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Bacterial Biofilm and Biofilm associated environmental protection

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Abstract

Biofilm is the syntrophic consortium of the microorganisms that mainly comprises of sessile groups of bacterial cells that adhere to the inanimate or living surfaces with the help of extracellular polymeric substance (EPS)and pili by forming a matrix composed of exopolysaccharide, proteins and DNA(polymeric conglomeration). The sessile group of cells forming the biofilm develop intimate connection by Quorum Sensing (QS). EPS forming the biofilm comprises glycocalyx and chemically made up of carbohydrates, proteins and nucleic acid. EPS helps those adherent cells by embedding them within a slimy layer and provides nutrients to the developing cells within the biofilm. Thus, it helps in pathogenesis of biofilm associated with infection and resistance due to the formation of impermeable layers to the penetration of antibiotics and drugs. Biofilm can be formed by the microbes depending on various different factors including cellular recognition of specific or non-specific attachment sites on surface, nutritional cures or by exposure of planktonic cells to sub-inhibitory concentration of antibiotics. Besides this pathogenicity the biofilms are able in bioremediation. In this review we are focused to exploit different strategies which are being used in bioremediation by the biofilms.

Keywords: Biofilm, EPS, Quorum sensing, Bioremediation.

1. Introduction

A biofilm is a multi-layered community of the cells that form a syntrophic consortium that remains embedded in hydrated extracellular polymeric substances (EPS). This EPS helps the cells to colonize upon the living, inert or the boundary surface. This influences the organisms that are living in the EPS matrix of the biofilm to become virulent due to that encapsulation which is responsible for its antimicrobial activity. This biofilm matrix is the preferred way for the bacteria to live in and hence it is the natural state of its existence. [1]

1.1 Different stages of Biofilm formation

Biofilm formation is a multistep process and it differs from species to different strains. There are mainly five generalized steps to describe formation of biofilm.[2]

Stage 1.a. The surface conditioning : For the initial attachment of the motile cells to the surface, which includes the formation of the conditioning layer that mainly comprises organic (proteins, electrolytes, surface active compounds and cholesterol) as well as inorganic (salts and ionic materials) compounds.

Stage 1.b. Cell-surface Interaction and attachment: It is the next step of biofilm formation which occurs rapidly. The primary colony interacts to the surface in two different ways: due to different forces like Brownian motion, gravity or diffusion or the flow of the liquid or air, or due to positioning mechanisms like flagella motility or surface appendages. The bacterial adherence to the surface may be reversible due to the interactive forces (hydrophobicity, electrostatic forces, charges interactions) involved to the single pole of the bacteria. Irreversible attachment is much more stable compared to the previous one as adherence proteins and extracellular proteins are expressed to cement the bacteria to the surface as the long axis of the bacterial cell is positioned parallel to the surface.

Stage 2 Cell accumulation and micro-colony formation: The second stage is cell accumulation which involves cell-cell co-adhesion. A multi-layer of bacterial cells forms microcolonies as mid-late colonisers adhere to primary colonisers. This occurs over a period of a few hours by the help of signalling molecules and quorum sensing pathways.

Stage 3 Extracellular polymeric substance production: After cell accumulation and adherence to the surface the bacterial cells develop an extracellular and multi-layered microcolonies which cover themselves with a layer of extracellular polymeric matrix (EPS)[3]. This extracellular polymeric matrix is consisting of polysaccharides, proteins, lipids, nucleic acid, multivalent compounds and inorganic substances. EPS is one of the major components of biofilm formation and can produce 50-90% of total biofilm mass [4]. It helps the bacterial colonies to communicate with each other and attach on any biomaterial surfaces.

In Gram negative bacteria the outside of EPS is anionic in nature due to presence of negatively charged compounds such as uronic acids and pyruvate, whereas, inside of EPS the compounds are positive in nature like calcium, magnesium. This compound gives structure straight to the biofilm to adhere strongly to the matrix. The major component present in EPS is extracellular DNA which provides the structure of biofilm.

Stage 4 Biofilm maturation: It is the fourth stage where biofilm gets matured. Biofilm is a complex architecture and has pores of different sizes where bacteria freely move within the EPS. As the biofilm gets more mature more voids spaces are produced through which nutrients, oxygen and other inorganic salts come and can freely move into the biofilm and the waste by products are removed through the void space[5]

Stage 5 Detachment: It is the separation of the bacterial cells from the biofilm layer by the physical and chemical mechanisms. Physical mechanisms like shear force that can cause erosion of biofilm. Chemical factors may stimulate detachment, for example substrate changes, nutrient changes and changes in the EPS.

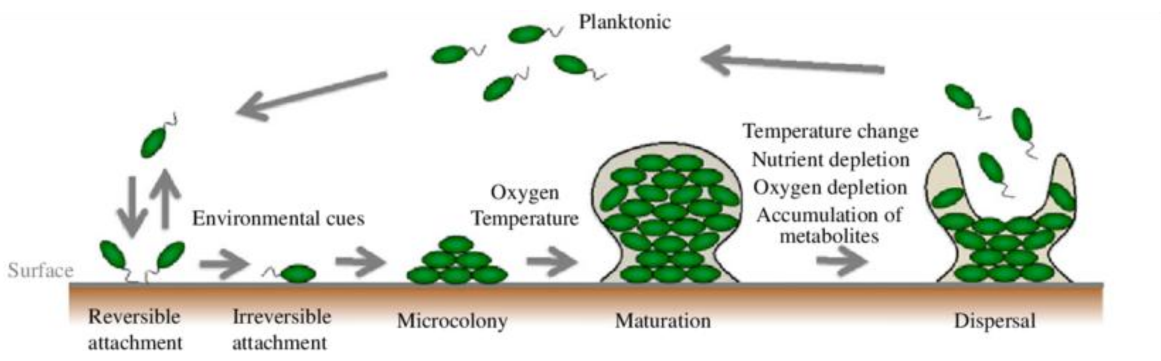
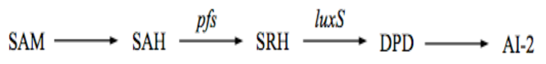


Fig 1 shows the various steps in biofilm formation. It involves irreversible adsorption of bacterial cells on the surface; production of cell signalling molecules by the biofilm forming cells; transport of substrates to or within the biofilm; substrate metabolism by the biofilm forming cells and transport of products out of the biofilm; this is accompanied by cell growth, replication and EPS production.**(**https://www.researchgate.net/figure/Environmental-factors-that-shape-biofilm-formations-Notes-The-image-shows-biofilm_fig1_279308459**)**

1.2 Role of Quorum sensing in bacterial biofilm

Quorum Sensing (QS) is the ability of the bacterial cells to sense bacterial density by cell-to-cell signalling using autoinducers (Autoinducers are signaling molecules that are produced in response to changes in cell-population density. Detection of signal molecules by bacteria acts as stimulation which leads to altered gene expression once the minimal threshold is reached (Davies 1998). These autoinducers cause aggregation of the biofilm forming cells with one another by EPS. QS is the mechanism by which both Gram positive and Gram- negative bacterial cells develop biofilm by producing signaling molecules such as small peptides, acyl-homoserine lactones (AHL) and Quinolones [6].

In Gram negative bacteria the bacterial systems employ the N-acyl Homoserine Lactones (AHL) synthesized by genes LuxI family of autoinducers and regulated by LuxR homologue which are response regulators, encodes a transcriptional activator protein detecting the cognate HSL inducing the appropriate phenotype. When AHL is bound the configuration of LuxR is altered, triggering the activation of transcription. There is another autoinducer called autoinducer-2 (AI-2), mediates quorum sensing in both Gram-positive and Gram-negative bacteria, and as such, in most cases, is referred to as the ‘universal autoinducer’[7]. AI-2 is a metabolic product of 4,5-dihydroxy-2,3-pentanedione (DPD)[8]as outlined in the pathway:



S-adenosylmethione (SAM) is converted to S-adenosylhomocysteine (SAH). The enzyme Pfs catalyses SAH to S-ribosylhomocysteine. The *luxS*gene encodes LuxS which acts on DPD to produce AI-2 and homocysteine[6] .The LuxS protein is responsible for the synthesis of AI-2, which accumulates extracellularly and then sensed and internalised by the *lsr*operon in the bacterial cell.

In contrast Gram positive bacteria use the secreted peptide as autoinducer for quorum sensing. Different bacteria have the different mechanism for quorum sensing for example *Bacillus subtilis* and *Streptococcus pneumoniae* use quorum sensing to develop bacterial competence. *Staphylococcus aureus* regulates virulence while *Enterococcus faecalis* employs quorum sensing to regulate communication among themselves. The peptide which is secreted through an ATP Binding Cassette transporter (ABC), increases in concentration with increasing cell density. The secreted peptide signals are detected by two component sensor kinases in the genetic circuit. The transcription of the target gene in quorum sensing gets altered by the DNA binding with the regulator protein which is generated due to the interaction of the peptide with the ligand molecules.[9]

1.3 Role of Bacterial Biofilm in environmental protection

Though bacterial biofilms have pathogenic effects, they possess useful effects on humans and other living organisms. For example, probiotic bacterial biofilms help in strengthening our immune system and digestion of foods. *Streptococcus thermophilus* can help to break down milk protein casein preventing allergies and promotes growth of healthy tissues in the small intestine and can be used in cancer treatments [10]. Some bacterial species such as *Bifidobacterium* sp., *Pediococcusacidilactici, Bacillus laterosporus, Lactobacillus* sp. help in neutralizing toxins, balancing blood sugar, supporting digestion and boosting immunity. Biofilms from *Alcanivorax*, *Marinobacter*, *Pseudomonas* and *Acinetobacter* can effectively degrade hydrocarbons which provide us an innovative way to deal with oil spills in the oceans[11].The bacterial cells coordinate their gene expression among their local population by cell-cell communication or quorum sensing.

1.3.1 Bioremediation of Waste-water:

The microorganisms are the main agents that cause decay in dead plants and animals as they feed themselves by the tissue of the dead plants and animals. The microbial communities break down into different nutrients like phosphorus and nitrogen containing compounds, carbonaceous materials and trap the other microorganisms present in wastewater. The chemicals released from the biofilm surface cause enzymatic disruption of the harmful micro-organism and remove them. If the waste water is passed through the biofilm, the micro-organism present on the biofilm would degrade the harmful organic materials. The biofilm treated water contains less amount of disinfectant (e.g. chlorine). In the biological treatment of the wastewater population of microorganism are placed in contact with the pollutant elements i.e. suspended solids and soluble substances of organic or mineral origin of the waste water inside the aerated tank to enhance the flocculation of the suspended solids with the micro-organism and the oxidation of soluble reducing substances. This procedure is called Activated Sludge treatment. After the removal of the pollutants the treated water is released into the environment or it can be used in agriculture.

Bioremediation associated with biofilm has the application in ground water treatment also. The design of the barrier material to accelerate bioremediation mainly depends on the contamination characteristics like concentration of contaminations, mixed contamination or the presence of the microorganisms. The presence of nutrients, electron acceptors or biocatalysts stimulates the formation of biofilm by the microorganisms in the barrier. The biofilm of the hydrogenotrophic bacteria is established on the barrier material containing the catalyst zero-valent iron has the synergistic effect on the abiotic and biological remediation of contamination containing nitrogenous and halocarbon compounds[12]. Also the supply of the dissolved hydrogen through the hollow-fibre membrane stimulate biodegradation of chlorinated solvents in groundwater.[13]

1.3.2 Polyolefin degradation:

Biological degradation is a phenomenon by virtue of which organic compounds get transformed into simpler form by agents like microbes and helps in maintaining the biogeochemical cycle. This mechanism helps the microbes to take carbon source from the degradable component and helps in their proliferation and on the other hand it results in the degradation of the waste material causing recycling of materials in the natural ecosystem.

Polyolefin can be degraded (reduction of the molecular weight of the polymer) by the microorganisms isolated from natural sources at a very slow rate. The microbial degradation of the polyethylene is associated with the formation of the biofilm which increases the hydrophobicity of the polyethylene and reduces its dry weight. Bacteria utilize plastic as their carbon source for their survival and degrade the plastic reducing their dry weight which leads the plastic to the ultimate degradation. The shelf-life of the synthetic polymers which are being widely used all around the globe is so high that itself it has become a threat to our ecology. The drawback of this mechanism is its slow rate of microbial activity thus slows down the rate of degradation of polymeric substances like PE, PET, PVC and many more. But, it has been found that this rate of degradation can be enhanced by the microbial biofilms formed over the substances. Research shows that strains of *Rhodococcusruber*, when form biofilms on the polyolefin materials can degrade upto 8% of the materials within 4 weeks increasing the rate of degradation upto 50%. Biofilm forming strain *Bacillus amyloliquefaciens* (mutant GA1) can also degrade poly(ethylene terephthalate) (PET) by attacking the amorphous region of the PET crystals, thus destroying the surface integrity of the polymer. The only major barrier seems to be the hydrophobic nature of the plastic materials which restricts bacteria's attachment to its substratum, thus restricting the biofilm formation and likewise the degradation of plastic. Mineral oil when applied on the surface of the plastic results in drastic decrease of hydrophobicity which results in the better adherence of bacterial cells effectively. This also causes effective uptake of the carbonyl groups present in the polymer and alters the physicochemical properties of the surface of the plastics, thus enhancing the degradation process. Also, various environmental factors viz. temperature, ionic strength and pH influence the interaction between microorganisms and polymeric substances upon these surfaces.[15][16]

1.3.3 Biofilm mediated heavy metal bioremediation:

Heavy metal creates havoc pollution when it gets sedimented into the water column. The microbial communities interact or can utilize the heavy metals for bioremediation purposes. Costley and Wallis 2001 reported that Cu, Zn, Cd had been removed by successive disruption cycles using biofilm. The diversity of the microbial colony enhance the biofilm activity in the packed-bed bioreactors for the treatment of the mercury-contaminated waste water.[14]. Increased microbial diversity establishes an ecological network and metabolic niches in order to allow biofilm associated microorganisms to survive in the environmental stress. Sulphate reducing bacteria present biofilm associated bioremediation are able to reduce sulphate and precipitate metal sulphides of Cu, Zn, Ni and Fe, which reduce 98% of these metals.[17]. The metal ions get immobilized to the EPS layer of the biofilm by the formation of the metal cations. This mechanism also forms complexes through electrostatic interactions between metal ligands and negatively charged biopolymeric components. The enzymes present in the EPS layer detoxify the heavy metals by enzymatic transformation. This EPS-metal interaction may be regulated by the complexity of the EPS layer as well as the bio-granules or activated sludge system.[18]

2. Conclusion:

From the present study it is observed that the microbial biofilm has a very good impact in the field of bioremediation and reducing environmental pollution. Based on the above bioremediation strategy of using biofilm we can implement it to the waste-water treatment, polyolefin degradation and remediation of the heavy-metal. It is anticipated that this approach will result in enhanced sustainability and thus a smaller environmental footprint for implementation of bioremediation solutions in the future.

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References

1. Hall-Stoodley L, Costerton JW, Stoodley P. "Bacterial biofilms: from the natural environment to infectious diseases". DOI:10.1038/nrmicro821. 2004.
2. Dominique H. Limoli,Christopher J. Jones,andDaniel J. Wozniak Nov,2015 “Bacterial Extracellular Polysaccharides in Biofilm Formation and Function” ,PMID: 26185074,doi: 10.1128/microbiolspec.MB-0011-2014.
3. Sutherland,Biofilm exopolysaccharides: a strong and sticky framework, PMID: 11160795 DOI:10.1099/00221287-147-1-3. 2001
4. Donlan, R. M., and J. W. Costerton. 2002. Biofilms: survival mechanisms of clinically relevant microorganisms DOI:10.1128/cmr.15.2.167-193.2002
5. J W Costerton, Z Lewandowski, D DeBeer, D Caldwell, D Korber, and G James, Apr Biofilms, the customized microniche.;doi: 10.1128/jb.176.8.2137-2142.1994.1994.
6. Brito, P. H., Rocha, E. P., Xavier, K. B. &Gordo, I. Natural genome diversity of AI-2 quorum sensing in Escherichia coli: conserved signal production but labile signal reception. Genome BiolEvol 5, 16-30, doi: 10.1093/gbe/evs122. 2013.
7. Schauder, S., Shokat, K., Surette, M. G. &Bassler, B. L. The LuxSfamily of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. Mol Microbiol 41, 463-476 DOI:10.1046/j.1365-2958.2001.02532.x. 2001.
8. Tavender, T. J., Halliday, N. M., Hardie, K. R. &Winzer, K. Lux Independent formation of Al-2 from ribulose-5-phosphate. Bmc Microbiology 8. DOI: 10.1186/1471-2180-8-98. 2008.
9. Bonnie L Bassler, How bacteria talk to each other: regulation of gene expression by quorum sensing, Current Opinion in Microbiology, DOI:10.1016/s1369-5274(99)00025-9.1999.
10. Whitford E. J., Cummins A. G., Butler R. N., Prisciandaro L. D., Fauser J. K., Yazbeck R; Lawrence A., Cheah K. Y. Wright T. H., Lymn K. A., Howarth G. S., Effects of Streptococcus thermophilus TH-4 on intestinal mucositis induced by the chemotherapeutic agent, 5-Fluorouracil (5-FU),pubmed/19305160. 2009
11. Bayat, Z., Hassanshahian, M., Cappello, S., Immobilization of Microbes for Bioremediation of Crude

Oil Polluted Environments: A Mini Review. .pubmed/19305160. 2015

12. Alvarez PJ, Till BA, Weathers LJ, Parkin GF, Schnoor JL. Fe(0)- based bioremediation of aquifers contaminated with mixed wastes. The University of Iowa Research Foundation USA. US Patent No. 6719902. 1998.
13. Fang Y, Hozalski RM, Clapp LW, Novak PJ, Semmens MJ. Passive dissolution of hydrogen gas into groundwater using hollow-fiber membranes. Water Res 36:3533–3542.2002.
14. von Canstein H, Kelly S, Li Y, Wagner-Döbler I. Species diversity improves the efficiency of mercury-reducing biofilms under changing environmental conditions. Appl Environ Microbiol 68:2829–2837.2002.
15. Jong T, Parry DL (2003) Removal of sulfate and heavy metals by sulfate reducing bacteria in short-term bench scale upflow anaerobic packed bed reactor runs. Water Res 37:3379–3389
16. Pal A, Paul AK Microbial extracellular polymeric substances: central elements in heavy metal bioremediation. Indian J Microbiol 48:49–64. 2008.
17. Lucas N, Bienaime C, Belloy C, Queneudec M, Silvestre F, Nava-Saucedo JE , Polymer biodegradation: mechanisms and estimation techniques. Sep;73(4):429-42. doi: 10.1016/j.2008.
18. I. Gilan (Orr) . Y. Hadar . A. Sivan, ApplMicrobiolBiotechnol ,Colonization, biofilm formation and biodegradation of polyethylene by a strain of Rhodococcusruber. 65: 97–104 DOI 10.1007/s00253-004-1584-8).2004.