Bacterial Biofilms: Deeper Insights into their Formation, Architecture and Applications

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Abstract
Biofilms are the consortium of microorganisms encased within the slimy sheath of Extracellular Polymeric Substance. These are rampant in nature, comprising a pivotal strategy used by microbes to endure severe environmental conditions. The microorganisms inhabiting the biofilms display a stratified structure, in which the role of every species is fixed and pre-determined as per the environmental suitability and synchronization. The role of quorum sensing in facilitating the communication processes in biofilms in magnificent. Biofilms can have positive impacts as well as negative repercussions, particularly with respect to industrial setting or on medical devices. In recent years, the role of biofilms in bioremediation has been realized and its potential for bioremediation is enormous and magnificent with respect to water, soil and air. The only pre-requisite for optimising such things is that the complete microbial profile, architecture and kinetics of biofilms should be known. The role of biofilms in bioremediation has attracted the role of microbiologists towards this field because of its widespread application in environment, industry and health.

Keywords: Bacteria, biofilm, quorum sensing

Introduction
Biofilms are complex microbial communities of self stabilized populations interwined together by the extracellular polymeric substrates (Douterelo *et al.,* 2018; Besemer 2015; Peipoch *et al.,* 2015; Findlay and Battin 2016; Nicholls and Crompton, 2017)), comprising of bacteria, fungi, microalgae, epiphytes, and detritus (Liu *et al.,* 2018a; Mai *et al.,* 2020; (Nadell *et al.,* 2009; Villeneuve *et al.,* 2013; Nadell *et al.,* 2016). In these communities, the autotrophic microbial assemblages play a major role in primary productivity and autochthonous carbon production (Calapez *et al.,* 2020). Moreover, heterotrophic communities can effectively biodegrade and remove organic pollutants, promoting the metabolism, mineralisation, and circulation of essential nutrients in aquatic ecosystems (Bondar-Kunze *et al.,* 2016; Mai *et al.,* 2020). The slimy sheath of biofilms is composed of

polysaccharides, proteins, DNA, lipids, humic substances etc (Pamp *et al.,* 2007). EPS constitutes 50-90% of the organic matter of biofilms, of which polysaccharides are the predominant compounds. In contrast to planktonic cells, biofilms form in an aqueous medium on a solid substratum (Flemming and Wingender, 2010). Biofilms form on the surfaces which are more hydrophobic, rough and continually conditioned by the medium. Additionally this attachment is also influenced by the hydrodynamic, turbulent and laminar forces.

The time frame of their existence dates back to 3.8 billion years and today is ubiquitous on Earth. Biofilms produce adhesive materials and exopolymers, so as to attach to surfaces and other neighbourhood cells. The matrix materials of biofilms defend the cells against various environmental stresses (Fux *et al.,* 2005; Singh *et al.,* 2009; Van and Halverson, 2004). Biofilm cells form a community which engage in intercellular activities (Davies *et al.,* 1998). The cells of biofilms are not static, but are involved in surface attached movements (Toole and Kolter, 1998). Biofilms have been compared to multicellular organs which differentiate with specialised functions (Costerton *et al.,* 1995). However the cells of biofilms are not only involved in communalism, but these also engage in competitive activities so as to antagonise the cells present in the vicinity (Moscosso *et al.,* 2011). Type 6 secretion is responsible for antagonism mechanism of biofilm microorganisms, particularly the bacteria, which comes into effect by injecting toxins, thus exhibiting both communal and competitive behaviour. In nature, the biofilm communities are stratified, wherein different organisms migrate to various positions for the access of light, air, nutrients, signalling compounds and secondary metabolites (Becraft *et al.,* 2011; Kim *et al.,* 2014; Ramsing *et al.,* 2000; Rani *et al.,* 2007).

Figure 1: Layer of biofilm on rock surfaces immersed in water (http://trollingwithlogic. com/bio-inspiration/biofilms-the-biology-of-slime-1-intro/)

Biofilm development

The biofilm developmental cycle encompasses many stages which are believed to include (1) initial attachment of microbial cell to the surface (2) microcolonies formation (3) biofilm maturation (4) dispersal (Toole *et al.,* 2000; Saucer *et al.,* 2002). The different biological mechanisms of biofilms as compared to planktonic cells can be pertained to varied microbial physiology and phenotypic response (Saucer *et al.,* 2002). The transition from planktonic lifestyle to communal biofilm community involves a change in microbes in such a manner that these starts secreting adhesins and extracellular polymeric substance which helps in binding the microbial cells together (Whitchurch *et al.,* 2002). The exopolymeric substance serves as a platform which plays a pivotal role in inter and intra-cellular communication and antimicrobial tolerance (Molin and Nielsen, 2003; Friedman and Kolter, 2003; Jackson *et al*, 2004; Ma *et al.,* 2006; Yang *et al.,* 2009). The initiation of biofilm formation occurs in response to an increase in the level of c-di-GMP, intracellular signalling messenger which regulates the bacterial lifestyle transition from planktonic cells to sessile forms (Ross *et al.,* 1987;Simm *et al.,* 2004;Ryan *et al.,* 2009;Gjermansen *et al.,* 2010;Romling *et al.,* 2013;). There is a correlation between the high c-di-GMP in the cell and biofilm formation or low c-di-GMP in the cell and motility, which has been demonstrated in several bacterial species such as *Escherchia coli, Pseudomonas aeruginosa* and *Salmonella enterica* (Simm *et al.,* 2004). It is assumed that cells use c-di-GMP as a checkpoint to proceed through distinct stages of biofilm development until they fully commit to the biofilm lifestyle, nevertheless these cells are still offered a choice to revert back any time to the original motile form (Romling *et al.,* 2013; Hengge, 2009). Biofilm determinants modulated by c-di-GMP range from flagella rotation to type-4 pili retraction, exopolysachharide production, surface adhesin expression, antimicrobial resistance, secondary metabolite production and dispersion of biofilm (Romling *et al.,* 2013).

Two varied classes of proteins with opposing enzymatic activities are involved in the synthesis and degradation of c-di-GMP in the bacteria (Henggee, 2009). Diguanylate cyclases harbouring GGDEF domains synthesize c-di-GMP from too GTP molecules, whereas phosphodiesterases harbouring HD-GYP domains degrade c-di-GMP. GGDEF and HD-GYP domains are frequently associated with sensory domains, which transcribe a wide range of environmental signals into c-di-GMP levels. Different c-di-GMP guanylate cyclases and phosphodiesterases are produces by bacteria and these are known to work in separate c-di-GMP circuits (Massie *et al.,* 2012). Additionally, biofilms formation is also regulated by small regulatory RNA's (sRNA) in many bacterial species (Chambers and Sauer, 2013). For example in *Pseudomonas aeruginosa*, the environmental signals attribute to the formation of exopolysachharides Psl and Pel which are sensed by sensor kinases **LadS** and **RetS ND** and a pair of sensor kinase/response regulator pair **GacS/Gac**A (Goodman *et al.,* 2004; Ventre *et al.,* 2006; Goodman *et al.,* 2009; Brencic and Lory, 2009;

Brencic *et al.,* 2009; Irie *et al.,* 2010). The adaptive responses play an important role in Biofilm formation and additionally, it can be terminated at any point in response to the surrounding environmental signals. In case of *Pseudomonas putida,* it has been seen that the biofilm grown on a flow chamber was able to disperse freely within few minutes of changing the nutrient medium containing carbon to the non carbon source (Gjermansen *et al.,* 2005). Biofilm formation in case *P. putida* is exclusively governed by LapA, large adhesive proteins (Gjermansen *et al.,* 2010; Nilsson *et al.,* 2011) and it has been established that its biofilm formation was regulated by c-di GMP signalling system in presence of LapA on their cell surface (Gjermansen *et al.,* 2010). Generally LapD and LapG proteins govern the presence of LapA in response to intracellular c-di-GMP level. When LapA is not repressed, the periplasmic proteinase, LapG cleaves the LapA off the cell surface. LapD protein surrounds the cytoplasmic membrane whose degenerate domains GGDEF and EAL bind to c-di-GMP. LapD protein also helps in regulating the activity of LapG proteinase by de-repressing it when the intracellular level of c-di-GMP is low and vice versa (Lopez *et al.,* 2013). A c-di-GMP phosphodiesterase gene in *P.putida* homologous to *P.aeruginosa bifA* gene has been responsible for starvation induced dispersal, which has recently been established.

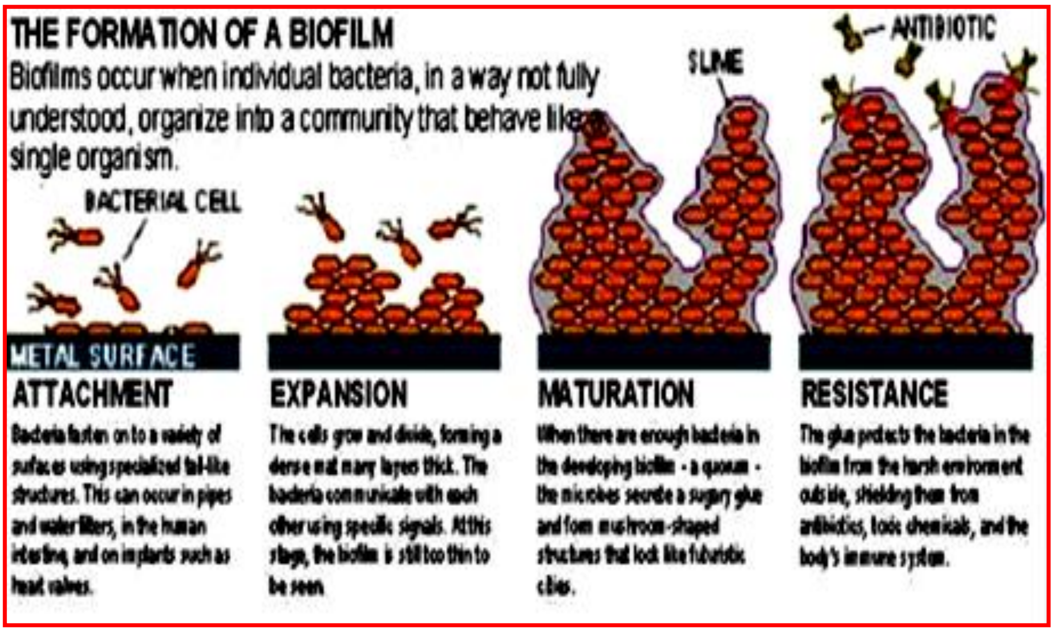

Figure 2: Biofilm developmental stages (http://thelymepost.blogspot.com /2015/01/biofilm-shield-for-lyme- disease-that.html)

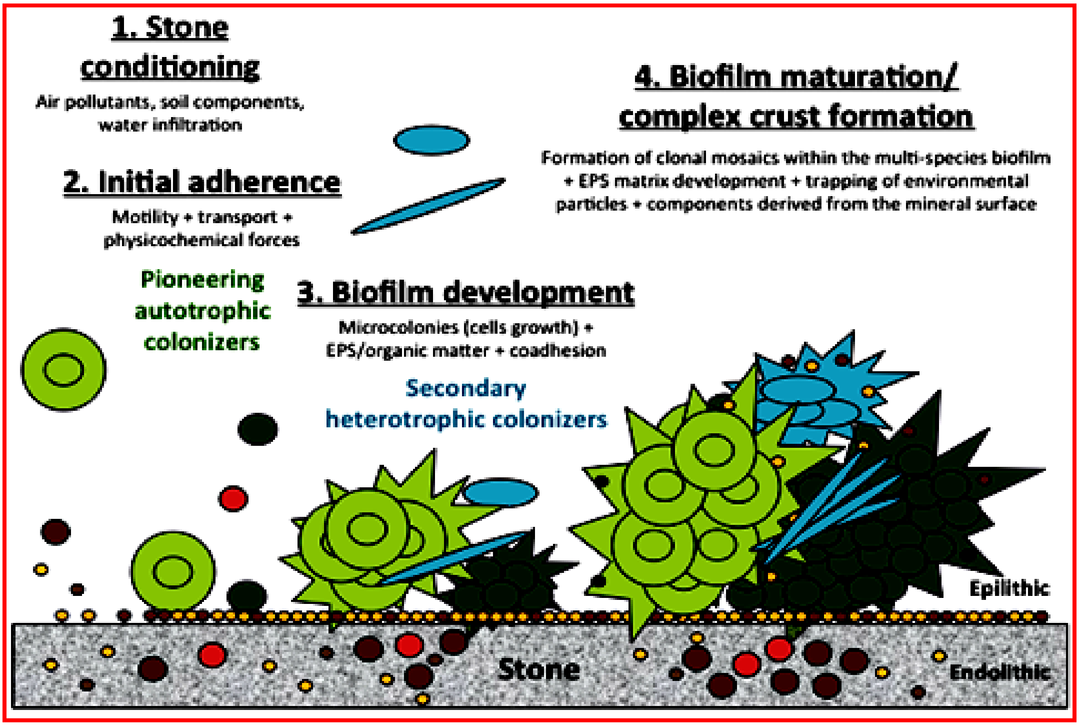

Figure 3: Biofilm development (https://www.researchgate.net/figure/fig-1-Different-successive-steps-in-the development-of-multispecies-biofilm-on-stone_fig1_301232453)

Role of quorum sensing in biofilm formation

Quorum sensing has been shown to play a pivotal role in biofilm formation for various bacterial species such as *P. aeruginosa* and *B. cenocepacia*. The flat and undifferentiated biofilms of *P. aeruginosa* were formed from a defective mutant strain of the said species in flow chambers under the conditions where large mushroom shaped colonies were formed by wild type strains (Davies *et al.*, 1998). There are certain chemicals such as Acylated homoserine lactone analogues, which inhibit the quorum sensing mechanism, thus impacting the biofilm formation of *P.aeruginosa* in flow chamber (Hentzer *et al.*, 2002). Many other studies have proven that quorum sensing signalling plays an important role in biofilm formation (Yang *et al.*, 2007; Yang *et al.*, 2009). Quorum sensing is responsible for the formation of extracellular DNA in P. aeruginosa biofilms (Holm *et al.*, 2006). In addition to this, large quantitiy of extracellular DNA is released at a later stage of biofilm formation that is regulated by *Pseudomonas* quinolone signal (PQS) based Quorum - sensing system. There is anevidence which shows that the DNA is released as a result of lysis of sub population of bacterial cells. The production of biosurfactant rhamnolipid, which also plays an important role in biofilm formation of *P. aeruginosa* is also controlled by Quorum sensing signalling (Davis *et al.,* 2003; Boles *et al.,* 2005; Pamp *et al.,* 2007). The production of siderophores, which are also important for biofilm formation, is also controlled by quorum sensing. Biofilm formation is thus a developmental process which is governed by adaptive responses of individual bacteria. This process basically involves Biofilm- specific genes that are a part of specific hierarchy pathway which is exclusively meant for regulating the transition through different biofilm stages.

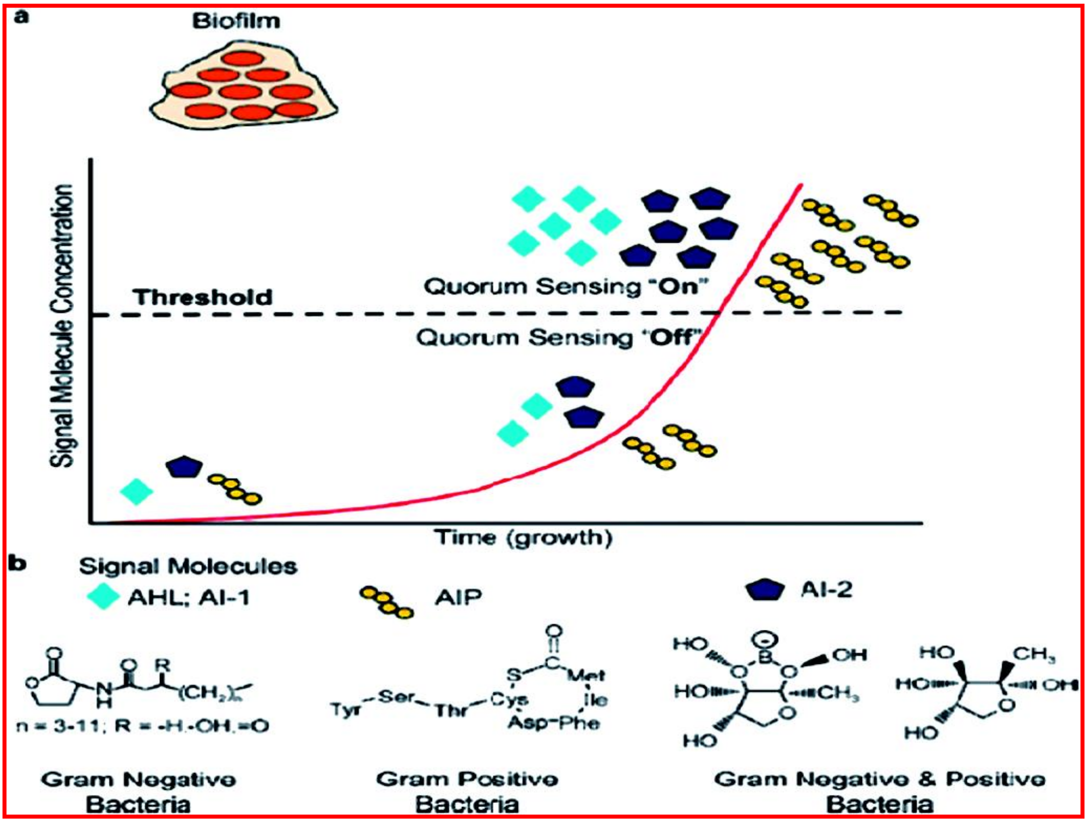

Figure 4: Quorum sensing and bacterial communication (https://pubs.rsc.org/en/content/articlehtml/2017/ra/c7ra02497e): (a) Signal molecules are generated by the bacteria and accumulate in the extracellular environment; b) Construction of AHL, AIP, and AI-2, which are signal molecules from various bacteria

The architecture of biofilms

Biofilm is composed of the microcolonies, which is entirely dependent upon the type of bacteria forming the biofilm. It has been seen that under identical conditions, *Pseudomonas putida* forms loose protruding microcolonies, while as *Pseudomonas knackmussi* forms spherical colonies (Tolker *et al.,* 2000). It has also been observed that when two *Pseudomonas* species were grown together in dual species biofilms, the individual Pseudomonas species still formed their characteristic microcolony structure without impacting each other (Tolker *et al.,* 2000). Thus the architecture of biofilms is dependent on biofilm forming bacteria. Different factors are involved in forming various structures of biofilms. In case of *Pseudomonas putida.* large adhesive proteins, LapA govern the biofilm formation in flow chambers (Gjermansen *et al.,* 2005; Gjermansen *et al.,* 2010; Nilsson *et al.,* 2011), whereas in case of *Pseudomonas aeruginosa,*biofilm formation is governed by exopolysachharides Psl and Pel (Jackson *et al.,* 2004; Matsukawa and Greenberg, 2004; Wozniak *et al.,* 2003).Different bacterial species form different structures under identical conditions, while as same bacterial species form different structures under different conditions e.g when *Pseudomonas aeruginosa* species are grown in glucose medium in flow chambers, it for mushroom shaped mini colonies and on contrary, *Pseudomonas aeruginosa* form flat biofilms when grown in citrate medium in flow chambers (Klausen *et al.,* 2003). However, the structure of biofilm can change in response to the change in nutritional condition. There are certain bacterial species that have the ability to interact metabolically when grown in flow chambers e.g. when *P. knackmussii and Burkholderia xenovorans* are grown together in chlorobiphenyl medium, the former species metabolize the chlorobenzoate produced by the latter. Additionally, when the dual species biofilm was fed with the medium infested with chlorobiphenyl, mixed species mini-colonies consisting of both the bacterial species were formed and on contrary, when the consortium was fed with citrate, that can be metabolized by both species, the two species formed separate microcolonies.when there was a shift of carbon source from citrate media to chlorobiphenyl, there was a change in the spatial structure of biofilm from separate colonies towards mixed colonies.This was optimised by the movement of *Psedomonas knackmussii* (Nielsen *et al.,* 2000). The construction and maintenance of a biofilm community depends solely on the production of extracellular polymeric substance (PSs) (Branda *et al.,* 2005; Sutherland, 2001). The PSs secreted by microbial cells vary greatly in their physical and chemical properties and thus the composition (Sutherland, 2001). Most of these molecules are polyanionic but some are neutral and polycationic.In most environments, PSs are found in an ordered hierarchy as well as composition with thin and long molecular chains. There are ample ways by which PSs can be elaborated such as environmental influence and association of PSs with other molecules such as proteins, lectins, bacterial and host extracellular DNA (e-DNA). Usually biofilms constitutes of a wide range of bacterial and fungal species in which the range of PSs may interact to generate a unique architecture (Jenkinson and Lamont, 1997). There has been a range of functional roles for PSs in microbial biofilms because of the diversity in PS structure. The biofilm landscape is formed because of the polysaccharides which are responsible for the formation of biofilm matrix. The inhabitants of the biofilm are protected by polysaccharides while maintaining an access to nutrients and also for responding to changing environmental conditions. PSs are useful for bacteria for a multitude of reasons such as adherance to different surfaces and host cells, protection from antimicrobials, acts as nutrient acquisition reservoir, thus creating an environment for the microorganisms to sustain.

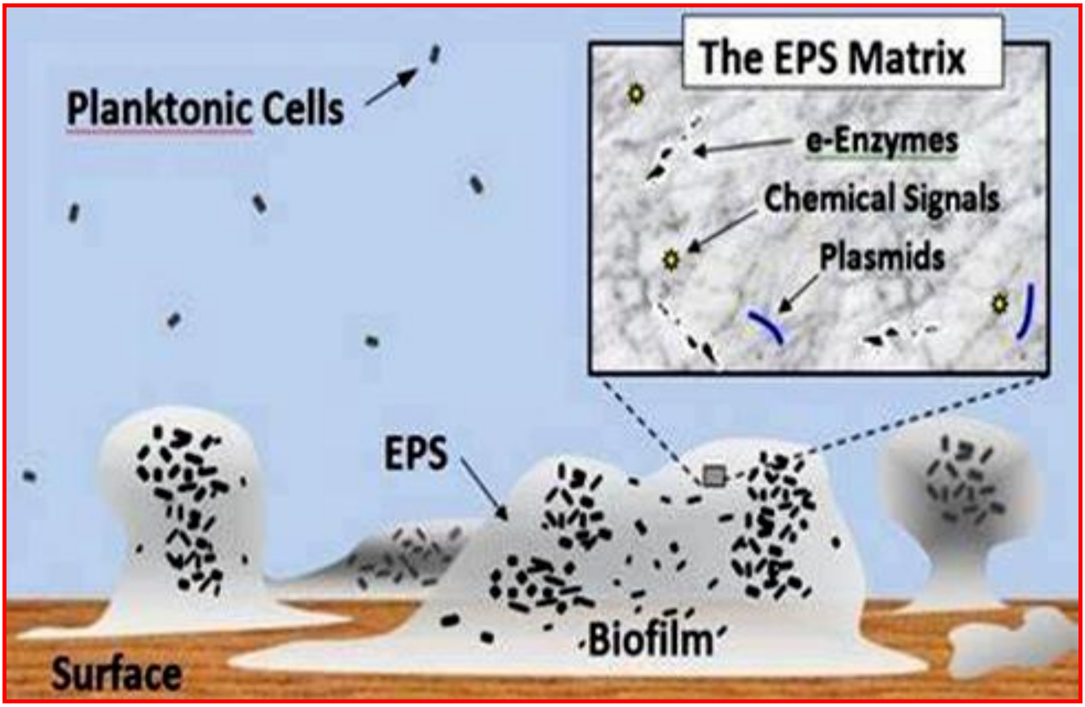

Figure 5: EPS matrix of biofilm (https://sc.edu/study/colleges_schools/public_health/research/research_areas/environmental_health_sciences/alan_decho_lab/)

Division of labour within biofilm cells

A crucial facet of the division of labor is specialization of cells for performing different tasks. Phenotypic variation can result from various contiguous mechanisms: responses to local environmental conditions, gene expression noise and genetic variation. Non genetic differences account for most of the phenotypic variation in monoclonal biofilms, also known as phenotypic heterogenity (Sclichting and Pigliu, 1998; Beldade *et al*, 2011). There is a sharp distinction between phenotypic specialization of biofilm cells and division of labour. Cell level property pertains to phenotypic specialization and colony level property pertains to division of labour.The presence of various cell types means that the cells will attribute the mechanism of division of labour but that does not imply that cells divide labour. In a biofilm, cell specialization accounts for the adaptive response to local environmental conditions but does not involve the cellular interactions between various cells. When the biofilm cells divide labour, the colony that consists of multiple cell types is able to perform better than the one which is formed solely of a single cell. This is known as an emergent property of biofilm cells which does not come into account because of local adaptation of cells to its environment. Division of labour allows cells to carry out specific tasks which avoids the metabolic burden of switching between various tasks. Consequently, one can visualise the division of labour from an emergent fitness benefits which occur at colony level due to cooperative interaction between the cells and in which different cell types are basically interdependent (Duarte *et al.,* 2011).

As we know that surface attached biofilms are heterogeneous in nature.When cells accumulate on surfaces, it results in the formation of gradients of nutrient sources, electron acceptors, waste products that are produced by the cells (Stewart, 2003; Rani *et al.,* 2007; Stewart and Franklin, 2008) and the cells of biofilms respond to such gradients in terms of physiological adaptation (Stewart and Franklin, 2008). Therefore it is expected that biofilm formation results in the physiological heterogenity of its composite cells and also that the organizing potential of biofilms can be afforded by the environmental gradients (Kolter and Greenberg, 2006). The cells of biofilms can organise and arrange themselves in spatial forms via cellular differentiation which can be attributed to the chemical gradients that have been found to be similar to morphogen gradients of Eukaryotic cells (Wolpert, 1969; Wolpert *et al.,* 2002). Subsequently this leads to the cooperative interactions between cell types and thus, the division of labour. The feedback between cellular response and enviromental conditions is a result of multicellular pattern formation with or without regulation pattern (Wolpert, 1969; Wolpert *et al.,* 2002; Bonner, 2001). The cells of biofilms differentiate in such a manner that these divide labour and also cooperate with one another. This cooperative interaction results in an emergent benefit at colony level. To characterize the potential ecological benefits of biofilm formation, one usually compares biofilms from planktons and in this two ecological differences have been discovered i.e. metabolic cooperation and protection from environmental stress. Biofilms are thus remarkable examples of biofilm construction. Life in aggregates is the most common trait founds in all life forms from animals to bacteria. This pertains to an effect, known as Allee effect, which is suggestive of the fact that it confers an inherent benefit to group on the whole, exemplified by the positive correlation between population density and mean individual fitness (Allee and Bowen, 1932). Nevertheless, grouping is beneficial most often, but its physiological consequences can lead to enhanced competition particularly when resources are low and there is high level of stress among the members of the community. Contrary to the competition, some sort of mutualistic behavior also develops between genetically different species occuring within the biofilm due to spatial proximity

(Taylor, 1992). Examples of such kind of interactions are mutual and cross- feed between two different species and efficient degradation of some pollutant by a blend of two or three species biofilm complex (Poltak and Cooper, 2011; Ramsay *et al.,* 2011;Burmolle *et al.,* 2006; Whiteley *et al.,* 2001).

Bioremediation using biofilms

With regard to the significant roles of biofilms in environment, industry, agriculture, and health, it has become an emerging topic for microbiologists. There was a late realization of the potential of microbes in biofilms for bioremediation. The synergistically beneficial relationship between different microbes present in the biofilm group makes it ideal for different contaminants to be bioremediated and for several pollutants to be degraded in the industrial sector as well. Bioremediation refers to the process of microorganism-based detoxification and degradation of harmful substances from air, water and soil. Since biofilm is made up of numerous microorganisms, it is imperative that the metabolic pathway of different microbes be distinct, which can be due to the ability of biofilm to individually or collectively degrade many contaminants (Gieg *et al.,* 2014; Horemans *et al.,* 2013). Due to the intrinsic potential of biofilms to efficiently adsorb, immobilize and degrade toxins, biofilm-mediated bioremediation has been found to be an eco-friendly, cost-effective and sustainable process for the environmental clean-up. Differential genes expressed in biofilms have been shown to be responsible for the degrading mechanisms of various microbial organisms. The distinguishing trait of biofilms is EPS, since it is primarily responsible for the immobilisation of contaminants (Sutherland, 2001). The EPS 3D structure with small oxygen concentrations towards the core carries aerobes, anaerobes, heterotrophs, sulphate reducers and nitrifiers closer to the center, thereby facilitating quicker engineered degradation of contaminants (Field *et al.,* 1995). Cyanobacterial EPS is a bioabsorbent which, in an aqueous process, can bind heavy metals (Micheletti *et al.,* 2008). Within the EPS, extracellular enzymes make it possible to maintain heavy metal concentrations and degrade other concentrations. (Flemming and Wingender, 2010). EPS of biofilms consisting of microorganisms that adsorb phosphorous and nitrogen serve as a reservoir for the recovery and thus the elimination from waste water of phosphorus and nitrogen (Zhang *et al.,* 2013). Municipal waste water is processed with the help of biofilms, which are optimized in activated sludge plants by flocular operation. The slow-sand filters that use biofilms are used to remove organic components and heavy metals from lakes and rivers (Logsdon *et al.,* 2002) Due to various metabolic processes in which ammonia is anaerobically oxidized to dinitrogen, biofilms that comprise planctomycetes in marine waters have the ability to extract nitrogen from waste water (Kartal *et al.,* 2010).

Biofilms play a significant role in water reclamation and reuse technologies and degrade toxic organic pollutants into harmless inorganic matter more commonly in water treatment systems. These constitute the intrinsic and integral part of membrane filters and biological reactors which are used for water treatment systems. Awareness of the creation, development and other facets of biofilm is very important for the optimization of water recycling facilities in order to design an efficient and sustainable scheme. With regard to water reuse technology, membrane bioreactors form an integral component which can be used for drinking water treatment, waste water treatment and water desalination. When there is an ever-increasing need for the availability of potable water along with strict standards for water safety around the world, the highest priority is various methods of decontaminating water. In such a situation, biological membrane filters can be effectively used to turn alternate sources into the supply of drinking water and also to deliver high quality water. The biological membrane filter is created by the combination of the active sludge system with the membrane filter, which removes the need for conventional gravity clarifiers. In addition, these technologies will substantially minimize waste water's nitrogen and phosphorous content (Di Giano *et al.,* 2004). One-quarter of the world's population is expected to face extreme water shortages by 2025 (Seckler *et al.,* 1999). In order to satisfy this ever rising demand, new technology for reusing and renovating water will be required. Membrane biofilters thus provide a viable method for securing a source of potable water. They can provide high-quality water, eliminate a number of pollutants, minimize the size of the required treatment plant, and can be used both for the treatment of water and waste water and for desalination.

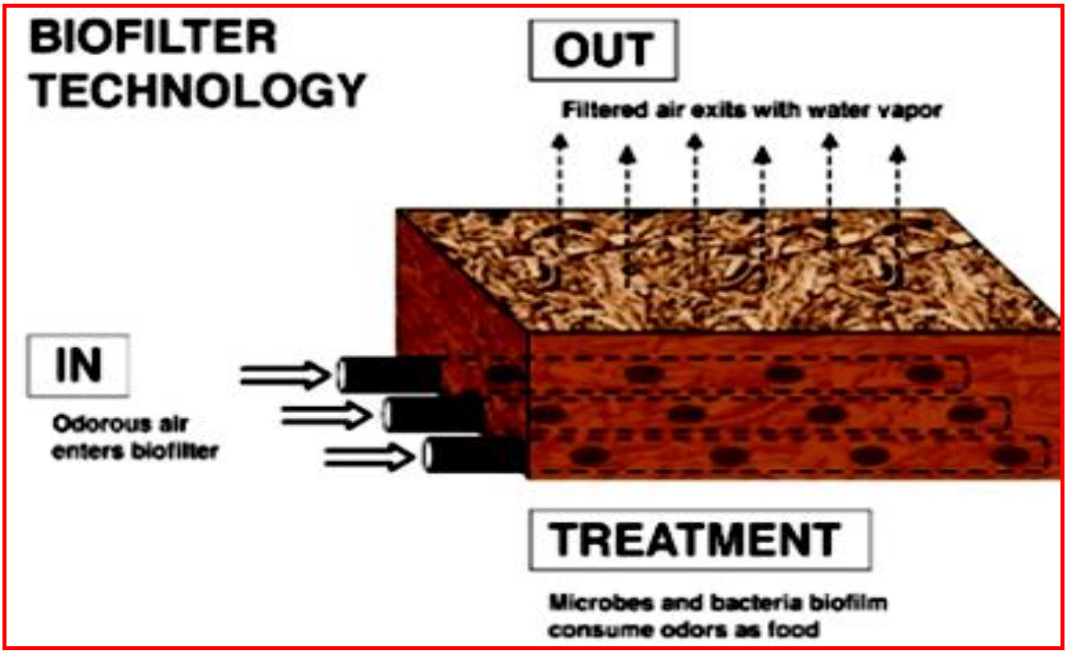

Figure 6: Biofilter technology using biofilms (http://www.seminarstopics.com/ seminar/8015/ biofilters- for-air-pollution-control/)

Air quality and biofilm

The human population spends more time indoors, which has resulted in urban air emissions in tandem with air-tight homes, increased insulation, and decreased ventilation. This has become a global epidemic, becoming a main cause of rises in mortality and morbidity. The primary form of air filtration technology currently focuses on mechanical filtration and dilution. (Fleck *et al.,* 2020). However, traditional methods of indoor air filtration have a limited range of uses for contaminants. Biological filtration alternatives are a rapidly increasing area of study that needs and improves biological systems that can serve a growing market that seeks to improve their performance and effectiveness. In such situations, biological filter systems will play a promising role in increasing the efficiency of indoor air in the interior of the home (Fleck *et al.,* 2020).

Biofiltration systems using different microbial biofilms can also be used to mitigate odorous gas emissions from livestock farming systems, which have been rising to meet the growing demand for meat consumption worldwide. This will emerge as a cost-effective and promising technology (Kumari and Tripathy, 2020).

Figure 7: Indoor air filter (https://www.slideshare.net/root66aquaponics/air-biofilters-indoor-overview121814)

Thus, we come to the view that in terms of bioremediation, biofilms have unparalleled functions. It can be used for decontamination purposes in various structures, and the only thing we need to know is its microbial composition, its organization, and the kinetics of how the biofilm culture functions.

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