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REVIEW ARTICLE

Microbial biofilms application in environmental monitoring, bioremediation and waste water treatment

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ABSTRACT

Increased contamination of the environment with toxic pollutants has paved the way for efficient strategies, which can be implemented for environment restoration. The major problem with conventional methods used for cleaning of pollutants is inefficiency and poor economics. Bioremediation is a growing technology having an advanced potential for cleaning pollutants. Biofilms formed by various microorganisms potentially provide a suitable microenvironment for efficient bioremediation processes. A biofilm is a microbially derived sessile community characterized by cells that are attached to an abiotic or living surface and embedded in a matrix of extracellular polymeric substances that they have produced. Biofilm communities provide a beneficial structure, possibility for nutrient, and genetic exchange to participating microorganisms as well as protection from the surrounding environment concerning for instance predation and chemical and shear stresses. Biofilms can also be utilized in other ways as biomarkers for monitoring of stream water quality from mine drainage. Biofilm systems are especially suitable for the treatment of recalcitrant compounds because of their high microbial biomass and ability to immobilize compounds. Bioremediation is also facilitated by enhanced gene transfer among biofilm organisms and by the increased bioavailability of pollutants for degradation as a result of bacterial chemotaxis. Many plant extracts contain phenol derivatives, terpenes, flavonoids, etc. which have ability to suppress the microbial cell attachment and biofilm growth. Strategies for improving bioremediation efficiency include genetic engineering to improve strains and chemotactic ability, the use of mixed population biofilms and optimization of physico–chemical conditions are discussed in this review.

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INTRODUCTION

Microbes adhere to the environmental surfaces extending from human tooth and intestine to the metal surfaces of the condenser tubes exposed to turbulent flow of water, for their physiological functioning, support and protection. Their association with these surfaces involves the synthesis of extra cellular homo- or hetero polymers of sugars called exo-polysaccharides (EPS). These extracellular polymers are fabricated and extended from the cells to the surfaces in a very systematic manner and they are tangled and channelized network of the polymeric fibres. The whole assemblage including the resident microbes and the channelized network of the EPS is termed as Biofilm. Because of its polyelectrolyte nature the microbial biofilms are highly absorptive and can gather significant quantities of silt, clay heavy metals and other detritus from their immediate environment. Biofilms give support to the high density of microbial biomass, which facilitates the mineralization processes by maintaining optimal pH conditions, localized solute concentration and redox potential in the vicinity of the cells. This is achieved by the unique architecture of the biofilm and controlled circulation of fluids within it. The phenomenon of mass transport in biofilms is influenced by its structure, which depends upon the local availability of the substrates. Solute transport in biofilm is driven by convective transport within pores and water channels and also by diffusion in the denser aggregates. Biofilms undergo dynamic changes during their transition from free living organisms to sessile biofilm cells, including the specific production of secondary metabolites and a significant increase in the resistance towards biological, chemical and physical assaults. Thus their unique physical, biological and chemical properties make them a very useful tool for environment cleanliness. Since the microbial biofilms are implicated in the natural and modulated environmental cleanliness systems being used for purification of the drinking water, biodegradation of organic pollutants, detoxification of the oil spills, removal of heavy metals and biodegradation of the hazardous xenobiotics in the contaminated waters and the soils, as well as environmental monitoring biosensor fabrications, here we discuss their utility in depth.

Microbial biofilm: Structure, characteristics and functions

Since the inception of microbiology, microorganisms have primarily been characterized as planktonic, freely suspended cells and have been described on the basis of their morphological and physiological properties and growth characteristics in nutritionally rich culture media. However, in most natural environments, microbes are commonly found in close association with surfaces and interfaces in the form of multicellular aggregates glued together with the slime they secrete (Wimpenny et al., 2000; Costerton et al., 1999) Biofilms are clusters of microbial cells that are attached to a surface. They occur in nearly every moist environment, where sufficient nutrient flow is available and surface attachment can be achieved. A biofilm can be formed by a single bacterial species, although they can also consist of many species of bacteria, fungi, algae and protozoa.

Basic structural units of a biofilm are microcolonies, separate communities of bacterial cells embedded into extracellular polysaccharide matrix (EPS). These microcolonies are in most cases mushroom-shaped or rod like and they can consist of one or more types of bacteria (Macleod et al., 1990). Depending on bacteria type, microcolonies consist of 10–25% of cells and 79–90% of EPS matrix (Costerton, 1999). EPS matrix protects biofilm cells from various negative environmental conditions, such as UV radiation, abrupt changes in pH values, draining. Between microcolonies, there are channels through which water flows. These water channels function in a biofilm as a simple circulatory system distributing nutrients to micro colonies and receiving harmful metabolites (Costerton, 1995). Biofilm is polymorphic and it can adjust its structure to changes in the amount of nutrients, which was demonstrated by experiments with different glucose concentrations. When glucose concentration is high, microcolonies grow fast and consequently biofilm thickness increases

significantly. When glucose concentration is decreased, biofilm biomass is reduced and the former structure is restored. Studies of biofilm in different hydrodynamic conditions, such as laminar and turbulent flow, have shown that biofilm structure changes depending on the flow type. In laminar flow bacterial micro colonies become round and in turbulent flow they extend in downstream direction (Stoodley et al., 1998). Biofilms occur in almost every humid environment, where there is sufficient nutrient flow and surface attachment is possible. It is now well known that in nature, most of the microorganisms attach to surfaces in a structured fashion on a biofilm (Chandki et al., 2011). Generally, the biofilm matrix is constituted of either water or a solvent bound to microbial cell capsules and dissolved solutes which generally determine the physical properties such as the viscosity of the matrix (Headley et al., 1998; Sutherland 2001). A biofilm's water-binding capacity and mobility in a matrix determines the diffusion process within it. The biofilm matrix is a complex of cell lysis products, particulate materials, metabolites and dissolved nutrients, secreted polymers, and debris from the immediate environment. The major macrobio-molecules (i.e., proteins, DNA, RNA, and poly-saccharides) may therefore be present in a biofilm environment with phospholipids, lipids, peptidoglycan, and other components (Sutherland, 2001; Vilain et al., 2009; Cortes et al., 2011). The anionic property of biofilms may be linked to the amount of uronic acids (e.g., D-glucuronic, D-galacturonic, and mannuronic acids). The microbial communities of a biofilm can be analyzed using microscopic and molecular methods, as has been determined by numerous studies (Azeredo et al., 2017). The more detailed structure of biofilm up to the nano scale level is possible now through new technologies (Neu and Lawrence, 2014). EPSs alone determine the living conditions of resident microorganisms in biofilms, as they affect the polarity, hydrophobicity, mechanical stability, density and water content in biofilms (Vu et al., 2009; Jain et al., 2013). The network matures with time to incorporate channels within the EPS; it enables nutrient and water transport, and electron acceptors such as oxygen or reduced compounds. EPSs can be very diverse, even in a single species of bacteria (Branda et al., 2005). The content of the EPS varies considerably depending on different factors. The nature of the biofilm depends on multivalent cations (e.g. Ca^{2+}) and is also determined by the EPS (Shukla and Rao 2013). Many microbes produce very important hydrophobic molecules for the adhesion phenomenon. These surface-active molecules, generally referred to as biosurfactants, influence the surface tension at the air-water interface and control the changing the surface tension to help to exchange gases. The EPS microbial production was reported to reduce salt stress in plants by chelating Na^{+} (Kumari et al., 2015). Several studies have shown that the biofilm matrix contains large amounts of surface proteins that play a crucial role in the architecture and stability of biofilms (Shukla and Rao, 2013). In the absence of EPSs, some of the biofilm proteins can form a mature biofilm. Biofilm proteins are generally divided into two subclasses: enzymes and proteins of structure. These enzymes, called EPS modification enzymes, are involved in EPS degradation, and act on substrates like biopolymers: proteins, nucleic acids, polysaccharides, and cellulose. The EPS nutrient storage is easily digested with the aid of these extracellular enzymes. These enzymes play a significant role in the dispersion of a biofilm, which easily releases and resettles microbial cells onto a new substratum. Biofilm-related proteins and homologous bap-like proteins are another type of relevant enzymes. Such type of proteins have a high molecular weight; tandem repeats domain, and an attachment which endorses the development of a biofilm (Latasa et al., 2006). Amyloids are the second protein component in the biofilm matrix. Amyloids are common proteins present in most biofilms. It is now clear that extracellular DNA (eDNA) is an essential part of the biofilm matrix consisting of large (~30 kb) fragments. Several reports state that eDNA support the exchange of genetic information, bacterial cell aggregation, antibiotic resistance, and determine biofilm mechanical stability. A recent study has demonstrated that DNA also facilitates cell self-organization of bacteria in biofilms (Tetz et al., 2009; Mulcahy et al., 2008).

Characteristics and functions of biofilms

Biofilms are remarkably heterogeneous. Many measurements and observations have been made of various biofilms; they all point to the diversity of individual biofilm colonies. As we have mentioned before, in typical, naturally occurring biofilms (as opposed to some that are grown in a laboratory for experimental reasons) there are nearly always a large number of different kinds of microorganisms living together. In addition, different biofilms seem to exhibit different internal structures, different chemical properties, different electrical properties, and, indeed, different properties of just about any other measurement or observation that can be made. Each of these properties seem to contribute to the characteristics of the biofilm as a whole that make it different (e.g., hard to kill) compared to dealing with each of the microorganisms in isolation (not in a biofilm, but in a planktonic environment. Here are some of the more evident characteristics common to all observed biofilms:

- Biofilms are dynamic and responsive to their environment; that is, they can adapt to changes in their environment.
- A phenomenon known as detachment seems to be common among all biofilms. Bacterial cells can detach from their biofilm colony individually or in clumps.
- When individual microorganisms detach from a biofilm, these isolated microorganisms are relatively easy to kill with chemicals designed for this purpose.
- When microorganisms detach from their biofilm colony in clumps, the clumps are pieces of the biofilm that are at the moment not attached to a surface; in this case they maintain the protective properties of the original biofilm and are thus much more difficult to kill.
- In the right conditions, biofilms can migrate across surfaces over a period of time in a variety of ways.
- Enhanced scavenging of nutrient from bulk liquid and surface
- Physiological alterations such as enhanced growth rates, higher DNA synthesis and RNA turnover rates, enhanced resistance, enhanced resistance, enhanced virulence and greater local diversity
- Physical protection and stabilization
- High densities–provide framework for coordinated and socialized behavior

In industry, biofilms cause food and drinking water contamination, metal surface corrosion, and clogging (Beech et al., 2005; Wong 1998). Due to the possibility of water contaminated with pathogens, biofilms present a serious hazard to the drinking supply. In the petrochemical industry, biofilms decrease the efficiency of equipment such as oil pipelines and platforms. Biofilms can also develop on ship hulls, fishing traps, and nets, representing a major economic problem. Although biofilms can be extremely costly to industry, positive aspects of the practical application of biofilms exist. For example beneficial biofilms are used in the production of industrial chemicals (e.g. ethanol, vinegar) and in the removal of toxic compounds during the treatment of wastewater and oily seawater. Biofilms are also greatly associated with vegetable life. The role of symbiotic nitrogen fixers and bacterial biofilms in legume–rhizobium interactions and metal tolerant microbes in the improvement of legumes is of great interest to professionals working in the field of agronomy.

Mechanism of microbial biofilm formation

Bacteria can attach to surfaces and thrive; this was first demonstrated by Zobell et al. (1937). One of the most important preconditions for biofilm is the existence of a surface. The general principle is that bacteria prefer to colonize on a physical surface in a hydrophobic environment. Extracellular compounds, such as EPSs and lipopolysaccharides (LPSs), and bacterial surface structures such as flagella, combined with environmental and cell signaling, are involved in the formation of biofilms (Schembri et al., 2001). The EPS plays a significant role in the formation of structures and the functioning of biofilm communities in various environments. The

extracellular polysaccharide matrix behaves as an anion exchanger and restricts the access to the biofilm of antimicrobial agents and also protects bacteria against environmental stresses (desiccation and osmotic shock, UV radiation, and pH change). Environmental signals trigger the process in commonly accepted models of biofilm formation, and it is necessary for the community to move across the surface (Costerton et al., 1995). The initial attachment steps are mediated by external membrane proteins (such as calcium-binding proteins), pili, or LPS. The generation of quorum sensing signals is necessary for the development of a mature biofilm after the formation of microbial colonies. Davies et al. (1998) noted that bacterial cell density was increased in biofilm through cell-to-cell signaling. This may lead to the secretion of low molecular weight molecules that are involved in population signaling; the process is called quorum sensing (Flemming and Wingender, 2010). Quorum sensing is a communication mechanism between microbes; some signaling will only be produced when microbes are together in groups (Li and Tian, 2012). Bacteria can regulate their behavior in accordance with population density by using autoinducers. Up to 40%–60% of genes in the bacterial genome are regulated by cell signaling mechanisms and the cells in biofilm become distinctly separate from their counterpart free cells, and are more resistant to stress. Bacterial treatment with sodium nitroprusside (a donor of nitric oxide) is also reported to lead to the formation of biofilms in bacterial cells (Vaishnav et al., 2018)

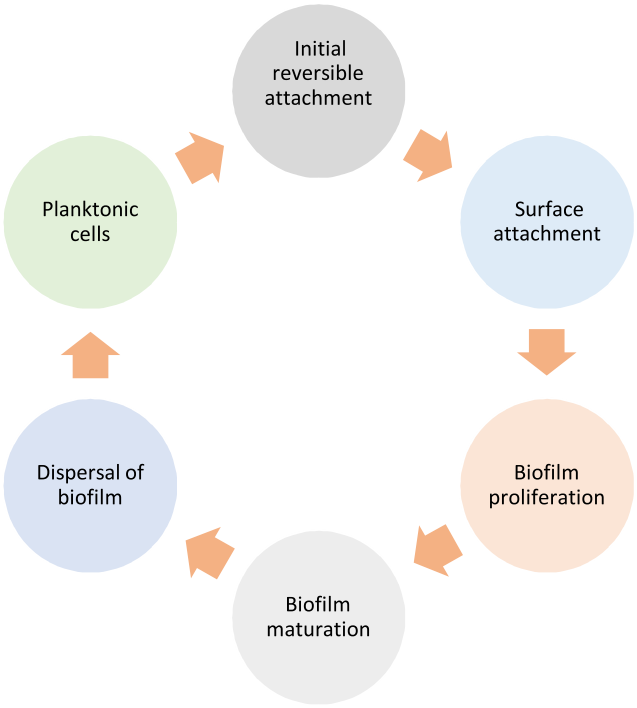

Figure 1: Different stages of microbial biofilm formation

The attachment of the microbes with the surfaces and the formation of biofilm is the consequence of the following processes:

1. **Conditioning of the surface-** The 'conditioning' of surfaces involves the adsorption of organic substrates to the physical surface so that it would be suitable for the adsorption of the microbes. The factors like the nature of the fabricating materials, surface tension, electrophoretic mobility, roughness and wettability of the surfaces control the conditioning process (Characklis and Cooksey, 1983). The adsorption of organic substrates brings a change in the physico-chemical properties of the surface, which facilitate the invasion of the microbes of the surface. The analysis of the organic substrates adsorbed to the surfaces indicated that the organic substance is the mixture of polysaccharides, proteins and glycoproteins.
2. **Adsorption of the cells to the conditioned surface-** The course of 'conditioning' follows the adhesion of surface by free floating or planktonic microbes (bacteria, algae, fungi). Initially the attachment of the microbes to the surface is reversible i.e., the microbes adsorbed to the surface at this stage are in Brownian motion and a mild rinsing could readily remove them from the surface. The attachment of the microbes with the conditioned surfaces at this stage is the result of some week forces like surface charge of the microbes, and the Van der Waals and electrostatic forces of the interacting molecules (Characklis and Cooksey, 1983).

3. **Formation of a tangled and channelled network of EPS-** A prolong microbial adhesion results an irreversible attachment of the microbes to the surface. This firm attachment of the microbes with surface requires severe mechanical or chemical treatment for its removal. Essentially, the irreversible or permanent attachment of the microbes is the consequence of the production of extra cellular cementing substances called EPS. The network of EPS fibres anchors the microbes to the surface and connects their cells like strands of spaghetti (Potera, 1996). With the passage of time, a diversified microbial consortium occupies the sticky web of the tangled polysaccharide fibres. The network of EPS provides cohesive forces within the biofilm, draws nutrients from the medium, protects microbes from sudden environmental shocks, and adsorbs heavy metals and other organic detritus from the surroundings. Aside from serving as means of intercellular communication within the biofilm, the network of EPS enhances the intercellular transfer of genetic material and acts as short-term energy storage house. The physical properties like diffusiability, thermal conductivity, and rheology of the microbial biofilms are also influenced by the EPS (Characklis and Cooksey, 1983). Chemical analysis showed that the EPS are mostly comprised of neutral and acidic sugars (e.g., glucose, galactose, mannose, rhamnose, ribose, fucose, uronic and gluconic acids, etc.).

The release of unwanted substances into the environment beyond stipulated limits, where adverse effects begin to manifest, is referred to as contamination. Since the preindustrial era, pollutants have been present in the environment. However, the rapid growth of industries and unscrupulous use of inborn synthetic chemicals such as PAHs and nitro aromatic compounds have led to serious pollution of the environment (Haritash and Kaushik, 2009). The chemical and pharmaceutical industries have discovered, synthesized, and used a large number of different new hydrocarbon molecules. Because of the hydrophobic nature and low solubility of PAHs, they are biodegradation-resistant and can accumulate through the food chain in the environment. The persistence and emergence of these organic pollutants are of major concern. Due to their use in various industries such as agriculture, clinical and general household usage, these chemicals dominate the human habitat (Seo et al., 2009). Several factors such as bioaccumulation, photo oxidation, volatilization, and transformation are associated with PAH toxicity, and are therefore a long-term hazard to human health. In our biosphere there are integrals of natural or anthropogenic organic complexes (~16 million molecular species), of which approximately 40,000 are under scrutiny because of their routine use. In addition to PAHs of fossil fuels, they are recorded environmental pollutants of soils, gas, and creosote wood treatment plants. As per the US Environmental Protection Agency and the EU Water Framework Directives, many PAHs are recognized as mutagens or carcinogens. Aquatic organisms can consume PAHs and be transported through food chains and accumulate in fatty tissues with potential long-term effects (Edwards and Kjellerup, 2013). Although PAHs are sorbed, volatilized, photolysed, and chemically degraded in nature, microbial degradation is considered to be the main process of degradation

Biofilm forming microbes involved in degradation

Microbial communities accumulate on the solid surface thereby forming a biofilm. Abiotic degradation allows chain scission to generate oligomers and monomers and their radicals followed by their biodegradation. (Gewert et al., 2015). Although, chemical and physical properties of the polymer, determine the degree of degradation. At the same, the floating plastic gets fouled due to the settling of biomass (Van Sebille et al., 2015). Biofouling involves adsorption, biomass immobilization followed by micro and macro fouling. Bacteria serve as primary a colonizer, which entraps other organisms such as fungi, diatoms, etc. Metabolic activity of the attached biomass leads to desorption, adsorption, and

fragmentation of polymer chain or degradation of the debris (Harrison et al., 2011). Other factors such as molecular weight, hydrophobicity, temperature chemical structure, elasticity, transition state, etc affect the degradation process. Microbial community composition varies in regions. A report on PCL degradation suggests the involvement of *Pseudomonas* and *Rhodococcus* sp along with two fungal strains. Together they have degraded PCL films up to 53% (w/w) in 30 days of incubation (Urbanek et al., 2018). Hence, *Pseudomonas* and *Rhodococcus* sp are salient bacterial candidate involved in biodegradation. Temperature plays a crucial role in degradation. During the degradation of biodegradable plastics such as PBS, PBSA, PLA and polyhydroxybutyrate (PHB), the microbial activity proceeded at 4°C but no degradation was observed at normal temperatures (Sekiguchi et al., 2011). Microbial genera responsible are *Pseudomonas, Tenacibaculum* and *Alcanivorax* sp where by *Pseudomonas* sp. strains were active at low temperature (Sekiguchi et al., 2011).

Role of biofilms in bioremediation of organic pollutants

Biofilm remediation is an environmentally-friendly and cost-effective way to clean up pollutants in the environment (Fida et al., 2012; Murali and Mehar, 2014; Gaur et al., 2014). Biofilm-forming bacteria are suitable for survival and bioremediation as they compete with nutrients and oxygen and biofilm tolerance observations in harsh environments. It can be expected that different microbial species, individually or in consortia of biofilms, each with different metabolic degradation pathways, can either individually or collectively degrade several pollutants. The use of biofilms is efficient for bioremediation because biofilms absorb, immobilize, and degrade various environmental pollutants (Role of some microbes are listed in Table 1). In indigenous populations near the highly polluted sites, bacterial biofilms exist to better persist, survive, and manage the adverse environment. Gene expressions vary in the biofilms and are distinctive when compared to free planktonic cells. In biofilms, differential gene expressions occurred due to the variable local nutrient and oxygen concentration in the biofilm matrix and the division of labor between microbes. Such a variable expression of the gene may be essential for the degradation of various pollutants by many metabolic pathways (Grace et al., 2011). Chemotaxis and flagellar-dependent motility are important considerations in the formation of biofilms in microorganisms. Responses such as swimming, swarming, twitching motility, chemotaxis, and quorum sensing in the presence of xenobiotics commonly found in soil and water help microbes to coordinate pollutant movement and improve biodegradation. Under natural environmental conditions, most bacteria persist in biofilm mode embedded in an EPS matrix that also provides a beneficial structure for microbes forming biofilms in bioremediation processes (Shukla and Rao, 2013). The biofilm matrix offers greater microbial resistance to environmental stress, shear stress, acid stress, antimicrobial agents, UV damage, drying, predation, solvents, and various concentrations of toxic synthetic chemicals and pollutants (Jain et al., 2013). Biofilm-forming microbes are especially adept at bioremediation because they are immobilized in an EPS that also removes pollutants during degradation (Shukla et al., 2014). Biofilm microbes also acquire relatively limited nutrients and oxygen, rather than convectional transport, compared to planktonic cells. The three-dimensional structure of EPS with reduced oxygen concentrations toward the center brings aerobics and anaerobic, heterotrophs with nitrifiers and sulfate reducers with sulfate oxidizers closer to each other, promoting faster degradation of various pollutants in natural and engineered technologies. The EPS from cyanobacteria acts as a biosorbent and removes aqueous heavy metals (De Philippis et al., 2011). An EPS containing surfactants may also aid solubilization of hydrophobic or other refractory substrates which would otherwise be inaccessible to microorganisms. Extracellular enzymes within the EPS of biofilms decontaminate pollutants such as heavy metals and organic compounds (Flemming and Wingender, 2010). An EPS serve as a trap for metal and metalloids due to the presence of many negatively-charged functional groups which enable

formation of complexes with heavy metals and organic contaminants and their subsequent removal. The EPS is known to bind a wide variety of metals including lead, copper, manganese, magnesium, zinc, cadmium, iron, and nickel. White and Gadd (1998) reported that sulfate-reducing bacterial biofilms were found to accumulate in the form of copper sulfide when it was exposed to a medium containing 20–200 mM copper grown in continuous culture. There was also a simultaneous increase in the biofilm's EPS content, suggesting the role of EPS and biofilms in trapping metal precipitates. In nature, biofilms are usually formed by a diverse mixed species that can promote enhanced biofilm formation and have a greater capacity to withstand environmental stress (Gieg et al., 2014). Biofilms are increasingly used as early warning systems or as a monitoring and evaluation indicator for heavy metal contamination in rivers and streams, as the structure and physiological changes of biofilms occur rapidly in the presence of toxicants. The choice of biofilms as an indicator system is beneficial, as biofilms can adsorb environmental pollutants and can be an easy sampling method. Biofilms are the first to interact with nutrients and pollutants in aquatic systems and can therefore be used as early monitoring of the environment in aqueous organisms. For the monitoring of environmental pollution, various indicator properties of biofilm can be assessed, including changes in biomass, composition of species, pigment production, photosynthesis, and enzymatic activities. Heavy environmental contamination can affect biofilm species diversity and microbial biofilm diversity estimates can also indicate environmental pollution. Valls and de Lorenzo (2002) observed that in variety of cases, genetic engineering can combine or improve a cellular trait present in the strains. Molecular approaches allow the construction of new recombinant strains with specific metal binding properties through the expression of various metal chelating proteins (Singh et al., 2011).

Role of microbial biofilm in bioremediation and detoxification of heavy metals

Heavy metal pollution has wreaked havoc in sediments and water columns since the industrial revolution with priority remediation practices relying mainly on dredging and *ex situ* treatment (Mulligan et al., 2001). Though very thorough techniques have been developed for extracting heavy metals from dredged soil, sediment, and wastewater, these processes are often expensive, invasive, and cause destruction to the overall ecosystem of the contaminated site. Heavy metal remediation is

Table 1: Microorganisms as biofilms for the removal of different types of pollutants

Microbes isolated from biofilms	Role in environmental cleaning	References
1. *Pseudomonas stutzeri, Aeromonascaviae, Sphingobacteriumthalpophilum, Fusariumudum* and *Rhodotorula mucilaginosa*	Reduction of BOD and COD values of wastewater	Bestawy et al. (2014)
2. *Pseudomonas stutzeri B. denitrificans* B79 and *A. hydrophila L6, Rheinheimera pacifica, Thauera* sp.	Biological denitrification of wastewater and the river water	SriuNaik and PydiSetty (2011)
3. *Nitrosomonas* sp. and *Candidatus kuenenia*	Removal of Ammonium from highly concentrated streams	Park et al., 2014
4. *Marine strain P. mendocina* NR802, *Pseudomonas paucimobilis*, *Sphingomonasbisphenolicum and Sphingomonas* sp.	Biodegradation of PAHs from polluted water and other micro-pollutants such as PAH and Bisphenol A.	Kwiatkowska and Zielinska 2016
5. *Acinetobacter sp*., *Graphium* sp., *Fusarium* sp., *Candida tropicalis*	Decontamination of phenol and m-cresol containing wastewater	Santos and Linardi (2004)
6. *Acinetobacter calcoaceticus, Erwinia herbicola, P. aeruginosa* and *Pseudomonas maltophilia*	Affinity for bio-sorption of Gold (Au)	Tsuruta (2004)
7. Microcystins degrading bacteria such as *Sphingpoyxis sp*. and *Sphingomonas* sp	Removal of some specific compounds such as microsytin-RR, aliphatic homopolyesters and aliphatic-aromatic copolyesters	Abou-Zeid et al., 2004
8. *Pseudomonas, Chryseomonas, Sphingomonas* and *Burkholderiae* species	Biodegradation of phenol and pyridine and the highest biodegradation capacity (1700 mg/L and 3000 mg/L of phenol and pyridine respectively) is shown by Pseudomonas MT1 isolate	Rakaiby et al., 2012

unique because, unlike organic pollutants, biological processes are not capable of completely removing the contaminants from the environment. Heavy metals exist in nature as dilute components of the geochemical cycles, thus it can be assumed that microbial communities can interact and utilize such metals. However, when heavy metals are present as contaminants, the concentrations are often higher than the natural concentration and mixed contamination can occur as well. Technologies that capitalize on the metal adsorptive properties of biomaterials have been heavily developed within the past decade. Costley and Wallis (2001) combined a biological immobilization/ sorption process with traditional acid extraction/ desorption in a rotating biological contactor (RBC) on which biofilms proved to be resistant to any malefactors of the desorption process (Costley and Wallis, 2001). It was reported that Cu, Zn, and Cd were removed in successive sorption–desorption cycles indicating the ability of the biofilm to repopulate after subsequent desorption cycles (Costley and Wallis, 2001). In packed-bed bioreactors for treatment of mercury-contaminated wastewater, mixed culture biofilms were able to retain higher amount of mercury and higher diversity in the presence of rapidly changing g mercury concentrations compared with monoculture biofilms (von Canstein et al., 2002). These results showed that more diverse biofilms were more efficient for bioremediation of metals thus being important for the development and implementation of biofilm-based solutions. Increased microbial diversity can result in establishment of a network of ecological and metabolic niches allowing biofilm associated microorganisms to survive in the midst of rapidly changing environmental stress, which results in greater performance whether that would take place in a bioreactor or *in situ* in the environment. In a moving bed sand filter designed to treat industrial wastewater containing high concentrations of heavy metals, biofilms of metal-resistant bacteria were formed (Diels et al., 2003). Biofilms not only served as biosorptive material but also lowered the pH enough to facilitate bio precipitation of some heavy metals. Another study, which employed a mixed species biofilm consisting of sulfate-reducing bacteria, capitalized on the oxidation–reduction potentials to precipitate metal sulfides of Cu, Zn, Ni, and Fe with concomitant precipitation of As (Jong and Parry, 2003).This treatment resulted in removal of 98%of Zn, Cu, Ni and 82 and 78 % of Fe and As, respectively. The overall biosorptive capacity of biofilms was limited by the resistance that some bacteria in the community showed toward metals such as *Stenotrophomonas* sp. exhibiting resistance to Cr (VI). All of these technologies were benefitting from the inherent resistance and adsorptive properties of biofilms to heavy metals. This effect is largely due to the presence of the EPS produced by the microbial biofilm community itself, which is in stark contrast to the lack of EPS production by planktonic cells (Teitzel and Parsek, 2003). The importance of EPS has been increasingly recognized and with the array of genetic engineering tools that are currently available, more creative ways to take advantage of biofilm produced EPS through engineered bacterial strains might occur in the future.

Role of biofilm and its EPS in heavy metal bioremediation

Removal of heavy metals from the environment using microbial biosorbents has been studied extensively as a major challenge in bioremediation. Extracellular polymeric substances produced by many microorganisms are of particular relevance to the bioremediation process because of their involvement in flocculation and binding of metal ions from solutions (Salehizadeh and Shojaosadati, 2003). The use of isolated biopolymers in biosorption phenomenon seems to be more economical, effective and safe alternative to chemical methods such as precipitation, coagulation, ion exchange, electrochemical and membrane processes. As a non-living sorbent, EPS is preferred for its easy availability in the treatment process and avoidance of pathogenicity issues of the organism concerned. EPSs and other biopolymers exhibit excellent metal-binding properties with varying degrees of specificity and affinity (Table 2). The binding of cations to bacterial biopolymers generally occurs through electrostatic interaction with negatively charged functional

groups such as uronic acids, phosphoryl groups associated with membrane components or carboxylic groups of amino acids. In addition, there may also be cationic binding by positively charged polymers or coordination with hydroxyl groups. Extracellular polymers surrounding the cells were able to chelate some metals and bind them to the cell surface (Santamaría et al., 2003).

Biofilm originates when a mass of microbial cells immobilized in the heterogeneous mass of EPS gets attached to a solid surface. They commonly occur in natural environments as coatings around the soil particles, in water-sediment interface, on surfaces of bioreactors, industrial production processes, sewers, etc. In nature monospecies biofilms are rare and majority comprised of a mixture of microbial species which may also include higher trophic groups of protozoans and metazoans (Wimpenny et al., 2000). Besides water (nearly 97%) and microbial cells, biofilm matrix is complexed with secreted polymers, nutrients and metabolites, cell lysis products, particulate material and detritus from the surrounding surfaces. Biofilm EPS containing all major classes of macromolecules are anionic in nature due to the presence of uronic acids or ketal-linked pyruvates and ionisable functional groups which interact with other molecules, minerals and heavy metals (Horn and Morgenroth, 2006). Several experimental findings have established that biofilm EPS plays a substantial role in the sorption of inorganic substances and assist in the biomineralization of metal ions (as sulphides, phosphates, carbonates, oxides and hydroxides), thus concentrating and depositing fine grained minerals within the matrix. Several stereoscopic as well as microscopic techniques are employed for describing the elemental composition and localization of the metal precipitate trapped in the biofilm matrix (Van Hullebusch et al., 2003). Metal binding to biofilm EPS is influenced by surrounding pH, metal concentration, presence of organic matter and biomass. In addition, carbohydrate: protein (C/P) ratio also plays a significant role in monitoring the state of biofilm process to remove heavy metals in the wastewater. In batch experiments, binding of Cu (II), Pb (II) and Ni (II) occurred with lowering of C/P ratio of EPS when exposed to Cu and Pb ions (Jang et al., 2001). Using a rotating-disk biofilm reactor it was demonstrated that biofilms were more resistant to Cu, Pb and Zn than the stationary-phase or logarithmically growing planktonic cells. The EPS encasing a biofilm protect cells from heavy metal stress by binding the metal ions or by retarding their diffusion within the biofilm. Metals (Fe, Au, La, and Cu) bound to biofilm of *Pseudomonas aeruginosa* PAO1 significantly exceeded those bound by planktonically grown cells (Jang et al., 2001). Autotrophic biofilms accumulate substantial quantities of metals from contaminated water. Biofilms in running water sorbed three to five times more Cd than in still water. Cd (II) sorption was 75% greater in high-biomass biofilm and light enhanced metal sorption by 40%. Metal toxicity to photosynthesis was evident in thin biofilms but photosynthesis by thicker biofilms occurred even at 100 ìg· Cd l–1 (Templeton et al., 2001). A long period x-ray standing wave technique was applied to probe the allocation of Pb (II) in *Burkholderia cepacia* biofilms growing on hematite (á-Fe2 O3) or corundum (á-Al2 O3) surfaces and determines quantitatively the partitioning of Pb (II) at the biofilm-metal oxide interface. The reactive sites on the metal oxide surfaces were activated by the formation of a monolayer biofilm and thus form a dominant sink for Pb (II) ions at submicromolar concentrations. Immobilization of precipitated metal sulphide inside a biofilm of sulphate reducing bacteria is considered an effective method to reduce the mobility of metal ion in waste water. In a stirred tank bioreactor growth of SRB biofilm with Cd (II) and sulphate led to accumulation of CdS and simultaneous increase in protein and polysaccharide content in biofilm, indicating sulphide precipitation and entrapment by biofilm EPS. Similarly binding of Cu took place through precipitation of CuS at the biofilm surface or in the liquid phase, followed by entrapment of precipitated CuS by the polymer (Webb et al., 1998).

Microbial biofilm in wastewater treatment

Biofilm system is a well-developed technology in which solid media are added to suspended growth reactors to provide attachment surfaces for

Table 2: Role of EPS components in environment restoration

EPS component involved	Application in biofilm	Role in bioremediation of heavy metal
1. Hydrophobic polysaccharides	Ion exchange, Sorption	Increase solubility and binding, hydrophobic organic compounds, metal binding, and detoxification.
2. Proteins	Adhesion, aggregation of bacterial cells, retention of water, sorption of organic and inorganic compounds, binding of enzymes, electron donor or acceptor, and protective barrier to cells	Play main role in flocculation and biosorption. The concentration and characteristics of the protein decide the fate of surface properties as well as biodegradability of the EPS.
3. Humic substances	Adhesion, Electron donor or acceptor	Minor role in the flocculation and biosorption. The impact of humic substances mainly depends upon its concentration as well as the nature of humic substances.
4. Neutral polysaccharides	Structural Component	Provide Stability
5. Amyloids	Structural component	Provide stability and confers resistance
6. Biosurfactant	Initial microcolony formation, development of biofilm, formation of water channels	Component cells within biofilms to solubilize and utilize substrates
7. Nucleic acids	Informative, gene transfer, structural	Transfer of genes useful for degradation of xenobiotics

biofilms, so as to increase the microbial concentration as well as rates of contaminant degradation biofilms to take advantage of a number of removal mechanisms, including biodegradation, bioaccumulation, biosorption and biomineralization (Pal et al., 2010). The microbial communities in the biofilm break down different nutrients, such as phosphorous and nitrogen-containing compounds, carbonaceous materials as well as trapped pathogens from the wastewater. Once pollutants are removed, treated water of a biofilter is either released to the environment or used for agriculture and other recreational purposes. Removal of the pollutants from wastewater by biofilm on the filter media is schematically represented in wastewater treatment with biofilm systems has several advantages, including operational flexibility, low space requirements, reduced hydraulic retention time, resilience to changes in the environment, increased biomass residence time, high active biomass concentration, enhanced ability to degrade recalcitrant compounds as well as a slower microbial growth rate, resulting in lower sludge production.

Microbial biofilm in enhanced phytoremediation of environmental contaminants

According to Cunningham and Berti (1993) phytoremediation is defined as the use of green plants to remove, contain, or render harmless environmental contaminants (Cunningham and Berti, 1993). In this process specially selected or genetically engineered plants are used which are capable of direct uptake of pollutants from the environment. Phytoremediation can be applied to both inorganic and organic pollutants present in solid and liquid substrate. Generally, phytoremediation of contaminants by a plant involves the following steps: uptake, translocation, transformation, compartmentalization, and sometimes mineralization (Schnoor et al., 1995). Factors affecting the uptake, distribution and transformation of organic compounds by a plant are mainly related to the physical and chemical properties of the compound (e.g. water solubility, molecular weight, octanol-water partition coefficient), as well as environmental conditions (e.g. temperature, pH, organic matter, and soil moisture content) and plant characteristics (e.g. root system, enzymes).

The rhizosphere provides a microenvironment possessing intense biological, chemical, and physical activities, harboring diverse microorganisms involved in various positive and negative plant-microbial interactions (Kamilova et al., 2006). Cell-to-cell signaling has been documented to play a key role in cell attachment and detachment from biofilms. Quorum sensing (QS) plays a vital role in root–microbe interactions, which facilitate bacteria to communicate and coordinate behavior via signaling molecules to colonize roots. QS is a regulatory process by which bacteria monitor their population density affecting population dynamics in association with host plants (He et al., 2003). These signaling molecules are unique among the microbial species. Direct observations of bacteria adhering to plant surfaces

have identified multicellular congregations, preferably microcolonies and cell clusters. During biofilm formation, the bacteria communicate chemically with each other by quorum sensing (De Kievit et al., 2001). Some examples of diffusible cues include N-acylhomoserine lactones (AHLs), 2-heptyl-3-hydroxy-4-quinoline, and autoinducer-2 that aid cell-to-cell communication within the bacterial community and facilitate synchronization of some actions.

In the rhizosphere, biofilms are formed on the surfaces of roots and soil particles, respectively, resulting in enhanced root colonization by the bacteria and cementing of soil particles. This can improve crop productivity and sustain physicochemical properties of soil. Biofilms can retain moisture and protect plant roots from a number of phytopathogens (Qurashi and Sabri, 2012). In case of leguminous hosts, biofilm mode of life offers a means for survival of bacteria as well as for establishment of symbiosis with the host legume. Successful biofilm formation has been reported in *Mesorhizobium* species-*M. huakuii* and *M. tianshanense*. There has been a current upsurge targeting roles and functions of biofilms in the plant host system and in the rhizosphere region. Matthysse (Matthysse 2014) has published an extensive review targeting the attachment of *Agrobacterium* to plant surfaces. It is also documented that *A. tumefaciens* binds to the surfaces of inanimate objects, plants, and fungi, and is an excellent root-surface colonizer. They can also effectively bind to soil particles and to other abiotic surfaces (Tomlinson and Fuqua 2009). Biofilm-inoculated plants showed significantly higher shoot and root nitrogen accumulation, indicating the efficacy of using nitrogen-fixing biofilms as inoculants that may promote soil nitrogen fertility and plant growth. Thus, an application of such a microbial association as a biofilm inoculum may prove to be effective for maintenance of soil fertility and survival of soil rhizobia in the absence of their hosts.

Microbial biofilms in environmental monitoring and assessment

Biofilm communities are one of the first victims of pollutants which can affect their structure and function. With increasing number of toxic molecules found in freshwaters (e.g., pharmaceuticals, nanomaterials, PAH, and metals), which can directly target heterotrophic organisms, recent research has focused on the bacterial biofilm responses to pollutants (Ricart et al., 2010). Direct effects of toxicants on bacterial community could lead to two biofilm responses, which may differ in their temporal pattern: short-term biochemical and physiological alterations and long-term changes in community structure. Short-term effects include increases in bacterial mortality and reduction in bacterial growth, production, respiration, and extracellular enzyme activities. In an experimental study Ricart et al. (2010) assessed the mortality of biofilm bacteria after their acute exposure to the bactericide triclosan, which inhibits bacterial fatty acid synthesis. Physiological effects of toxicants on bacteria have been also shown such as the significant decrease in bacterial production (as measured by thymidine and leucine incorporation) after the biofilms being exposed to metals (Blanck et al., 2003). On the other hand, recent studies on the effect of pharmaceuticals showed significant effects on biofilm extracellular enzyme activities, thus compromising the biofilm organic matter cycling role in the ecosystem. (Boninneau et al., 2010) showed that leucine-aminopeptidase activity was significantly inhibited after 6-h exposure of biofilm bacteria to the b-blockers propranolol and atenolol, reflecting the negative effect of these pharmaceuticals on the bacterial ability to hydrolyze peptides. The presence of pollutants in lotic systems may lead to a change in microbial biomass or to a shift in community structure. It is widely accepted that chemical pollution could reduce bacterial diversity. Nevertheless, the effects of pollutants on bacteria may also favor the selection of certain species. Bacterial species composing the biofilm may have ranging sensitivities and responses toward various anthropogenic pressures Blanck et al. (2003).

During exposure to toxic agents such as metals or antibiotics, the most sensitive organisms may be overtaken by the more resistant or more tolerant ones. On the other hand, in case of prolonged exposure to pollutants, the processes of bacterial adaptation may also lead to stimulation of biodegradation capacities of these compounds. Indeed, because of their species-rich communities and the possibility to adapt their metabolisms, biofilm bacteria provide a great potential for the removal of contaminants from water systems by using the pollutants as a source of organic matter. Some studies have explored the degradation pathways of pollutants by microbial communities taken from rivers and confirmed their important role in elimination process of pharmaceutical or other chemical residues. Pieper et al. (2010) investigated the degradation of the pharmaceuticals phenazone, a pyrazolone derivate in widespread use, by bacterial isolates from natural biofilms. Results indicated that some bacterial strains were able to metabolize phenazone in its metabolite 1,5-dimethyl1,2-dehydro-3-pyrazolone over the sampling period of 8 weeks. In conclusion, two complementary approaches coexist to assess direct effects of pollutants on bacterial communities. The first one is based on structural analysis of communities (biomass, taxonomy, and diversity), and the second one is a functional approach generally based on the metabolic activity measurements (respiration, enzymatic activities, and potential for biodegradation). The use of bacterial communities in epilithic biofilms as bioindicator might therefore allow an important choice of descriptors, adapted to different types of contaminants (Tlili et al., 2010).

Strategies for combating biofilms by the help of medicinal plant extracts

Several medicinal plant extracts and their active ingredients were extensively investigated to eradicate the *Propionibacterium acne* biofilm (Coenye et al., 2012). This study demonstrated that among 119 plant extracts, five (*Epimedium brevicornum, Malus pumila, Polygonum cuspidatum, Rhodiola crenulata* and *Dolichos lablab*) showed potent antibiofilm activity. The researchers also reported that extracts of *E. brevicornum* and *P. cuspidatum* and their active ingredients (icartin and resveratrol) exhibit a significant antibiofilm activity even when used at levels below the minimum inhibitory concentrations (MICs). Bark extracts of *Melia dubia* (30 mg/ml) showed strong suppression of hemolysis, swarming motility, hydrophobicity, and biofilm formation of *E. coli* (Ravichandiran et al., 2012). Similar results were also reported by Abraham et al (2011), concerning *Capparis spinosa* extract (2 mg/ml) significantly inhibited the biofilm formation and EPS production in *E. coli, Serratia marcescens, Pseudomonas aeruginosa, and P. mirabilis* (Issac et al., 2011). The study by Faraz et al., (2012) showed that both dandasa and green tea (6.2 and 12.5 mg/ml, respectively) had good antibiofilm effects on *Streptococcus* mutans and also on on *E. coli* (12.5 and 3.1 mg/ml, respectively).

Harjai et al., (2010) reported that fresh garlic extract 6 log unit's *P. aeruginosa* biofilm. The in-vitro screening of anti-*Staphylococcus epidermidis* biofilm activity of 45 aqueous extracts from 24 Caatinga medicinal plant species was published (Trentin Dda et al., 2011). The most promising extracts were isolated from *Bauhinia acuruana* branches, *Chamaecrista desvauxii* fruits, *B. acuruana* fruits, and *Pityrocarpa moniliformis* leaves, reduced biofilm formation significantly even though they were tested at a 10-fold lower concentration. Moreover, the researchers also reported that *Commiphora leptophloes* and *Senna macranthera* fruit extracts reduced biofilms by 67.3% and 66.7%, respectively. Biofilm formation by *Mycobacterium smegmatis* was examined using various qualitative and quantitative techniques (Syed et al., 2014). The researchers studied four plants (*Azadirachta indica, Hippophae rhamnoides, Juglans regia,* and *Vaccinium oxycoccus*) and other plant spices to search for effective biofilm-controlling natural substances (antimicrobial activity). The test of efficacy of the plant extracts as antibiofilm agents revealed that the extract of *A. indica* was most efficient in reducing and removing *M. smegmatis* biofilm. These findings might be

extrapolated to other pathogenic biofilm-forming Mycobacteria. This notion may ensure effective mycobacterium biofilm control. The ability of casbane diterpene isolated from the extract of *Croton nepetifolius* to inhibit biofilm formation of two Gram-positive species of bacteria (*Staphylococcus aureus* and *S. epidermidis*), five Gram-negative bacteria (*Pseudomonas fluorescens, P. aeruginosa, Klebsiella oxytoca, Klebsiella pneumoniae*, and *E. coli*), and three yeast species (*Candida tropicalis, C. albicans*, and *C. glabrata*) has been extensively evaluated (Carneiro et al., 2011). The latter authors reported that biofilm formation was inhibited at MICs even when planktonic growth was not reduced. Another study showed that *Candida biofilm* formation was reduced significantly by *Boesenbergia pandurata* oil. Biofilms were reduced by 63% to 98% when sub-MIC levels (from 4 to 32 µL/ml) were used. Recently, diverse plant extracts were screened against enterohemorrhagic *E. coli* (EHEC) O157:H7 biofilm (Lee et al., 2013). This study showed that among 498 plant extracts, 16 inhibited biofilm formation of EHEC by more than 85% without the growth of planktonic cell. Several essential oils have also been reported as anti-biofilm agents. Among them are cumin oil (Safoura et al., 2010), cinnamon oil (Oliveira et al., 2012) and oregano oil (Nebahat et al., 2010) etc.

Factors affecting microbial biofilm formation

Biofilm formation varies under diverse nutrient conditions ranging from high to almost nondetectable. However, they are more abundant and dense in a nutrient-rich environment as it promotes the transition of bacterial cells from planktonic to biofilm state, while depletion of these nutrients causes detachment of biofilm cells from surfaces. There are different means by which bacterial biofilms obtain nutrients: (i) concentrating trace organics on surfaces through extracellular polymer, (ii) using the waste products from secondary colonizers and (iii) pooling the biochemical resources with the help of different enzymes to break down food supplies. Any change in pH greatly affects the growth and development of bacterial and biofilm formation as it can overwhelm different mechanisms and have negative or killing effects on the microorganisms. In response to internal or external changes in pH, bacteria quickly adjust the activity and synthesis of proteins that are associated with different cellular processes. However, some of the cellular processes, including excretion of exopolymeric substances or polysaccharides, do not adapt to pH variations so easily. The optimum pH for polysaccharide production varies among different species, but for the majority of bacteria, it is around 7 (Oliveira et al., 1994). Microbial activities are very sensitive to change in temperature. Optimum temperature results in healthy growth of bacterial populations, whereas a slight variation may reduce bacterial growth efficiency. The reason for this is a reduction in bacterial enzyme reaction rates. For many bacteria found in cooling water systems, the optimum temperature for maximum growth is about 40°C.

Surface topography greatly influences the ability of bacteria to adhere to a surface. During the initial steps of colonization, surface roughness at nanoscale and microscale levels enhances the adhesion of bacteria to substrates by providing more surface area for cell attachment. Surface roughness reduces the shear force on bacterial cells and communities present in flowing liquids at high flow rates, such as water pipes in industrial plants. A material surface exposed in an aqueous medium will inevitably become conditioned or coated by polymers from the medium and the resulting chemical modification will affect the rate and extent of microbial attachment. Moreover, other factors such as charge, hydrophobicity and elasticity are also influential in microbial attachment (Prakash et al., 2003).

The area from the surface where no turbulent flow is experienced is known as the boundary layer. Within this area, the flow velocity has been shown to be insufficient to remove biofilms. The area outside this layer is characterized by high levels of turbulent flow and has an influence on the attachment of cells to the surface. The size of the boundary layer is dependent on the flow velocity of water. At high velocities, the boundary layer

decreases in size and the cells are exposed to a high turbulence level. Hydrodynamic conditions can influence the formation, structure, EPS production, thickness, mass and metabolic activities of biofilms (Simoes et al., 2007).

Studies have shown that up-regulation and down-regulation of a number of genes are involved in the initial attachment of cells with the substratum. Approximately 22% of genes were upregulated and 16% were down-regulated in the biofilm formation of *Pseudomonas aeruginosa*. In addition, *algD, algU, rpoS* and genes controlling polyphosphokinase synthesis were also up-regulated in the biofilm formation of *P. aeruginosa*. Biofilms of *Staphylococcus aureus* were up-regulated for genes encoding enzymes involved in glycolysis or fermentation, such as phosphoglycerate mutase, triphosphate and alcohol dehydrogenase (Becker et al., 2001). Cell-to-cell signaling, also termed QS, has recently been proven to play a significant role in cell attachment and detachment from biofilms. Growth and development of biofilms on different surfaces are mediated by a density-dependent chemical signal released by bacterial cells densely packed with an EPS matrix. QS makes use of a transcriptional activator protein that acts in concert with small autoinducers (AIs) signaling molecules to stimulate expression of target genes, resulting in changes in chemical behavior. After accumulation of sufficient AIs, this form of intercellular communication serves to coordinate gene expression, morphological differentiation and the development responses of bacterial cells (Xiong and Liu, 2010).

Extracellular polymeric substances (EPSs) are a complex mixture of high-molecular-weight polymer (Mw = 10,000) excreted by microorganisms, products from lysis and hydrolysis as well as adsorbed organic matters from wastewater. Generally, EPSs have been shown to be a rich matrix of polymers, including polysaccharides, proteins, glycoproteins, DNA oligomers, phospholipids and humic acids. EPSs are also highly hydrated because they can incorporate large amounts of water into their structure by hydrogen bonding. EPSs are typically reported to aid in the formation of a gel-like network that keeps bacteria together in biofilms due to bridging with multivalent cations and hydrophobic interactions. In addition, EPSs also cause the adherence of biofilms to surfaces, flocculation and granulation, protect bacteria against noxious environmental conditions and enable bacteria to capture nutrients from the surroundings. Different biofilms produce different amounts of EPSs, and the amount of EPSs increases with the age of biofilms (O'Toole, 2011).

Extracellular DNA (eDNA) has been reported to be a major constituent of various single and multispecies biofilms. eDNA or naked DNA is a central part of bacterial self-produced extracellular polymeric substances (EPSs) and has similarity to chromosomal DNA in its primary sequence. Its role is very important in various stages of biofilm formation, such as initial bacterial adhesion, aggregation and microcolony formation that favors wastewater treatment. eDNA also helps strengthen biofilms, provides protection to biofilms from physical stress, antibiotics and detergents as well as serves as an excellent source of nutrients for biofilm growth (Mulcahy et al., 2008). In addition, eDNA can be utilized in engineering of biofilms for beneficial purposes, such as remediation of environmental pollutants and electricity or fuel production in bioelectrochemical systems or bioreactors.

Challenges and future prospects

In recent times, bioûlms have gained attention for the bioremediation of many toxic compounds and environmental pollutants. Bacterial bioûlms can be efûciently employed in the bioremediation process, as they provide protection to microbes under changing environmental conditions. Bioûlm is composed of a numerous structural components which are helpful in immobilization and solubilization of number of hydrophobic compounds. Bioûlm provides an optimum microniche which encourages growth of diverse microbes. Moreover, the inherent stress-resistant and metabolic dynamic qualities of bioûlm make its use in bioremediation of assorted industrial and environmental pollutants. Bioûlm-mediated

bioremediation represents a more economic technology. Adding signals of QS to bioremediation can come up with more advanced bioremediation practice. Involvement of QS in bioûlm growth cycle has been extensively studied in some bacterial groups, but its successful application in bioremediation technology is limited to date. Thus, there is a need to understand more on QS molecules to combine bioûlm and QS features of bacteria to use for proûcient biodegradation of poorly degradable compounds. Bacterial QS system has immense capability of signal diffusion, recognition, and ampliûcation which confers them the unique property of communication among themselves as well as with different species. This kind of communication helps in developing consortium with enhanced bioremediation potential, biofilm-based systems have potential advantages. Microbial Biofilm can also be used in Wastewater Treatment also for better designing of biofilm wastewater treatment systems, knowledge about the composition of filter media and developing biofilms is highly necessary. The composition and quantification of different elements in filter media can be determined using spectroscopic techniques. Complete biofilm community profiling is carried out by advanced techniques such as microbial sequencing, clone library generation, genetic fingerprinting, DNA microarray, denaturant gradient electrophoresis (DGGE) and next-generation sequencing (NGS), to increase the performance, stability and robustness of biofilm.

Genetically modified organisms (GMOs), a future prospective can be produced by manipulating the genome of an organism as per the requirements when applying genetic engineering technology. Bacteria that are present in the contaminated sites have adapted to that habitat by developing an internal resistance power. The recombinant strain produced from such native resistant bacterium by introducing a single gene or gene cluster can be a highly potential strain that can provide an opportunity for better bioremediation and biodegradation of many environmental pollutants.

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