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Review

Biofilm mediated decontamination of pollutants from the environment

Arindam Mitra [1,*] and Suman Mukhopadhyay [2]

[1] Department of Microbiology, Adamas University, Kolkata 700126, West Bengal, India
[2] Division of Microbiology and Infectious Diseases, NIAID/NIH/HHS, Bethesda, MD, USA

* **Correspondence:** Email: arindam.mitra@adamasuniversity.ac.in.

Abstract: In this review, we highlight beneficial use of microbial biofilms in remediation of environmental pollutants by bioremediation. Bioremediation is an environment friendly, cost effective, sustainable technology that utilizes microbes to decontaminate and degrade a wide variety of pollutants into less harmful products. Relative to free-floating planktonic cells, microbes existing in biofilm mode are advantageous for bioremediation because of greater tolerance to pollutants, environmental stress and ability to degrade varied harsh pollutants via diverse catabolic pathways. In biofilm mode, microbes are immobilized in a self-synthesized matrix which offers protection from stress, contaminants and predatory protozoa. Contaminants ranging from heavy metals, petroleum, explosives, pesticides have been remediated using microbial consortia of biofilms. In the industry, biofilm based bioremediation is used to decontaminate polluted soil and groundwater. Here we discuss conventional and newer strategies utilizing biofilms in environmental remediation.

Keywords: bioremediation; biofilms; *in situ* bioremediation; pollution control; biodegradation; wastewater treatment; biofilm reactors

1. Bioremediation

Bioremediation refers to the environment friendly process of detoxification of harmful pollutants from soil, water and air using microorganisms [1,2]. In bioremediation, harsh chemicals are not used and as such, damaging effect on the environment is reduced [3]. Bioremediation offers several advantages over other methods including no or minimum disruption of land or wildlife surrounding the treated area, reduction of noise and dust during treatment as well as avoidance of harsh chemicals. Bioremediation is economical when applied to large areas compared to conventional decontamination methods. The process of bioremediation employs diverse microbes for degradation or treatment of xenobiotic compounds, aromatic hydrocarbons, volatile organic compounds, pesticides, herbicides, heavy metals, radionuclides, crude oil, jet fuels, petroleum products and explosives [4]. Even though bioremediation has been known for several decades, it's effectiveness has been the subject of public attention following a major oil spill by Exxon Oil in the shorelines of Prince William Sound, Alaska in the year 1989 [5,6]. This incident is followed by an era of development in the field of bioremediation across the world, particularly in the US, making the field one of the fastest growing sector in the hazardous waste industry [7,8].

Microbes particularly bacteria can be easily grown and genetically manipulated which makes them suitable for bioremediation. Besides bacteria, fungi are also used for pollution removal [9,10,11]. Importantly, microbes are extremely efficient in degrading natural organic compounds or waste pollutants via diverse catabolic pathways as microbes adapt to persist in diverse environments [12,13]. Some microbes used in bioremediation are extremophiles that can withstand acidic or heavy metal-contaminated or radioactive environment. Success of bioremediation relies on maintenance of conditions or factors that expedite biodegradation of pollutants by microbes [14]. The process of bioremediation depends on enzymatic activities of microbes for transformation and degradation of environmental pollutants or wastes into less toxic or harmless constituents such as carbon dioxide and water [15]. The process of metabolic pathways requires transfer of electrons from electron donors to electron acceptors. The electron donors act as food for microbes which is usually limited in a non-contaminated site. However, in a contaminated site release of an organic electron donor may stimulate microbes to compete for available acceptors to restore the balance of the system. Microorganisms can degrade pollutants without and with oxygen in anaerobic and aerobic mode of degradation respectively. In aerobic degradation, microbes use oxygen as final electron acceptor to convert organic and inorganic pollutant into harmless products, often carbon dioxide and water. In anaerobic degradation, oxygen is not present or limited; microbes use other electron acceptor such as nitrate, iron, manganese, sulphate, etc. to break down organic compounds often into carbon dioxide and methane. Generally, aerobic microbes are capable of faster contaminant degradation than anaerobic ones. Sometimes some microbes may break contaminants by fermentation. Pollutants that are electron donors may be readily degraded in presence of oxygen by aerobic microbes, whereas those contaminants that are poor electron donors may degrade pollutants under anaerobic conditions. Depending on the pollutant, both electron donor and acceptor may be supplied to facilitate the degradation process. Many redox reactions also immobilize trace elements found in the contaminated sites. A change in oxidation potential of metals is also associated with change in toxicity or solubility as evident in the case of uranium and chromium [16,17,18]. In case of heavy metals, the conversion of sulphate to sulphide changes solubility and facilitate immobilization and removal of sulphate from wastewater by sulphur reducing bacteria [19].

Bioremediation is primarily of two types depending on location of pollutant treatment. In case of *in situ* bioremediation, the contaminated sample is treated in the original location whereas in *ex-situ* remediation the sample is treated typically off-site [20,21]. *In situ* bioremediation is an attractive choice as movement of contaminants from the site is not required and as a consequence it minimizes transportation cost and site disruption. A general approach for bioremediation is optimization of physical and chemical conditions (such as pH, aeration, moisture) for the bacteria in the contaminated site to degrade pollutant faster along with supplemented nutrients. An alternative strategy is to choose microbial strains that can serve as efficient degraders or use genetically modified microorganisms that can modify pathways for efficient pollutant degradation [22,23,24]. Importantly, a wide variety of microorganisms present in a community mode of "biofilms" are utilized to degrade diverse pollutants in the natural environment and in engineered systems.

2. Role of Biofilms in Bioremediation

A biofilm may be defined as an assemblage of microorganisms comprising of microbial species attached to a biological or inert surface and encased in a self-synthesized matrix comprising of water, proteins, carbohydrates and extracellular DNA [25]. It may be anticipated that different microbial species present in consortia of biofilms each with different metabolic degradation pathway are capable of degrading several pollutants either individually or collectively [26,27]. Biofilm forming bacteria are adapted to survive and suited for bioremediation as they compete with nutrients and oxygen and observations of tolerance of biofilms towards harsh environment found way in the process of bioremediation. Biofilm mediated remediation is environment friendly and cost effective option for cleaning up environmental pollutants. Use of biofilms is efficient for bioremediation as biofilms absorb, immobilize and degrade various environmental pollutants. Bacterial biofilms exist within indigenous populations near the heavily contaminated sites to better persist, survive and manage the harsh environment. Expressions of genes vary within the biofilms and are distinctive relative to free floating planktonic cells. Differential gene expressions within biofilms are owing to variable local concentration of nutrients and oxygen within biofilm matrix and division of labour among microbes. Such variable gene expression may be important for degradation of varied pollutants by numerous metabolic pathways. An important consideration for biofilm formation in microorganisms is chemotaxis and flagellar dependent motility [28]. Responses such as swimming, swarming, twitching motility, chemotaxis, quorum sensing in presence of xenobiotics commonly present in soil and water assist microbes to coordinate movement towards pollutant and improved biodegradation [29].

Under natural environmental conditions, most bacteria persist in biofilm mode encased in an extracellular polymeric substance (EPS) matrix which also provides a beneficial structure to biofilm forming microbes in bioremediation [30,31,32]. The EPS is made of both bound and secreted form of polysaccharides, proteins, lipids, nucleic acids, humic substances and water [33]. The composition of EPS varies between species and is dependent on growth conditions, surface on which biofilms are formed and environmental stress among others. Bacterial biofilms and EPS production can undergo changes both in structure and content depending on the environmental conditions in which the microbes are found [34,35,36]. Biofilms appear filamentous and mushroom-like shapes in fast moving and static water respectively [37,38]. In presence of predatory protozoa, bacteria often adapt to form biofilms in form of large inedible microcolonies that enable survival and persistence under harsh environment [39]. The biofilm matrix offers greater resistance than planktonic cells to microbes from environmental stress, shear stress, acid stress, antimicrobial agents, UV damage, desiccation, predation, biocides, solvents, high concentration of toxic chemicals and pollutants [40,41]. In contrast, free floating planktonic cells decontaminate environmental pollutants by metabolic activity but such cells are not stationery and not adapted to persist under mechanical and environmental stress. Biofilm forming microbes, in contrast, are particularly adept in bioremediation as they are immobilized in an EPS which also immobilizes pollutants during degradation [42]. Biofilm microbes also acquire relatively limited nutrients and oxygen as compared to planktonic cells because of diffusional mode of transport instead of convectional transport. The three-dimensional structure of EPS with reduced oxygen concentrations towards the centre brings in close proximity of aerobes and anaerobes, heterotrophs with nitrifiers, and sulphate reducers with sulphate oxidizers which promotes faster degradation of varied pollutants in natural and engineered systems [43]. EPS from cyanobacteria act as biosorbent and removes heavy metals from the aqueous phase [44,45,46]. EPS containing surfactants may also aid solubilisation of hydrophobic or other refractory substrates which would otherwise be inaccessible to microorganisms [47]. Presence of EPS also makes nutrient exchange and removal of by-products possible in biofilm community. Extracellular enzymes within EPS of biofilms decontaminate pollutants such as heavy metals and organic compounds [30]. EPS serve as traps for metal and metalloids due to presence of many negatively charged functional groups which enable formation of complexes with heavy metals and organic contaminants and their subsequent removal [48,49]. EPS is known to bind a wide variety of metals including lead, copper, manganese, magnesium, zinc, cadmium, iron and nickel [50]. Nutrient limitation can also increase EPS synthesis which can further absorb metals and pollutants from the environment. EPS of biofilms comprising of phosphorous-accumulating microorganisms act as reservoir and help removal and recovery of phosphorous from wastewater [51,52].

In nature, usually diverse mixed species biofilms are formed which may promote enhanced biofilm formation and greater capacity to withstand environmental stress [53]. Coexistence of multiple microbial species within biofilms in close proximity promotes interaction among its members. Biofilms can be found in natural and engineered systems. Common place natural environmental biofilms exist in soil, aquatic plants, sediments, covering rocks in streams and plants, lakes and rivers and in wetlands. Natural biofilms in the environment can degrade and remove pollutants from soil and river. Biofilms formed on the surface of water consists of bacteria, protozoa, fungi and algae. Naturally developed biofilms by algae such as *Nitzschia* in organic sediments can aid recovery of sediments by production of oxygen which in turn facilitates the biodegradation of aerobic bacteria present in the biofilm. Natural biofilms are an important part of food chain where predatory protozoa or grazers may feed on biofilm material. The protozoa in turn may be engulfed by small fish and subsequently small fish by larger fish and terrestrial animals. Microbial aggregates in aquatic systems such as microbial mats consisting of phototrophic bacteria and algae are formed on sediment surface under extreme conditions to survive predation from grazers and stress. Fragile structures called flocs are formed during bloom periods. Treatment of municipal wastewater is based on the floc activity in activated sludge plants. Slow-sand filters utilize biofilms formed on the surface of sand to remove organic compounds and metals from lakes, rivers and reservoir water [54]. Planctomycetes present in biofilms of seaweed in marine waters have the potential to remove nitrogen from wastewater because of its capacity to perform anammox reactions in which ammonia is anaerobically oxidized to dinitrogen [55,56].

Biofilms are increasingly used as early warning systems or an indicator for monitoring and evaluating heavy metal contamination in rivers and streams as structure and physiological alterations of biofilms occur rapidly in presence of toxicant. The choice of biofilms as an indicator system is beneficial as biofilms can adsorb pollutants from the environment, develops rapidly and offer easy sampling methods [57]. Biofilms are the first to interact with nutrients and pollutants in aquatic systems and as such biofilms can be used as early environmental monitor in aqueous bodies [58]. Various indicator properties of biofilm may be assessed for monitoring environmental pollution including change in biomass, species composition, pigment production, photosynthesis and enzymatic activity. Species present in river biofilms varies seasonally and on the level of pollution [59]. Heavy environmental contamination such as Zinc and Cadmium can influence the species diversity within biofilms and as such species diversity estimation of microbial biofilms can also indicate environmental pollution [60]. Apart from convention techniques, modern molecular biology methods such as denaturing gradient gel electrophoresis, ribosomal spacer analysis and terminal restriction fragment length polymorphism has been used to estimate species diversity within biofilms [61–64]. Biofilm sampling has been shown to provide an improved indicator of heavy metal contamination of aquatic microbial community [65]. Biomass change of natural algal biofilm community upon exposure to heavy metals and herbicides has also been assessed and may be used as an environmental pollution indicator [66]. Furthermore, as pigment composition among members of biofilm community can alter following exposure of toxic chemicals, analysis and patterns of pigment composition can serve as a biomarker [67,68]. Often, short term toxicity tests on multiple parameters affecting biofilm structure and function are assessed including photosynthetic activity and pollution induced community tolerance [69].

3. Strategies for Use of Biofilms in Remediation

Biofilm mediated remediation *in situ* may be performed in various ways. The process of natural attenuation relies on natural processes without the use of engineered steps or intervention including addition of specific strains for bioremediation. For example, microbial biofilm community present in the soil can biotransform certain pollutants into less harmful components. Natural attenuation is based on premise that under favourable conditions, certain contaminants can be degraded, transformed, immobilized and detoxified without any human intervention [70]. This passive remediation process requires resident profile of microbes which may be present in biofilm mode capable of degrading pollutants and requires long time. Extra nutrients such as carbon and phosphorus compounds, air to improve oxygen availability and additives may be added to increase growth and faster degradation of the pollutant in a process is called biostimulation. Natural attenuation may be monitored at defined times [20,71]. Natural attenuation strategy is typically used when the level of contaminant is relatively low and has been quite widely used in remediation of petroleum hydrocarbon sites [72]. This process is also known as monitored natural attenuation.

Bioaugmentation or bioenhancement, on the other hand, relies on inoculation of specific competent microbes or consortia of microbes in contaminated sites to perform degradation [73]. It is also likely that indigenous microbes capable of degrading pollutants may be present but requires substrates and nutrients to be added to assist the process. Microbial populations formed near the contaminated site can often degrade the pollutants found in the contaminated sites. Such microbial consortium can be stored under laboratory conditions and subsequently added to contaminated sites for cleaning the pollutant. Bioaugmentation is preferred method for freshly contaminated sites where indigenous population of microbes might lack or exhibits reduced capacity for efficient degradation. Such environment also facilitates development of microbial biofilms which increases the efficiency of degradation. The process of bioaugmentation may be monitored using biomarkers based on *gfp* or *luc* to track the efficacy of the inoculated microbes [74]. Bioaugmentation may be improved by incorporation of conventional genetic engineering techniques or by methods to increase the nutrient concentrations or persistence of microbes or by airventing and biostimulation methods [75]. In biostimulation, stimulus such as nutrients, growth substrates, electron donors and acceptors are provided to enhance the activity of microbes that are present near the site to better biodegrade the pollutant from the site [76]. A study on comparative bioremediation strategies showed that use of bioaugmentation and biostimulation were more efficient in remediation for petroleum hydrocarbon oil [77]. In another study, bioaugmentation and biostimulation have been shown to enhance nitrification performance [78]. In air venting, air is pumped into the site of contamination present below the soil surface to enrich aerobic microbial community and establish biofilms.

Contaminated sites lacking suitable endogenous microbial degrading population or favourable conditions for degradation that accelerate degradation of a pollutant in question may be subjected to *ex situ* remediation frequently in a reactor. In engineered systems, biofilms are used in a bioreactor in an inert support. The biofilm bioreactors are used for sorption and biochemical conversion of pollutants particularly for heavy metals, hydrocarbons and industrial and municipal wastewater [79]. Bioreactors based on biofilms particularly have been used commercially for cleaning up industrial wastewaters for decades [80,81]. Biofilm reactors offer many advantages over conventional treatment processes; high concentration and retention of biomass for long periods of time, enhanced metabolic activity, increased process flow rates, greater tolerance to harsh pollutants, large mass transfer area, enhanced volumetric biodegradation capacity, coexistence of anoxic and aerobic metabolic activity and reduced interruption in the bioreactor. In industrial set up, biofilm reactors are used in situations where free floating microorganisms in suspension do not produce adequate biomass or where biomass may not be retained long enough for efficient volumetric conversion. This can happen when microorganisms grow very slowly in suspensions or when a diluted feed streams are used in bioreactors. In a typical biofilm reactor, a support medium is needed for adhesion of microbes and development of biofilms. Biofilm bioreactors are of different types including batch, continuous stirred tank, trickle bed, air-lift reactors, upflow anaerobic sludge blanket, fluidized bed, expanded granular sludge blanket, biofilm airlift suspension batch reactors [80,82,83]. Biofilm reactors can be used off-site or used near the site of contamination. A schematic diagram of important types of bioreactors is shown in Figure 1.

Fixed-bed reactor, also known as packed bed reactor (Fig. 1A) is a common biofilm based reactor in which solid supports (media) are packed tightly where biofilms are colonised and provide high interface between the biofilm mass and the liquid. A packed bed reactor containing a biofilm of mercury resistant strains has been successfully employed in bioremediation of mercury [84]. However, fixed-bed reactors achieve high biofilm mass, which at times can clog the fixed-bed. Trickle-bed biofilm bioreactor (TBR), a special type of fixed-bed reactor, is one of the oldest types of biofilm reactor which has been extensively used for treatment of wastewater. Media used in TBR are usually plastic, rock, ceramics and other materials where biofilm develops. In TBR, wastewater trickles downward from the top via distribution system over the biofilm surface held on a fixed media. Pollutants in the water get metabolized as it diffuses through the biofilms. Oxygen may be supplied upward or downward which diffuses through the water to reach the biofilm. A net production of suspended solids in TBR requires a liquid-solid separation via a clarifier. The biofilm in the reactors may not have enough feed in certain areas and may cause reduced productivity.

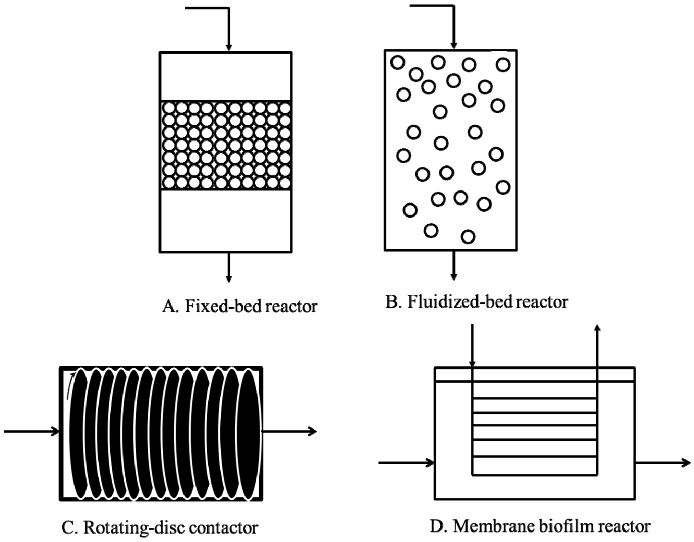

Figure 1. Various types of biofilm reactors. A. Fixed-bed reactor B. Fluidized-bed reactor C. Rotating-disc contactor D. Membrane biofilm reactor.

Fluidized-bed reactor (Fig. 1B) employs a column of biofilm coated beads in which polluted water is slowly pumped upward and keeps biofilm beads suspended during treatment of contaminated water [85]. This is in sharp contrast to fixed-bed reactor, where the media is not suspended. Solids are suspended either by flow of liquid or gas at certain velocity. Fluidization enables biofilms to develop on a large surface area and produce high biomass. Aeration is done either via oxygenator or supplied from the bottom of the reactor. Fluidized-bed reactor has been used for the treatment of streams contaminated with organic and inorganic compounds [85,86,87]. In Rotating Biological Contactors (RBC) or modified forms of RBC (Fig. 1C) has been used globally for wastewater treatment by reducing chemical oxygen demand and biochemical oxygen demand and also for nitrification and denitrification process [88,89,90]. RBC uses a thin biofilms of aerobic microbes which are grown on a rotating cylinder or biodiscs. Typically, the discs are partly submerged in effluent and slowly rotated such that biofilm microbes are alternately exposed to the effluent and air during which the attached biofilm on the disc degrades the pollutant. Excess biomass may slough off the RBC media and may be removed by clarifiers. Rotating biological contactors are economical in functioning and do not require much space or land. Some of the parameters that affect pollutant removal are rotational speed of the discs, disc submergence and hydraulic retention time. RBCs may be used for treatment of water contaminated with heavy metals, degradation of dyes, volatile organic compounds and PAH [88,91,92,93]. Membrane biofilm reactor (MBfR) (Fig. 1D) delivers pressurized air or oxygen via gas permeable membranes to the attached biofilms formed on the membrane exterior. Such bubble-free, high transfer of oxygen prevents stripping of volatile organic compounds and greenhouse gases and foaming when surfactant is being used. MBfR is particularly suited for treatment of high oxygen demanding wastewater. The membrane may also act as support media for development of biofilms. In some case, hydrogen-based MBfR, hydrogen may be delivered to the a biofilm comprising of autotrophic bacteria, which then oxidizes hydrogen and use electron donor to various pollutants such as chlorate and nitrate [94,95]. A methane fed membrane biofilm reactor has been used to remove nitrate and pesticides from contaminated water [96].

Various new biofilm bioreactors have been designed for treatment of recalcitrant pollutants. In sequential biofilm reactors, different bioconversion processes may be performed in separate stages. For example, a sequential aerobic-anaerobic two-stage biofilm reactor has been used to degrade polychlorinated hydrocarbons [97]. Simultaneous nitrification and denitrification was made possible due to presence of aerobic and anoxic biofilms in a novel air-lift internal loop biofilm bioreactor [98]. An intensified biofilm-electrode reactor combining autotrophic and heterotrophic denitrification has been demonstrated to remove nitrate from contaminated groundwater [99]. Biofilm reactors using sulfate-reducing bacteria entrap or precipitate metals such as copper and zinc at the interface of biofilms [100,101,102]. Studies are performed to better understand and optimize the processing conditions and parameters for biofilm based bioremediation in bioreactors [103,104,105]. Modeling and simulation studies are conducted to better design and optimize the biodegradation processes [106,107,108].

4. Types of Pollutants Remediated by Biofilms

Microbial biofilm mediated bioremediation are being increasingly used in removal of different types of pollutants including persistent organic pollutants, oil spills, heavy metals pesticides and xenobiotics. Biofilm remediation has been particularly useful in treatment of heavy metal contaminated samples from groundwater and soil for frequently encountered heavy metals such as cadmium, copper, uranium and chromium [109,110]. Phosphatase enzyme in presence of biofilm matrix facilitates metal precipitation both for aerobic bacteria and for anaerobic conditions [111]. In certain cases, biofilm formation is induced by addition of carbon sources in contaminated ground water to create a barrier or reduce flow of pollutants away from the site of contamination minimizing its spread.

Persistent organic pollutants (POP) are the most persistent of the pollutants with long half-lives due to hydrophobicity and can be found in air, water and sediments. Examples of POPs include polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls, polychlorinated ethenes, all of which are listed as toxic or harmful pollutants in the United States Environmental Protection Agency [112–115]. Many bacterial biofilm formers have been isolated from the environments that degrade POP which may be further modified and used in bioremediation [113]. POPs are hydrophobic and can be toxic when moved to the food chain [116]. Bacterial biofilms have been engineered for detoxification of POP. Studies have shown that biofilm formation and cometabolism in biofilms are key factors in remediation of polycyclic aromatic hydrocarbons [117,118,119].

Increasing use of petroleum in the industry is associated with rise in its accumulation in the water environment causing toxicity to marine life. Oil spills can itself shift the production of hydrocarbon degrading microbes in the soil [120]. Oil spills in the marine environment can be also decontaminated using hydrocarbon degradation bacteria exhibiting speedy growth [5,121]. In this context, several microorganisms including *Pseudomonas*, *Arthrobacter*, *Rhodococcus*, *Bacillus*, *Alcanivorax* and *Cycloclasticus* spp. of gamma proteobacteria have been employed [122]. Biofilm formation has been stimulated by turning off unused oilfields. New microbial strains capable of biofilm mediated oil degradation are being continuously screened [123]. In case of petroleum degradation, a microbial consortia comprising of *Bacillus subtilis* and *Acinetobacter radioresistens* with a surfactant producing strain has been shown to better degrader than microbial consortia consisting of degraders alone [124]. Use of water-insoluble fertilizers such as uric acid can provide the required nitrogen source for hydrocarbon degraders to facilitate the biodegradation of oil in open environment [125].

Heavy metals such as copper, zinc, nickel, cadmium, cobalt have been remediated using diverse biofilm reactors. Biofilm forming sulphate reducing bacteria (SRB) are particularly useful in mines for scavenging metals from metal contaminated water into precipitates of metal sulphides [102,126]. Recent studies suggest that certain bacterial strains are capable of forming electroactive films or electrochemically active biofilms (EAB) which directly exchange electrons with a conductive solid surface [127]. EAB are increasingly explored in the field of bioremediation and used for reduction of heavy metals from contaminated groundwater and soil [128,129].

5. Conclusions

Biofilm based bioremediation have certain limitations. Bioremediation is relatively slow as compared to the chemical treatment for pollutant degradation. A major limitation of bioremediation is reliability which limits its application in certain situations particularly during heavy contamination. Not all chemicals are amenable to biodegradation, particularly the man-made unnatural recalcitrant compounds such as plastics and certain halogenated aromatic compounds. Another limitation of bioremediation is that some metabolic toxic products could be generated post microbial degradation. The strategy of bioremediation is ideally useful in situations when the level of pollution is relatively low or does not require immediate restoration or where chemical treatment is not ideal. Bioremediation may be slow or ineffective when essential nutrients for supporting microbial growth are limiting, particularly when the level of pollution is high. Bioavailability of pollutant to microbes also determines the efficiency of bioremediation and pollutants that are not enclosed by other materials such as clay are more amenable to biodegradation. Adequate technical expertise and interdisciplinary approach from different field such as environmental microbiology, civil engineering soil science is also required for successful performance of biofilm mediated bioremediation. Biofilm reactors may not be used with rapid growing microorganisms where the reactor capacity is dependent on oxygen diffusion. Because of these limitations, bioremediation may be assessed for effectiveness relative to other methods available for decontamination of environmental pollutants [130].

While biodegradation by microbes has been taking place since the beginning of life, the field of bioremediation is relatively new. Novel approaches are constantly being tested and employed in the field of bioremediation. Improved strains are continuously being designed that energetically favour pollutants as preferred substrates over other available compounds for effective bioremediation [109]. A novel approach for improving bioremediation uses natural transformation process within biofilms utilizing uptake of DNA harbouring catabolic genes that facilitate biodegradation of selected pollutants [131]. Genetically modified microorganisms (GEM) have been constructed with the capacity to degrade diverse pollutants including halogenated aromatic compounds [132,133]. Horizontal transfer of genes capable of biodegradation from GEM to members of biofilm populations can further enhance biodegradation process. Cloning of genes for biosurfactant synthesis and chemotactic ability of GEM can further enhance the biodegradative capability of modified microbes. Nevertheless, the release and use of GEM in the nature and transmission is under much debate and controversial. However, the majority of organisms usually have other disabling mutations that will not permit the microbes to grow outside a given environment. Reengineering of secreted proteins in biofilm matrix is also an area for further development in the field of bioremediation [134]. Cell-cell interaction among members of biofilm community can be explored further to improve the bioremediation process. Combined processes are increasingly used such as trickling filters with RBC or with activated sludge to maximize the strengths and minimize the weaknesses of these individual processes [135,136]. In certain cases of recalcitrant pollutants, biofilm mediated bioremediation can be used in combination with phytoremediation or with chemical treatments. Still in other cases, microbial consortia of bacteria-fungi may be used in combination to degrade xenobiotic compounds. Overall biofilm mediated bioremediation remains an attractive choice in mitigating environmental pollution despite of its limitations.

Conflict of Interest

The authors declare no conflicts of interest regarding this paper.

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