numerous and diverse bacteria, including gram positive (*Bacillus* spp., *Listeria monocytogenes*, *Staphylococcus* spp. and lactic acid bacteria, including *Lactobacillus plantarum* and *L. lactis*) and gram-negative species (*Escherichia coli*, or *Pseudomonas aeruginosa*). However, cyanobacteria form biofilms in aquatic environments (**Orell *et al.*, 2017**).

Table 1 Biofilm forming microorganisms isolated from different ecological niches

S.No	*Microbial group*	*Source of isolation*	*References*
A.	**Bacterial group**		
1.	*Fusobacterium nucleatum, Porphyromonas gingivalis*	Human teeth	**Noguchi *et al.*, 2005**
2.	*Streptococcus mutans S. sanguinis, S. mitis S. oralis* and *S. salivarius*	Oral cavity	**Hegde and Munshi, 1998; Bagg *et al.*, 2006; Krzyściak *et al.*, 2014**
3.	*Staphylococcus aureus* and *S. epidermidis*	Cardiovascular devices	**Otto, 2009**
4.	*Staphylococcus aureus*	Mucosal biopsies	**Shields *et al.*, 2013**
5.	*Enterococcus faecalis, S. aureus, S. epidermidis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas mirabilis and P. aeruginosa*	Urinary catheter	**Stickler, 1996**
6	*Listeria monocytogenes*	Pharmaceutical and food industries	**Mogha *et al.*, 2014**
7.	*Enterobacter, Micrococcus, Listeria, Streptococcus, Bacillus* and *Pseudomonas*	Dairy industry	**Waak *et al.*, 2002; Sharma *et al.*, 2003;Salo *et al.*, 2006**
B.	**Fungal group**		
1	*Candida albicans*	Denture stomatitis patient	**Chandra *et al.*, 2001**
2	*Exophiala dermatitidis*	Respiratory tract of cystic fibrosis (CF) patients	**Kirchhoff *et al.*, 2017**
3	*Candida. albicans* strain GDH2346, *C. albicans* strain M61, *Candida parapsilosis* A71	Denture stomatitis Intravascular line culture & Sputum culture	**Kuhn *et al.*, 2004**
4	*Trichophyton rubrum* and *T. mentagrophytes*	Clinical samples	**Costa-Orlandi *et al.*, 2014**
5.	*Cryptococcus laurentii*	Skin lesions	**Ajesh and Sreejith, 2012**
6.	*Candida glabrata, C. parapsilosis, C. dubliniensis, C. tropicalis, C. lusitaniae, C. krusei*and *C. pelliculosa*	Bloodstream infections	**Pannanusorn *et al.*, 2013**
7.	*Aspergillus* sp.,*Botrytis* sp., *Alternaria* sp., *Cladosporium* sp., and *Penicillium* sp.	Drinking water distribution systems	**Siqueira and Lima, 2013**
C.	**Actinomycetes group**		
1.	*Steptomyces albus*	Marine sediments	**You *et al.*, 2007**
2.	*Streptomyces* strainA4	Endocervical swabs of IUD wearers	**Shanmughapriya *et al.*, 2012**
3.	*Streptomyces clavuligerus*	KulonProgo River, Yogyakarta	**Waturangi *et al.*, 2016**
4.	*Nocardia* strain C15 and *Nocardia* strain C17	Intrauterine contraceptive devices	**Shanmughapriya *et al.*, 2012**
5.	*Arthrobacter mycorens*	Telaga Biru Lake, Cibodas	**Waturangi *et al.*, 2016**

Plant-colonizing bacteria also formed biofilms. For instance, *Pseudomonas putida, P. fluorescens*, and associated pseudomonads, which are commonly found on leaves, roots and soil, and most natural isolates form biofilms (**Rossi and Philippis, 2015**). A few nitrogen fixer symbionts like *Rhizobium leguminosarum* and *Sinorhizobium meliloti* in legumes form biofilms on legume roots. Archaea and a variety of eukaryotic organisms, like fungi such as *Cryptococcus laurentii* and microalgae (e.g. diatoms, which colonize fresh and marine environments), are also producing biofilms along with bacteria (**Orell *et al.*, 2017**). Various examples

of other microorganisms isolated from various sites that can form biofilms are depicted in Table 1.

WHEN DO MICROBES DECIDE TO FORM BIOFILM?

1. When they have to recognize specific or non-specific binding sites on a surface.
2. When they get nutrition signs.
3. Disclosure of planktonic cells to antibiotic concentrations, which are sub-inhibitory.

BIOFILM FORMATION

Over the past 20 years, biofilms have been the subject of many studies that have led to a bounty understanding of the process of microbial attachment and biofilm formation. To understand the attachment- the foremost step in biofilm formation, the properties of the substrate and the cell surface need to be looked at carefully. The substrates range from highly charged hydrophilic materials such as glass, and various metals to hydrophobic materials such as Teflon (dupont), various plastics, latex and silicone. Some materials are rather coarse or textured (for example, water pipes, environmental surfaces), whereas others are much softer (for example, silicone or Teflon catheters). Some materials also have antimicrobial properties that need to be considered (e.g. catheters impregnated with antibiotics or heart valve sewing rings, and copper-containing metal tubes or alloys). The substrate properties can have a momentous effect on the rate and degree of adhesion of microorganisms. Usually, the toughest and most hydrophobic materials (with a few exceptions) can develop biofilms more quickly (**Fletcher and Loeb, 1979; Pringle *et al.*, 1983; Characklis *et al.*, 1990; Quirynen *et al.*, 2000**). The situation becomes more complicated when one considers any substrate placed in a fluid environment (whether the sea, the blood or the urinary tract) acquires a film or coating comprising protein material present in the environmental fluid. Conditioning provides chemical properties to the substrate surface, which can completely mask the properties of the underlying substrate.
The properties of the cell surface are also important, besides substratum properties. For instance, the existence of flagella, pili, fimbriae or glycocalyx may affect the microbial binding rate. Once the microbial cell is removed from the surface, it needs to resolve the repulsive forces common to all materials. Such attachments allow cells to remain adhered to surfaces until additional stable attachment mechanisms are formed. Comparing wild-type and mutant species, **Korber *et al.*, (1989)** demonstrated that the existence of flagella allowed the binding of gram-negative bacteria to surfaces. In another study, **Rosenberg *et al.*, (1982)** demonstrated the importance of fimbriae (the bacteria's external protein constructs) for binding. It has also been found that the hydrophobic aspect of the cell surface is important for binding.

STEPS INVOLVED IN BIOFILM FORMATION

The transformation of free- floating planktonic for microorganism to associative but genetically distinct form represents the onset of biofilm formation, as depicted in Figure 1 (**Barnes *et al.*, 2012**). A precondition of biofilm formation is that bacteria should be sufficiently close to the surface. Several forces, both attractive and repulsive, come into play as bacteria reach the surface. The negative charges present on both the bacterial surface and most environmental surfaces repel each other at a distance of about 10–20 nm (**Rabin *et al.*, 2015; Palmer *et al.*, 2007**). However, attractive van der Waals forces between the bacterial cells and the surface could overcome this repulsion, in addition to the use of fimbriae and flagella for providing mechanical attachment to the surface (**Palmer *et al.*, 2007**). Following are the major steps involved in biofilm formation (**Costerton *et al.*, 1999**).

(i) Initial binding to a surface
(ii) Formation of micro-colony
(iii) Formation of three-dimensional structure
(iv) Dispersal (biofilm detachment)

(i) Initial binding to a surface

Once a microbial cell reaches the vicinity of any substratum so much that its motion is sluggish, a rescindable relation is formed either with the substratum and/or with some other microbial cell already attached to the surface (Figure 1). A solid-liquid interface system (e.g. blood, water) provides an optimal environment for biofilm formation to bind and evolve micro-organisms (**Costerton *et al.*, 1999**). Rough, hydrophilic, and coated surfaces typically provide a better condition for most regular attachment and formation of biofilms. An increase in attachment may also occur because of increases in flow velocity, water temperature, or concentrations of nutrients but not exceeding critical level. Also important is the presence of locomotive structures on cell surfaces, such as flagella, pili, fimbriae, proteins or polysaccharides, and may provide an advantage in biofilm formation when there is a mixed community (**Donlan, 2002**).

(ii) Formation of micro-colony

As bacteria attach to the surface of any physical or biological material, the emergence of a micro-colony is accompanied by its permanent attachment to the surface led to the formation of a complete micro-colony. Bacterial multiplication starts in the biofilm thanks to chemical signals. The genetic mechanism of exopolysaccharide production is activated when the signal intensity crosses certain thresholds (**Costerton *et al.*, 1999**). Henceforth, the proliferation of bacterial cells that occur within the entrenched EPS matrix ultimately results in the formation of micro-colonies by using such a chemical signal (**Mckenney *et al.*, 1998**).
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(iii) Formation of three-dimensional structure and maturation

The expression of genes linked to biofilm is triggered past a micro-colony forming stage of biofilm. The products of these genes are indispensable for the main structural component of biofilm, *i.e*. EPS. Adherence of bacterial cells itself is documented to be associated with the formation of extracellular polymeric matrix. Once the matrix is formed, the transport of nutrients occurs through water-filled channels formed within biofilms. Researchers have compared these water channels with the circulatory systems where transport of nutrients and removal of waste/ toxic materials within the micro- colonies of biofilms are carried out by these channels (**Parsek and Singh, 2003**). After achieving critical population density, biofilm starts producing planktonic microorganisms. These free-floating organisms escape the biofilm and colonize on other surfaces. Scarcity of nutrients, low pH and build-up of toxic metabolites led to the inactivation or death of these bacterial cells (**Dunne, 2002**). In this phase, past matured biofilm formation, the microbial cells became ready to disrupt from the surface.

(iv) Dispersal

Owing to the depletion of the nutrients in mature biofilms, the dispersion and migration of bacterial cells from biofilms began**.** Finally, the microorganism detaches from the macrocolony (**Veerachamy *et al.*, 2014**). Scientists have often seen that generally, bacteria leave biofilms themselves regularly after biofilm formation. In this way, bacteria multiply and disperse at a faster rate and bacterial cell's segregation from mature biofilms can be viewed as a naturally learned function (**Costerton *et al.*, 1999**). Occasionally, because of some mechanical stress, bacteria are detached from the colony to the surrounding area. In most cases, however, some bacteria stop EPS production accompanied by release into the environment. Biofilm cell dispersion occurs either by detachment of newly formed cells from growing cells or by dispersion of biofilm aggregates because of flowing effects or by quorum-sensing (**Baselga *et al.*, 1994**). Extraction of cells in biofilm becomes possible because of an enzyme that acts on alginate for its digestion (**Costerton *et al.*, 1999**). Apparently, the process of biofilm dispersion affects the phenotype of species. The cells dispersed from biofilms have the potential to keep antibiotic in-sensitivity like properties of biofilms. Because of growth, cells dispersed from the biofilm will return quickly to their usual planktonic phenotype (**Baselga *et al.*, 1994**).

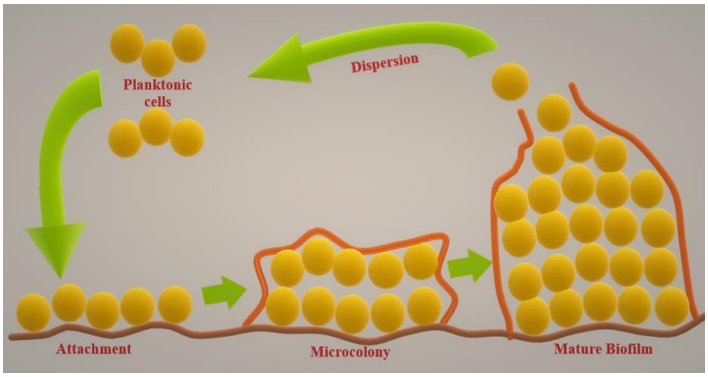

Figure 2 Process of biofilm formation **(Barnes *et al.*, 2012)**

STRUCTURE ELUCIDATION OF BIOFILMS

Biofilms have a common trait, i.e. the matrix of biofilms. Unlike liquid plankton cells, biofilm cells are integrated into a self-produced extracellular matrix, i.e. EPS that clamp them together. The major volume of biofilm is EPS (80-85 per cent) while only 15-20 per cent of the cells make up the rest of its volume.
As depicted in Figure 2, the formation of EPS matrix of biofilms consists of one or more extracellular polysaccharides, DNA and proteins (**Flemming *et al.*, 2007**). Channels in the biofilm permit the passage for air, water and nutrients to all other parts of the structure (**Zhang *et al.*, 1998**). The EPS matrix components are described herein below:

(i) Exopolysaccharides

Exopolysaccharides are either extracellularly or intracellularly synthesized or secreted into the external environment (**Nwodo *et al.*, 2012**). The electron micrographs of EPS reveal that the long linear or branched strands that attach to the cell surface resemble and extend to form large networks. Exopolysaccharides serve as a framework for the attachment of other carbohydrates, lipids, proteins, and nucleic acids. The exopolysaccharides elements, forms, and characteristics, however, differ from each other.

Nelson and his colleagues analyzed the compositions and bindings of the EPS matrix by *Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *E.* spp. Biofilms (**Bales *et al.*, 2013**). The most common carbohydrates are mannose, galactose and glucose, followed by N-acetyl glucosamine, galacturonic acid, arabinose, fucose, rhamnose and xylose. Majority of exopolysaccharides are not specific to biofilms, but their synthesis escalated under stress conditions like production of carbonic acid in *Escherichia coli* (**Prigent-Combaret *et al.*, 1999**) and the synthesis of alginate in *P. aeruginosa* (**Davies and Geesey, 1995**).

(ii) Extracellular proteins

Another important component of the EPS matrix is extracellular proteins. Some proteins bind to the surface of the cells and polysaccharides to help form and stabilize biofilms (**Frolund *et al.*, 1996**). One example is glucan-binding proteins (Gbps) in the biofilm of *Staphylococcus mutans* (**Lynch *et al.*, 2007**). By linking bacteria to exopolysaccharides, Gbps assists in maintaining the architecture of biofilms. Biofilms synthesized by Gbps mutants have been observed with drastically reduced height. Amyloids (insoluble fiber proteins) are also known to play an indispensable role in biofilm architecture. Another example is the *Pseudomonas* spp. Fap amyloids. Over-expression of Fap amyloid leads to cell aggregation and increase biofilm formation (**Dueholm *et al.*, 2013**). The Amyloid protein tasa is one of the vital components of the biofilms of *B. subtilis* (**Romero *et al.*, 2010**). Tasa forms solid fibers that hold together the biofilm cells and can tolerate severe destructive forces.

Dawson *et al.* (2021) demonstrated that *Clostridioides difficile* bioflms are composed of extracellular DNA (eDNA), cell surface and intracellular proteins, which form a protective matrix around *C. difficile* vegetative cells and spores and protect them against the effect antibiotic vancomycin.

(iii) Enzymes

Some enzymes handle biofilm degradation processes. The polysaccharides like cellulose, nucleic acids, proteins, lipids, and other components entrapped in the EPS matrix serve as substrates for enzymes. These enzymes act upon these biopolymers to provide carbon and energy sources to the biofilm cells, especially during hunger (**Zhang and Bishop, 2003**). The elimination and propagation of biofilm also require enzymatic functions. The enzyme-led internal degradation of the EPS matrix releases biofilm cells and initiates a new life cycle for biofilms. For instance, the dspb protein removes the surface of *Actinobacillus pleuropneumoniae* biofilm (**Kaplan *et al.*, 2004**).

(iv) Extracellular dnas (ednas)

Formerly, these were regarded as lysed cell debris until Mattick and his colleagues discovered that DNAse could prevent *P.aeruginosa* biofilm formation (**Whitchurch *et al.*, 2002**). The fact that eDNA not only comes from lysed cells but is also actively secreted indicates its momentous contribution in biofilm formation (**Hamilton *et al.*, 2005**). It turns out to be crucial for the attachment of the biofilm. Its negative charge acts as a repulsive force in the initial fixation, but when the interspace between the cell and the surface becomes a few nanometers, the eDNA connects with receptors on substrate's surfaces to ease adhesion (**Das *et al.*, 2010**). Additionally, eDNA found to coordinate cell movement during motility-induced *P. aeruginosa* biofilm expansion (**Gloag *et al.*, 2013**). Because it is negatively charged, edna can chelate metal cations, and some positively charged antibiotics. Ednacan chelate Mg^{2+} and enables two-component systems phopq/ pmrab, leading to resistance to antimicrobial peptides in *P. aeruginosa* (**Lewenza, 2013**) and other gram-negative bacteria. For *S. epidermidis*, eDNA has also been found to inhibit vancomycin transportation within biofilms and thus protect the bacteria embedded in the biofilm (**Doroshenko *et al.*, 2014; Otto, 2014**).

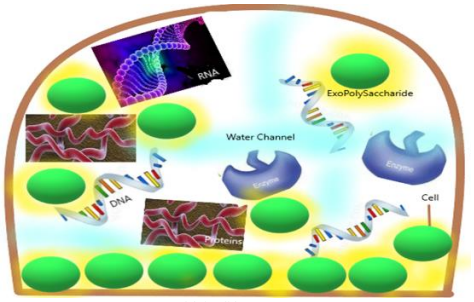

Figure 3 Structure of biofilm **(Flemming *et al.*, 2007)**

WHY DO MICROBES FORM BIOFILMS? (Wilking *et al.*, 2013; Rabin *et al.*, 2015)

i. Biofilm can enhance the tolerance of bacteria to harsh environmental conditions. Bacteria can avoid being washed away by water flow or blood stream by simply attaching to a surface or tissue.

ii. In deeper layers, the EPS matrix defends bacterial cells from antibiotics like other antimicrobials and potentially from the immune system by restricting their diffusion rate.

iii. Besides providing a suitable environment for bacterial cells, ample nutrient supply in the biofilms enables cells to bind to surfaces while holding them in optimal spaces.

iv. The proximity of bacterial cells in biofilms not only facilitates communication but also enhances the probability of genetic material exchange/ horizontal gene transfer among them.

v. The nutrient supply channel system of mature biofilms facilitates the distribution of those nutrients to different parts of biofilm, which is otherwise not possible through the process of simple diffusion.

BENEFICIAL IMPACTS OF BIOFILMS

Biofilm communities provide several advantages to their members, including easy access to food and nutrients. A few of them are described herein below:

(i) Biofilms as cell factories

Microbial biofilms serve the purpose of cell factories as they may involve either in the production of various industrially important chemicals like lactic acid, ethanol, succinic acid, butanol etc. through fermentation or in the processes of wastewater treatment and bioremediation. Generally, monospecies biofilms have been used so far for the production of industrial chemicals as they enable to regulate the controlled growth conditions required to enhance the yield of the desired compound (**Maksimova, 2014; Ercan and Demirci, 2015**).

(ii) Biofilms in food

Biofilm formation confers numerous advantages to the microbial cells in a food industry environment, such as physical resistance (against desiccation), mechanical resistance (against liquid streams in pipelines) and chemical protection (against chemicals, antimicrobials and disinfectants used in the industry) (**Flemming *et al.*, 2016).** Activities of a mixture of microbial communities that form biofilms on the surfaces of diverse food substrates, such as the olive skin, decide the fate of fermentation (**Benítez-Cabello *et al.*, 2015**). Natural olive fermentation environment comprising a complex blend of various LAB, gram-negative bacteria, and yeasts (**Botta and Cocolin, 2012**). Throughout the initial step of the cycle, gram-negative bacteria play a crucial role and achieve maximum population density on the second day after the olives are put in brine. Subsequently, the population density of yeasts or LAB, or both, slowly escalated subject to the nature of fermentation, replace the gram-negative bacterial populations and finally excrete citric or lactic acids and other volatile compounds while, absorbing the nutrients in the medium (**Hurtado *et al.*, 2012**). Of the latter, ethanol is the most abundant, tailed by methanol, while propanol, ethyl acetate, 2-butanol, acetaldehyde and dimethyl sulfide were noticed in lesser quantities (**Grounta *et al.*, 2016**). Ethanol serves as the parent to ethyl esters, the most common being ethyl acetate in terms of olive palatability. The propanol, being the principal metabolic product of yeasts, together with ethanol, imparts secondary odour to fermented olives (**Osborne *et al.*, 2000**). Therefore, irrespective of the method of olive processing, the quality, flavour, safety and taste of the final produce are determined by biofilm-inhabiting microbial cells. Cocoa (**Lima *et al.*, 2011**) and coffee (**Lee *et al.*, 2015**) represent another classical example of common foods that rely on the activities of biofilm entrenched, complex microbial communities.

(iii) Antibiotic resistance

Biofilm formation confers the cells inside with an additional advantage of resistance not only to the body's own antimicrobial agents but also to the antibiotics

that are administered normally. Indeed, bacterial cells inside biofilms as compared to planktonic (free-floating) bacterial cells of the same species might be up to a thousand times more resistant based on organism and nature of antimicrobial and investigational setup (**Mah, 2012**). There is a remarkable correlation between antibiotic resistance and biofilm formation. The strength of biofilm formation in antibiotic-resistant strains is higher than compared to the sensitive strains (**Karimi *et al.*, 2021**).

(iv) Biofilms and dormancy

Microbial biofilm communities may enter a dormant stage (unculturable but viable state) amid hostile conditions like nutrient deprivation (**Oliver, 2010**). As documented by **Epstein (2009)**, members within microbial biofilm communities wake up from the dormancy state periodically in a process similar to "hiring out spies" to "protect the biosphere" and subsequently test its feasibility for the growth, development and establishment of microbial population. Under this situation, once the resurgent cells realize that now the favourable conditions have replaced the former hostile environment for their growth, these cells then signal rest of the cells to re-surge.

(v) In natural environment

To sustain life on earth in a protected and safe environment, microbial biofilms have a significant role to play. Much of earth's living biomass comprises microorganisms that play indispensable roles in nutrient recycling necessary for the sustenance and survival of life as documented in a report cited in "Global Environmental Change: Microbial Contributions, Microbial Solutions". It has been erudite that those microbes frequently live in EPS- matrix entrenched microbial communities within biofilms on the surface.

(vi) Wastewater treatment

Biofilm formation can be viewed as well - structured automated technology where solid media components suspended in the growth vessels (fermenters/bioreactors) serve as bushings for the attachment of biofilm microbial communities so that their concentration along with removal of contaminants from biofilms through bioaccumulation, biomineralization, biodegradation and bio-sorption could be increased (**Sehar and Naz, 2016**). Various biofilm reactors have been developed for wastewater treatment, such as membrane reactors, moving beds, fluidized beds, and rotating contactors (**Huang *et al.*, 2019**). Microbial communities embedded in the biofilm matrix (bio-filter) carry out the breakdown of carbon, phosphorus and nitrogen containing nutrients along with entombed wastewater pathogens. After passing through a bio-filter, the filtered water thus got could either be released into the ecosystem or used for other farming/ agriculture like recreational purposes. A schematic representation of biofilm – mediated removal of pollutants from wastewater is illustrated in Figure 3. Low operating cost and efficiency, low hydraulic retention period, small space necessity, tolerance to environmental changes, enhanced biomass concentration and persistence time, augmented potential for the degradation of recalcitrants but reduced rate of microbial growth for minimized output of sludge are some advantages conferred by biofilm-mediated wastewater treatment process.

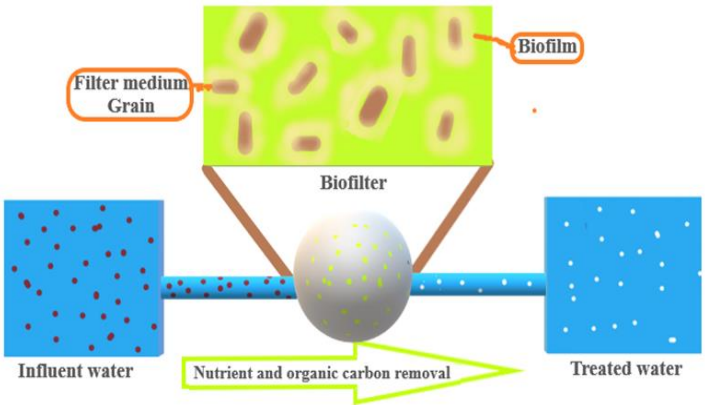

Figure 4 Biofilm-mediated removal of pollutants from wastewater **(Sehar and Naz, 2016)**

(vii) Microbial leaching

Biofilms are organized communities of mono or polyspecies of microorganisms, adhere to a solid substratum and enclosed in an extracellularly produced EPS matrix. Biofilms act like a shield for protecting embedded microbial cells within it from the action of various antimicrobials like metals thus, providing additional advantage of concentrating Fe^{3+} (oxidizes the metal sulfide bond) ions during metal leaching process. The prerequisite step in biofilm formation is surface attachment and is very important for mineral oxidation. *Acidithiobacillus ferrooxidans*, *A. caldus* and *Ferroplasma acidarmanus* are some of the examples of acidophilic microorganisms that have been documented to involve in biofilm formation (**Edwards *et al.*, 2001**). Attachment to the surface is not spontaneous, as it ensues at interrupted sites like cracks and the boundaries of mineral grains (**Gehrke *et al.*, 1998**). A number of genes (structural and genetically encoded) that regulate the process of biofilm development have been identified. Biofilm formation in some bacteria is mediated by N-acyl homoserine lactones (HSLs) like quorum sensing molecules, which enable cell-cell communication (**Stanley and Lazazzera, 2004**) and HSL production has been characterized in *A. ferrooxidans* (**Farah *et al.*, 2005**). The EPS matrix facilitates the physical binding to the mineral surface accompanied by complex formation of Fe^{3+} with exoplymer and subsequent attachment to mineral through electrostatic forces in *A. ferrooxidans*. Neutral sugars and lipids are the main constituents of *A. ferrooxidans* EPS (**Kinzler *et al.*, 2003**) that are produced from UDP-glucose, UDP-galactose, and dTDP-rhamnose precursors (**Barreto *et al.*, 2005**).

(viii) Remediation of contaminated soil and groundwater

One of biofilm's less apparent beneficial uses is to clean up oil and fuel spills because some naturally occurring bacteria love to use oil and gasoline in soil as their food source. Once noxious organic pollutants like diesel, fuel oil, and chlorinated solvents are by chance released underground, the bacterial communities' native to soil change their ecological composition to such an extent that organic pollutants could be utilized as a source of their food. The name given to this process is bioremediation and, if effective, likely has the potential to transform formerly toxic organic materials into non-toxic by-products (**Yadav, 2017**).

Bioremediation results in (i) a reduction in both the concentration of contaminants and the mass of certain subsurface contaminants (e.g. petroleum hydrocarbons and chlorinated organics) and/or (ii) a beneficial speciation shift in the biofilm bacteria that allows them to treat other contaminants, such as heavy metals (e.g. mercury).

DETRIMENTAL IMPACTS OF BIOFILMS

Biofilms have some detrimental impacts too (**Coughlan *et al.*, 2016**), which are described below:

(i) In natural environment

Microbes can negatively affect environments on a global level, including producing and consuming atmospheric gases that affect climate; mobilizing toxic elements such as mercury, arsenic and selenium; and producing toxic algal blooms and creating oxygen depletion zones in lakes, rivers and coastal environments (eutrophication). Additionally, the prevalence of infectious diseases like influenza, plague, cholera, Lyme disease, and West Nile virus are correlated with climate change.

(ii) Industrial environments

In the current scenario, the most persistent and costly problems that industries are facing due to biofilms include biofouling and bio-corrosion that led to equipment damage and product contamination (**Garrett *et al.*, 2008**). In mussels and bird farms, biological microbial species block nets and cages and ultimately transport the species to space and food.

(iii) Health

Biofilms may also be responsible for a wide variety of nosocomial (hospital-acquired) infections. Biofilm-related causes of infection may include catheter surfaces, wound dressings, medical implants and many other types of medical equipments (**Sitges-Serra *et al.*, 1985; Linares *et al.*, 1997**). **Chakraborty *et al.* (2021)** demonstrated that biofilms play a crucial role in the establishment of *Mycobacterium tuberculosis* infection and protect residing bacilli from immune response and antimycobacterial agents.

Most domestic surfaces in the kitchen and bathroom, including toilets, sinks, countertops and cutting boards, are only some of the examples that biofilm populations can avidly colonize. An increase in the disease incidence on account of pathogen's presence in the domestic environment directly reflects inadequate and ineffective practices employed for disinfection and cleaning purposes.

(iv) In the aquatic environment

Aquatic biofilms are an ever-present potential health hazard in water containing and circulating systems. While Legionnaires disease is perhaps the most widely known of these hazards, a multiplicity of other microbes is regularly found in salt water, industrial water and in domestic water supplies. *Pseudomonas aeruginosa*, for example, is a common contaminant of domestic water (**Whiteley *et al.*, 2001**). Biofilms are also a preferred habitat of Mycobacteria in aquatic systems. Contamination of water distribution systems has been reported for *Mycobacterium kansasii* and *Mycobacterium flavescens* (**Schulze-Robbecke and Fischede, 1989**).

(v) Food production and preparation environments

Several food spoilage and pathogenic bacteria have been reported to attach to and from biofilms on food contact surfaces (**Zottola and Sasahara, 1994; Flint *et al.*, 2001**). The consequence of this can be food spoilage and/or food poisoning. Even with cleaning procedures consistent with good manufacturing practice, these biofilm microorganisms can remain on equipment surfaces.

CONCLUSION

Microbial biofilms is such an aggregate of microbial communities where cells remained embedded in an EPS matrix comprised of DNA, proteins, polysaccharides, RNA, enzymes and water. In almost every submerged surface, biofilms occur in both natural and man-made environments, providing an adequate and optimal Environment for the growth, activity, and interaction of different bacterial organisms. Biofilms are formed many different bacteria including gram positive and gram-negative species. Biofilm formation is a complex process, which begins with a transition from the plankton form of the microorganism (free swimming) to its genetically different linked form and is generally considered as the tactic for the survival of pathogens. Although some biofilms are beneficial, some may have detrimental impacts too.

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