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MINI-REVIEW

Applications of biofilms in bioremediation and biotransformation of persistent organic pollutants, pharmaceuticals/personal care products, and heavy metals

Sarah J. Edwards · Birthe V. Kjellerup

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Abstract In this review, the strategies being employed to exploit the inherent durability of biofilms and the diverse nutrient cycling of the microbiome for bioremediation are explored. Focus will be given to halogenated compounds, hydrocarbons, pharmaceuticals, and personal care products as well as some heavy metals and toxic minerals, as these groups represent the majority of priority pollutants. For decades, industrial processes have been creating waste all around the world, resulting in contaminated sediments and subsequent, far-reaching dispersal into aquatic environments. As persistent pollutants have accumulated and are still being created and disposed, the incentive to find suitable and more efficient solutions to effectively detoxify the environment is even greater. Indigenous bacterial communities are capable of metabolizing persistent organic pollutants and oxidizing heavy metal contaminants. However, their low abundance and activity in the environment, difficulties accessing the contaminant or nutrient limitations in the environment all prevent the processes from occurring as quickly as desired and thus reaching the proposed clean-up goals. Biofilm communities provide among other things 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 for instance mine drainage. The durability and structure of biofilms together with the diverse array of structural and metabolic characteristics make these communities attractive actors in biofilm-mediated remediation solutions and ecosystem monitoring.

S. J. Edwards · B. V. Kjellerup (✉)
Department of Biological Sciences, Goucher College, 1021 Dulaney Valley Road, Baltimore, MD 21204, USA
e-mail: birthe.kjellerup@goucher.edu

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Introduction

For decades, industrial processes have created waste all around the world, resulting in contaminated sediments and subsequent, far-reaching dispersal into aquatic environments (Lucas et al. 1993). Dredging, capping, and monitored natural remediation have remained the priority methods for remediation (EPA 2005). Though removing tons of sediment to designated waste sites eventually reduces the risk of exposure to indigenous organisms, it is an expensive process that only dislocates the problem (Perelo 2010). Dredging and capping reduce the bioavailable portion of contaminants in pore water, but during implementation these solutions might cause detrimental resuspension of sediment and thus negative effects on benthic fauna due to the release of contaminants to the water phase (Cho et al. 2009). In addition, the pollutants still remain in the environment despite the short-term benefits caused by reduced exposure to benthic organisms (Samuelsson 2012). As persistent pollutants have accumulated in the environment for decades and new types are still being created, the incentive to apply a suitable solution to effectively detoxify and reduce the exposure in the environment is even greater.

Though indigenous bacterial communities are capable of metabolizing persistent organic pollutants and oxidizing heavy metal contaminants, their low abundance and activity often together with a lack of access to the contaminants and limitations in available nutrients in the environment prevent these processes from occurring at sufficiently rapid rates to reduce the exposure in the aqueous environment (de Lipthay et al. 2003; Petrie et al. 2003). Free-living planktonic bacteria can metabolize the same pollutants or toxins as their biofilm counterparts. However, while

their survival in the environment is less likely due to decreased protection, the low metabolic activity combined with low bioavailability of the pollutants in the water phase cause insignificant transformation by planktonic bacteria (von Canstein et al. 2002). By contrast, attached and sessile microorganisms located in biofilm communities provide structure and protection because of their growth in a self-produced and complex polymeric matrix (Vu et al. 2009). In addition to genetic diversification in single species biofilms, biofilms can harbor diverse species of both aerobic and anaerobic organisms allowing them to metabolically complement each other and survive in the presence of varying nutrients (von Canstein et al. 2002; Boles et al. 2004). This durability and metabolic range makes them very attractive actors in bioremediation. In the environment, indigenous biofilms constantly perform bioremediation, particularly in soils and sediments, which is a part of the global nutrient cycling process and a part of the global self-purification system. However, the question is always how the kinetics of biodegradation of organic pollutants or the immobilization of toxic metals can be influenced to overcome the limitations and thus obtain the best outcome.

Bacterial communities have been utilized for the past century to neutralize, degrade, and mineralize many xenobiotic compounds in wastewater-activated sludge (Byrns 2001; Bertin et al. 2007). Despite these advances, the importance of facilitating biofilm growth to enhance detoxification as well as the longevity of microbial diversity and abundance has just recently been actively appreciated and thus applied (Boon et al. 2003; Accinelli et al. 2012). The isolation and characterization of bacteria like that of the phylum *Chloroflexi* (*Dehalococcoides*, *Dehalobium chlorocoercia*, and *o-17*) capable of organohalide respiration has shed light on the potential use of microbial processes for the degradation of halogenated hydrocarbons (Löffler et al. 2013). Furthermore, members of the genus *Dehalococcoides* have been recognized as valuable residents of a biofilm reactor community for reductive dechlorination of trichloroethene (Chung et al. 2007). Utilization of bacterial communities has not only been applied towards organic contaminants but also towards metals. Passive oxidation of arsenic and iron by biofilms was successful at gold-quartz mining sites (Guezennec et al. 2012), selenium has been reduced and subsequently concentrated in biofilms on tubes containing nutrients (Williams et al. 2013), and in coal mine drainage regions, biofilm enzymes have been employed as biomarkers for stream water quality (Pool et al. 2013). These instances exemplify the diverse potential of biofilm-mediated remediation and pollution monitoring services.

The objective of the mini-review is to provide an overview of the strategies for bioremediation and biotransformation that have been applied based on the benefits from the biofilm mode of growth. The limitations of the employed biofilm solutions will also be discussed. The priority pollutants such as persistent organic pollutants (e.g., PCBs and dioxins), pharmaceutical and personal care products, as well as toxic metals are in focus. In addition, the utilization of enzymatic mechanisms of biofilms will be discussed as they can provide a more detailed information on the quality and status of contaminated ecosystems.

Why biofilms?

The ability for microorganisms to congregate in sessile biofilm structures allows for many advantages compared with their free-living planktonic counterparts, such as protection from the surrounding environment, ability to communicate and exchange genetic material, nutrient availability (from the environment and each other), and persistence in different metabolic states (Davies et al. 1998; Costerton 1999) (Table 1). This array of characteristics exemplifies a complex and durable mechanism akin to different types of cells functioning as an organ (Fux et al. 2005). Biofilms can consist of single or multiple species of microorganisms originating from one or more kingdoms such as bacteria, fungi, algae, and archaea and with varying environmental requirements with regard to electron acceptors/donors and nutrient concentrations (Ferrera et al. 2004; Baker et al. 2009). This versatility results in the development of physical biofilm structures that reflects all the environmental conditions that biofilms are exposed to. Encased within extracellular polymeric substances (EPS) secreted by the involved microbes, the biofilm structures grow and incorporate water channels, which allow transport of nutrients, electron acceptors such as oxygen or other more reduced compounds to occur. Thus, cells on the outer layers of these structures are able to thrive, whereas those located further away might experience limitations in nutrient availability, etc. (Picioreanu et al. 2000; Chen et al. 2013). Additionally, the EPS contains surfactants, which can aid in solubilizing hydrophobic or other recalcitrant substrates that would otherwise be inaccessible. This has been well documented in biofilms found in diverse geographic regions such as tidal flats, streambeds, corroded pipes, and even sites of infection (Latch et al. 2003; Seo et al. 2009; Wang et al. 2011).

The tolerance toward toxic and hazardous chemicals is of particular interest, when biofilms are applied for bioremediation and biotransformation purposes, as the chemicals in question can be present in such high concentrations that would be detrimental to planktonic microorganisms. Aggregation of microorganisms in sessile or floating biofilms (such as activated sludge) has the major advantage of increased tolerance towards changes in environmental conditions such as nutrients, predation, exposure to toxic chemicals (e.g., antibiotics or pollutants in high concentrations) or other environmental stressors as for instance changes in pH, temperature, salt concentration, and water content (Heipieper et al. 1991; Beveridge et al. 1997; Hall-Stoodley et al. 2004). This increased tolerance does not only occur in environmental

Table 1 Major characteristics of biofilms with importance for bioremediation

Property	Characteristic	Reference
Tolerance towards environmental stressors (e.g., toxic chemicals, pH change, predation, and dehydration)	Extensive genetic diversification of biofilm bacteria	Boles et al. (2004), Vu et al. (2009), Boon et al. (2003), Accinelli et al. (2012), and Hall-Stoodley et al. (2004)
Communication (quorum sensing)	Critical cell density for biofilm formation	Hentzer et al. (2003) and Sarkar and Chakraborty (2008)
Exchange of genetic material	Horizontal transfer of genetic material between species, DNA sharing	Beaudoin et al. (1998), Ghigo (2001), and Wolcott et al. (2013)
Metabolic diversity and symbiosis	Utilizing waste products and/or accumulated products from the environment or other microorganisms	von Canstein et al. (2002), Boles et al. (2004), Picioreanu et al. (2000, and Chen et al. 2013)
Redox and electron acceptor diversity	Different metabolic functions with respect to electron-acceptor reduction	Falkentoft et al. (2002), Zhang et al. (2010), and Ontiveros-Valencia et al. (2012)
Varying growth rates in the biofilm	Inducing biofilm persistence due to different metabolic states in the biofilm	Wentland et al. (1996), Davies et al. (1998), Costerton (1999), and Woo et al. (2012)
Porous physical structure with water channels	Allow for transport of nutrients, electron acceptors, and waste products	Latch et al. (2003, Hall-Stoodley et al. (2004), Wijeyekoon et al. (2004), Seo et al. (2009), Wang et al. (2011), and Xiao et al. (2012)
Surfactants	Aid in solubilizing hydrophobic or recalcitrant substrates	Rodriguez and Bishop (2008) and Seo et al. (2009)
Microcolony and gradient formation	Redox potential and nutrient cycling because of aerobic and anaerobic processes	Galiana et al. (2008) and Verhagen et al. (2011)

biofilms, but it can also be observed in biofilms in the human body. The antibiotic concentration required to kill planktonic bacteria often is 100–1,000 times lower than required for biofilms if these at all respond to treatment (Mah et al. 2003). Examples of this are *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* biofilms present in cystic fibrosis patients (Waters and Ratjen 2012; Wu et al. 2013) but also *Staphylococcus aureus* biofilms in chronic wounds show increased tolerance towards antibiotics (Secor et al. 2011). A general stress adaptation strategy for gram-negative bacteria in biofilms is the release of membrane vesicles (MVs) (Manning and Kuehn 2011). It has been shown for *P. aeruginosa* that predatory MVs can eliminate neighboring bacteria and destroy the competition thus making nutrients from the lysed cells available for the surrounding biofilm bacteria otherwise exposed to starvation stress (Beveridge et al. 1997). In studies using *Escherichia coli*, Manning and Kuehn (2011) showed that MVs could neutralize environmental agents such as polymyxin B targeting the outer membrane of the cells (Manning and Kuehn 2011), whereas exposure to hydrocarbons resulted in release of MVs thus altering the cell surface charge and hydrophobicity for *Pseudomonas putida* DOT-T1E (Baumgarten et al. 2012).

Recent advances in bioremediation have been taking strides to understand and improve the utilization of biofilm communities as the participating microorganisms in biofilms naturally complement each other's metabolic needs and demonstrate enhanced resistance to environmental stresses (Hall-Stoodley et al. 2004). In heavily contaminated sites, it has been shown that cells predominantly grow in biofilms to manage the harsh environmental conditions (Gross et al. 2007). This natural characteristic and thus the robustness of biofilms can effectively be utilized to develop strategies for bioremediation in situ (groundwater and other aquatic systems) or ex situ in biofilm reactors (Table 2).

Successes with bioremediation of persistent organic pollutants

Organic compounds that mostly have been produced for industrial applications are some of the widest spread and most persistent pollutants found in air, water, and sediments (Ritter et al. 2002; Roots et al. 2010). These persistent organic pollutants (POPs) comprise several of the most prioritized compounds on the USEPAs lists of harmful and/or toxic contaminants (Buth et al. 2009). They include but are not limited to: polycyclic aromatic hydrocarbons (PAHs), polychlorinated ethenes (PCEs), polychlorinated dibenzo-*p*-dioxins and difurans (PCDD/Fs), and polychlorinated byphenyls (PCBs). In addition to these pollutants, most of which have been banned for over three decades, other xenobiotic compounds such as pharmaceutical and personal care products (PPCPs) as well as fertilizers, pesticides, and herbicides are produced and distributed in massive amounts contaminating water and sediments world-wide (Karlaganis et al. 2001; Lammel and Lohmann 2012). Given that many POPs are hydrophobic, they can accumulate in the food chain to toxic levels as carcinogens and endocrine disruptors (Hardell et al. 2004;

Table 2 Current biofilm-based approaches for bioremediation and biotransformation of organic and inorganic contaminants and the existing limitations

Approach	Pollutant	Organisms	Efficiency	Limitations	References
Organic contaminants					
Mulch biowall barrier	PAHs (phenanthrene and pyrene)	PAH degraders: mix of activated sludge and PAH-contaminated soil enriched on phenanthrene	Phenanthrene, 97–99 % and pyrene, 99.9 %	Access to contaminant (surfactant added), abundance of organisms, and presence of electron acceptor (O_2 and NO_3^-)	Seo et al. (2009)
	Naphthalene	Naphthalene degraders: mix of activated sludge and PAH-contaminated soil enriched on naphthalene	94–100 %	Access to contaminant (surfactant added); Abundance of organisms, presence of electron acceptor (O_2 and NO_3^-)	Seo and Bishop (2008)
Bioactive granular-activated carbon	Polychlorinated biphenyls (PCBs) mixed PCB contamination	*Dehalobium chlorocoercia* DF1 and *Burkholderia xenovorans* strain LB400 together with activated carbon	56 % (120 days)	(1) Abundance of organisms with potential for PCB dechlorination an (2) access to adsorbed PCBs	Payne et al. (2011)
	PCBs: Aroclor 1248	*D. chlorocoercia* DF1 and *B. xenovorans* strain LB400 biofilms on activated carbon	33 % (200 days)	(1) Abundance of organisms with potential for PCB dechlorination and (2) access to adsorbed PCBs	Edwards and Kjellerup (2013)
Sand aquifer	Raw water from Lake Malaren, Sweden (natural organic matter and humic acids)	Raw water from Lake Malaren, Sweden	TOC, 10 % and AOC, 87 %	Contaminant recalcitrance	Langmark et al. (2004)
Fractured porous media (rock)	Compounds in groundwater of drinking water quality	Inoculum from groundwater	Reduced porosity due to biofilm, 6–52 %	Abundance of organisms, access to contaminants, and nutrient availability	Charbonneau et al. (2006)
Hydrogen-based membrane biofilm reactor	Perchlorate and nitrate, nitrate, trichloroethene, trichloroethane, and chloroform	In situ groundwater inoculums and autohydrogenotrophic denitrifying bacteria (in situ)	Perchlorate and nitrate, 99.5 % and nitrate, 97–100 %	(1) Hydrogen if sulfate reduction occurs; (2) inhibition by chloroform and TCE; and (3) H_2 water solubility	Ziv-El and Rittmann (2009), Xia et al. (2010), and Chung and Rittmann (2008)
Hollow-fiber membrane biofilm reactor	Toluene;	*Pseudomonas putida* strain To1 1A	17 g m^{-3} min^{-1} (98 %)	Access to contaminant (increased mass transfer)	Kumar et al. (2010)
	Para-chloronitrobenzene (*p*-CNB)	Inoculum from bioreactors treating nitrate contaminated drinking water	*p*-CNB, 95.7 %	Competition with electron acceptors (nitrate and sulfate) for H_2 (electron donor)	Li et al. (2013)
Packed-bed biofilm reactor	Acenaphthene, phenanthrene, and pyrene	Micro flora from bioreactor adapted to acenaphthene/ phenanthrene	Acenapthene and phenanthrene, >99 % and pyrene, 90 %	Diluted source/access to contaminant and competition with other organic contaminants for electron acceptor (O_2)	Guieysse et al. (2000)
Sequencing batch biofilm reactor	Phenol and 2-chlorophenol	In situ groundwater inoculum	Phenol and 2-chlorophenol, 99 %	Reaction time (recirculation required) and co-metabolism	Farabegoli et al. (2008)
Inorganic contaminants					
Rotating biological contactor	Cadmium (Cd), copper (Cu), and zinc (Zn)	Enrichments (metal) from activated sludge	Cd_2^+, 30–35 %; Cu_2^+, 74–85 %; and Zn_2^+, 50–57 %	Nutrient limitation and dilute concentration of contaminant	Costley and Wallis (2001)
Horizontal rotating tubular bioreactor (HRTB)	Chromium (Cr), manganese, and cobalt	Enrichment culture from heavy metal-contaminated soil	Cr, 100 %; manganese, 94 %; cobalt, 69 %; overall metal removal, 2.5–8.3 mg L^{-1} h^{-1}	Toxicity of metals and transport/ diffusion (highest affinity for Cr)	Zeiner et al. (2012)
Membrane feeding substrate bioreactor	Nitrate	*Alcaligenes eutrophus*	Nitrogen removal rate (1.6–5.4 g $Nm^2day^{-1})^{-1}$	Nutrients (H_2 and CO_2)	Ho et al. (2001)
Moving bed sand filter	Cd, Zn, Cu, Pb, Hg, Ni, Co, and nitrate	Heavy metal-resistant bacteria	–	Nutrients and carbon source and reaction time with biofilm	Diels et al. (2003)
Sequential anaerobic–aerobic moving-bed biofilm reactor	Landfill leachate (municipal solid waste)	–	Nitrogen, >97 % and COD, 93 %	Contact time/access to contaminants	Chen et al. (2006)
Anaerobic upflow sludge bed	Pentachlorophenol (PCP)	*Desulfitobacterium frappieri* PCP-1	4 mg PCP g^{-1} ·VSS day^{-1}	Abundance of degrading organisms	Guiot et al. (2002)
Sand aquifer	Strontium	*Halomonas* sp (Sr resistant)	Strontium (Sr), 80 %	Abundance of urease positive organisms with tolerance to Sr and calcite precipitation	Achal et al. (2012)

Bonefeld-Jorgensen et al. 2006). It is known that bacteria exist in the environment with specialized capabilities of metabolizing and mineralizing many of these POPs (Chua et al. 2001; Zhang et al. 2010; Kataoka and Takagi 2013). Accordingly, the science of bioremediation is actively taking advantage of these naturally existing microbial biofilms to engineer systems that more effectively can overcome the current limitations and thus detoxify and recycle POPs in the environment.

Polycyclic Aromatic Hydrocarbons

A large obstacle slowing the process of bioremediation of PAHs is their low bioavailability as well as low solubility once adsorbed into soil and sediment. PAHs can be consumed by benthic organisms and make their way up the food chain, bioaccumulating in fatty tissues with potential mutagenic and carcinogenic effects. In 2004, Johnson et al reported that biofilms increased the solubility of PAHs and subsequently their mass transfer from recalcitrant crystals to cells for biotransformation (Johnsen and Karlson 2004). They showed that biofilm formation was the principal mechanisms by bacteria to overcome mass transfer limitations for recalcitrant PAHs and that the presence of biosurfactants resulting in "pseudo-solubilization" did not directly enhance the bioavailability of the PAHs. Later, Rodriguez and Bishop (2008) demonstrated that mixed biofilms formed with activated sludge inoculum achieved higher biodegradation efficiencies on mixed PAH substrates than if it was combined with a surfactant (Rodriguez and Bishop 2008). The results from these studies showed that increased solubility of PAHs due to the presence of surfactants was not the most important factor for increased biodegradation. Instead, co-metabolic mechanisms enabled degradation of multiple PAHs simultaneously. This is important given that over 100 PAH compounds exist and that a mixture of these compounds most often is found in the contaminated environment. Co-metabolism was shown in biofilms in the engineered "Biozo" process by Plosz et al (2010). Here, a combination of ozone and biological treatment was applied to remediate PAH and xenobiotic micro-pollutant contamination from landfill leachate (Plosz et al. 2010). A staged moving-bed biofilm reactor (SMBBR) combined with ozone that alternated between a pre-anoxic zone, where the majority of the PAH removal occurred, and an aerobic zone exhibited optimal PAH and nitrogen removal (Plosz et al. 2010). This technology exemplified the concept that nitrate reduction was related to PAH degradation in reduced, anoxic environments and emphasizing that co-metabolism can be a very successful bioremediation solution (Heidler and Halden 2007; Lolas et al. 2012).

Chlorinated ethenes

Another POP that often threatens drinking water sources is chlorinated ethene. Löffler et al. (2013) described a group of bacteria (*Dehalococcoides*) that are the only known microorganisms to reduce chlorinated ethenes by specializing in reductive dechlorination (Löffler et al. 2013). This was reflected in results by Chung et al (2007), where a hydrogen based, denitrifying membrane biofilm reactor (MBfR) was utilized to reductively dechlorinate trichloroethenes (TCE) (Chung et al. 2007). It was reported that *Dehalococcoides* was naturally present at the beginning and end of the study and that they were enriched for by exposure to TCE (Chung et al. 2007). This indicated that the biofilm reactors already being applied might be utilized for multi-purposes.

In a different arena of bioremediation, work has been done to evaluate electron donor amendment (N-lactate) intended to stimulate the reductive dechlorination by *Dehalococcoides ethenogenes* in a TCE contaminated aquifer (Conrad et al. 2010). The results showed that the added electron donor might also have stimulated co-metabolism by the obligate aerobic methane-oxidizing bacterium *Methylosinus trichosporium*. The change from methanogenesis to methane oxidation was indicated in part by dissolved methane and confirmed by microarray analysis of 16S rRNA from groundwater plumes. Furthermore, Lohner et al (2011) demonstrated sequential reductive and oxidative co-metabolic degradation of PCE stimulated by electrochemical processes originating from a coupled anode-cathode bio-electro setup (Lohner et al. 2011). Complete degradation was observed after two months with removal of up to 1.5 μmol/day PCE demonstrating the feasibility of applying this system to contaminated groundwater or wastewater (Lohner et al. 2011).

Polychlorinated biphenyls and dioxins

PCBs and dioxins are other POPs that are among highly toxic compounds. Despite their ban over thirty years ago, PCBs can still be found in soil, sediment and even air all around the world (Roots et al. 2010). Dredging and capping still remain priority methods for sites deemed contaminated with PCBs. Recently, attention has been paid to the group of microorganisms called dechlorinating *Chloroflexi*, because of their ability to reductively dechlorinate highly chlorinated PCB congeners under anaerobic conditions thus leaving less chlorinated structures available for aerobic degradation (Fagervold et al. 2005). Payne et al (2011) reported that bioaugmentation by the anaerobic dechlorinating bacterium *D. chlorocoercia* DF1 to sediment contaminated with weathered PCBs affected doubly flanked chlorines as well as stimulated indigenous dechlorinating microbial communities to dechlorinate other PCBs (Table 2). On PCB oil droplets, significant microbial degradation of penta-chlorobiphenyls was noticed in the presence of a mature biofilm (Macedo et al. 2005). Coupling the concept of bioaugmentation with that of activated carbon sediment amendment, it has been shown that mature biofilms of dehalogenating bacteria can develop on granular activated

carbon (GAC) surfaces (Mercier et al. 2013). Furthermore, several studies have shown that formation of a mature biofilm on GAC does not hamper the adsorption properties of GAC and that adsorption of PCBs does not affect biofilm formation (McDonough et al. 2008; Mercier et al. 2013). Since the techniques for delivering GAC to sediments through water columns have been in place for almost a decade (McLeod et al. 2004; Cornelissen et al. 2006; Werner et al. 2006), the field of PCB bioremediation might greatly benefit from a combination of GAC amendment with biofilm engineering, which has been evaluated on a mesocosm scale for contaminated sediment (Edwards and Kjellerup 2013).

Currently, dioxins such as dibenzofuran or 2-chlorodibenzofuran are considered some of the most toxic pollutants. They are unintentionally created as by-products from the mixing of various chemicals in industrial processes such as the production of herbicides, the bleaching of wood pulp for paper and the incineration of medical and municipal waste (Glasser et al. 1991; Lohman and Seigneur 2001). The preferred methods of remediation include high energy thermal and chemical incineration, but biodegradation is looking ever more attractive as studies enhance the understanding of the microorganisms available to metabolize such compounds (Hiraishi et al. 2001; Wang and Oyaizu 2011). Using quinone-profiling to study microbial communities, Hiraishi et al (2001) were able to obtain 22 % degradation of PCDD/Fs within three months in highly polluted soil microcosms (Hiraishi et al. 2001). A promising study, which employed *Burkholderia sp.* NK8 together with *P. aeruginosa* PA01, showed enhanced ability of the dual species biofilms to completely degrade chlorinated benzoates (Yoshida et al. 2009). More recently, biofilms consisting of *Comamonas sp.* Strain KD7enhanced by their relationship with *Trifolium repens* (white clover) roots showed significant reduction of existing dioxins in soil samples (Wang and Oyaizu 2011).

Pharmaceutical and personal care products

In addition to the aforementioned pollutants, there are scores of other xenobiotic chemicals that fall under the category of PPCPs. Many of these compounds are not regulated in terms of wastewater and are therefore emitted into the environment as part of the effluent. One of particular concern is triclosan, a commonly prescribed and industrially used antimicrobial that can be photo transformed into dioxin congeners in water columns (Latch et al. 2003; Buth et al. 2009). Not only does triclosan cause detrimental effects to native biofilm communities that have the capacity to degrade dioxins due to its antimicrobial nature, but dioxins are thereby being unintentionally replenished. The threat of pollution to waterways increases, when changing climates reduce the flow of water in some areas, concentrating pollutants in the water. In a study that looked at the effects of draught on river ecosystems, it was reported that biofilms periodically exposed to environmentally relevant levels of triclosan and the herbicide, diuran, have the capacity to return to homeostatic conditions within a few days to weeks (Proia et al. 2011). However, long-term exposure exacerbated by drought threatens the survival of even established biofilms justifying the development of more effective wastewater treatment (Proia et al. 2013). Still, the biodegradation of triclosan in conventional sewage treatment facilities is inadequate resulting in the contamination of agricultural land from recycled sludge fertilizer (Heidler and Halden 2007). Currently, the initial understanding of the microbial influence has begun together with identification of the microbial populations responsible for triclosan degradation. Further studies within this area will hopefully inform and subsequent improve the wastewater treatment process (Lolas et al. 2012). In terms of other common PPCPs in wastewater, it was shown that membrane biofilm reactors proved more useful than traditional activated sludge in terms of removing several PPCPs, such as diclofenac, trimethoprim, metoprolol, and gemfibrozil (Sui et al. 2011). This study also reported that the concentration of these PPCPs increased in wastewater effluent during the winter months indicating that the bacterial biofilm communities responsible for their degradation were less abundant and/or active during the winter. Another study demonstrated the efficacy of in situ remediation of PPCPs, when wastewater sludge was applied as inoculum to form biofilm on sand column filters (Onesios and Bouwer 2012). In this study, 10 of the 14 PPCPs tested (biphenylol, *p*-chloro-*m*-cresol, chlorophene, 5-fluorouracil, gemfibrozil, ibuprofen, ketoprofen, naproxen, triclosan, and valproic acid) were completely degraded. Though the remaining compounds were more recalcitrant, this experiment showed that biofilm-based treatment can significantly reduce the impact of PPCPs in the receiving waters thus preventing further environmental contamination.

Biofilms and heavy metals: bioremediation and assessment

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 (Kwon and Lee 1998). Heavy metal remediation is unique because, unlike organic pollutants, biological processes are not capable of completely removing the contaminants from the environment (Fig. 1). 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

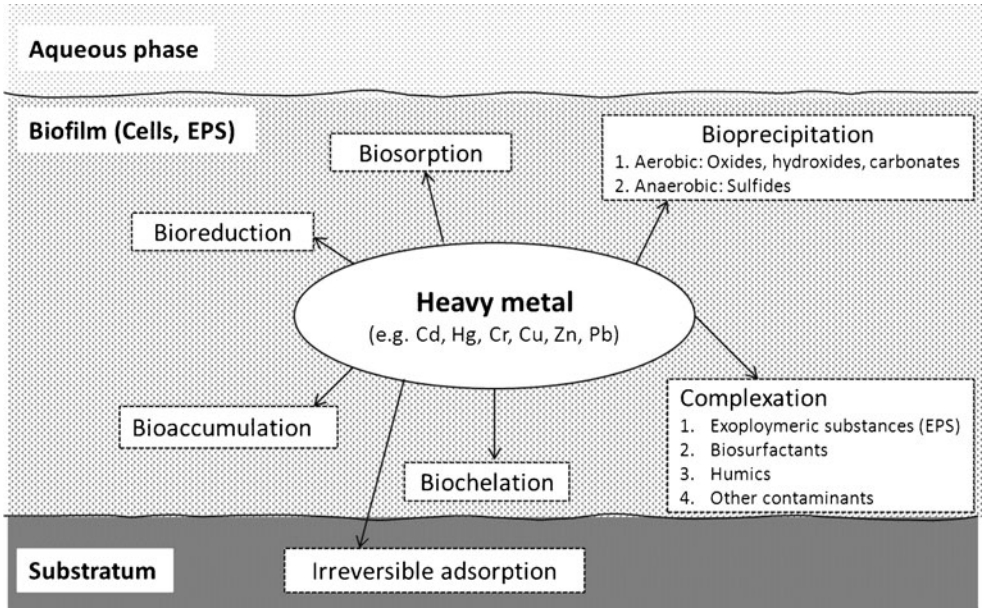

Fig. 1 Mechanisms influencing the mobility and biotransformation of heavy metals in the presence of biofilms

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). Though this study did not evaluate the species richness of the RBC biofilm, its ability to cope with such environmental stress and thrive on multiple metallic species suggested that it was composed of a diverse community. 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 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). Here, it was reported that the biofilms not only served as biosorptive material but also lowered the pH enough to facilitate bioprecipitation of some heavy metals (Diels et al. 2003). 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) (Morel et al. 2009). 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 benefits of EPS for bioremediation of heavy metals were described in detail for specific bacteria in a review by Pal and Paul (2008). 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.

Bioremediation limitations

Lack of success with bioremediation can largely be attributed to the following issues (Table 2): (1) absence or too few microorganisms holding the potential to degrade the contaminant, (2) lack of access for the microorganisms to the

pollutant due to adsorption, and (3) growth and activity limiting factors such as availability of electron donors/acceptors (e.g., oxygen, nitrate, or sulfate), water activity and possible benefit from co-metabolism (Dua et al. 2002; Frascari et al. 2013).

The challenges for bioremediation of recalcitrant POPs are often the lack of indigenous microorganisms capable of performing bottleneck processes as for instance reductive dechlorination of PCBs. Here, congeners containing four to five chlorines have to undergo anaerobic reductive dechlorination in order for aerobic PCB-degrading organisms subsequently breaking down and mineralizing the biphenyl structure completely. Only a limited number of bacterial species are currently known to perform this process and the in situ abundance is low (Fagervold et al. 2007; Kjellerup et al. 2008; Löffler et al. 2013). In addition, the dechlorination process yields little energy for the organisms thus making it slow and sensitive to inhibiting factors (May et al. 2006). The bioavailability of hydrophobic compounds (for instance PCBs) is often limited due to strong adsorption to inorganic surfaces in the environment (Choi and Al-Abed 2009). Thus, biofilm-based bioremediation approaches involving bioaugmentation to supply organisms located on a surface that can compete with the adsorption to inorganic surfaces might be a solution for this group of contaminants, which the use of activated carbon combined with bioaugmentation has shown (Payne et al. 2011; Kjellerup et al. 2013).

The lack of available electron acceptors has been experienced for several bioremediation strategies and contaminated environments (Table 2). An example is the subsurface, where *Geobacter* species play an important role in bioremediation of metals, and it is known that Fe(III) availability as electron acceptor is a limiting factor (O'Neil et al. 2008). Transcript analysis has revealed that limited Fe(III) availability also limited assimilatory functions even though large amounts of Fe(II) were produced by reduction of Fe(III) and sequestered in solid phases in the subsurface. Instead, *Geobacter* utilizes iron–sulfur proteins to satisfy the assimilatory requirements for iron. Similarly, it was shown by microarray analysis that phosphate might be limiting the activity of *Geobacter sulfureducens* under culture conditions (N'Guessan et al. 2010). However, stimulation of dissimilatory U(VI) reduction was not obtained, when phosphate was added to subsurface sediments. Instead phosphate adsorbed to the sediment and reduction of U(VI) occurred even when phosphate was limited. A strategy to overcome the issues of nutrient limitation for *Geobacter sp*. would be to form biofilms. As described previously numerous advantages can be obtained by living in a biofilm (Table 1). Specifically for *Geobacter* sp., enhanced access to Fe(III) or iron–sulfur proteins and phosphate from other species in the biofilm or the environment would be obtained (Babauta et al. 2012). Several studies have applied the biofilm properties of *G. sulfureducens* for instance in microbial fuel cells (Reguera et al. 2006; Rollefson et al. 2009).

Biofilm strategies for bioremediation

Numerous solutions for *in situ* and ex situ bioremediation have been evaluated and applied the last several decades (Fig. 2; Table 2). The in situ biofilm barrier has been applied for bioremediation of groundwater with nitrate (Williamson et al. 2012) and natural organic matter (Langmark et al. 2004). Depending on the contamination characteristics such as the concentration of contaminants, mixed contamination, presence of microorganisms, etc., the barrier material can be designed to accelerate the bioremediation effort. Biostimulation can occur, when the barrier is loaded with nutrients, electron acceptors, and/or biocatalysts (for instance Fe0) (Alvarez et al. 1998), resulting in the development of a biofilm on the barrier as the microorganisms are already present. If there is a lack of required microorganisms (abundance or diversity), the barrier can be put in place with an already existing biofilm or the active microorganisms can be added simultaneously but not attached (Payne et al. 2011) or after the barrier has been installed, so the biofilm can be established on the barrier material (Seo et al. 2009). Application of a hollow-fiber membrane, passively supplying dissolved hydrogen (H_2), was recently developed to stimulate the biodegradation of chlorinated solvents in groundwater (Fang et al. 2002), whereas barrier material containing the catalyst zero-valent iron together with hydrogenotrophic bacteria has been evaluated for synergistic abiotic and biological remediation of contamination containing nitrogeneous and halocarbon compounds (Alvarez et al. 1998). Another possibility can be to utilize surfaces already in place such as fractured porous rock or sand formations and establish the biofilm barrier in situ (Langmark et al. 2004; Charbonneau et al. 2006).

Biofilms as monitoring and assessment tool

Biofilms have in addition to more traditional bioremediation approaches recently been applied as tools for monitoring and assessment of heavy metal pollution in water columns and streams. Early on, Fuchs et al (1997) recognized the potential to detect heavy metal pollution in freshwater ecosystems by sampling biofilms. It was determined that biofilms demonstrated pollution profiles similar to sediments and thus, with an easy technique and low sample variability, provided a great resource for in situ monitoring of heavy metal pollution (Fuchs et al. 1997). With emerging technologies, it is now possible to analyze the enzyme activity in biofilms to obtain an improved understanding of the community structure of a biofilm in times of increased contamination (Sabater et al. 2007). A field application based on extracellular enzyme activity (EEA) of biofilms for assessment of acid mine drainage showed positive trends indicating ecosystem recovery in one case. In another case, variable EEA results were observed that inferred resistance to the recovery of the EEA. In both instances, the EEA results were in

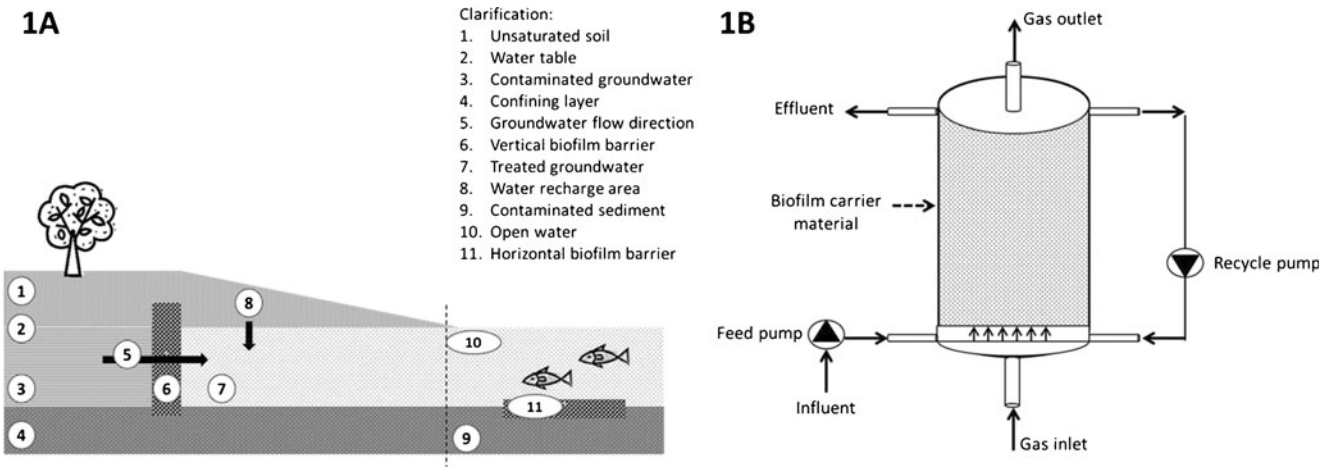

Fig. 2 Applied biofilm strategies for bioremediation and biotransformation. **a** In situ: biofilm barriers for treatment of contaminated groundwater and sediment. **b** Biofilm reactor design for generic biotransformation of contaminants. Different barrier materials can be utilized in situ (**a**) and ex situ (**b**) depending on the contamination (see Table 2 for specific information)

agreement with data from periphyton index scores (Pool et al. 2013). The results from another EEA study suggested that metal contamination may yield EEA results from fluvial biofilms, which might mask the changes due to seasonal variability (Bonet et al. 2013). In this case, it was argued that a multi-biomarker approach utilizing EEA as well as physicochemical markers in the water would be optimal to assess and monitor the pollution and ecosystem recovery. Still, in situ biofilm sampling will provide an improved assessment of the overall effect of heavy metal pollution on aquatic biota than sediment sampling alone in terms of the variations of bacterial and ciliate communities (Ancion et al. 2013). A different study assessing the recovery of periphytic biofilms after the exposure to Zn and Cd contaminated industrial effluent, demonstrated that even after 56 days of decontamination during which the chemical state of the hydrological system improved, the biofilms still exhibited low diversity resulting from the exposure to Zn and Cd (Arini et al. 2012). This indicated that the selective pressures of metal contaminants on biofilms had a lasting effect, which hampered the system's ability to handle future variable stresses. More investigation on how to facilitate the recovery of biofilm diversity is needed. Nevertheless, biofilms can to a great extent provide information about the status of an ecosystem and the consequence of exposure to heavy metal contamination. Therefore, biofilms can be used as an indicator "organism" for the health and function of the ecosystem exposed to heavy metal contamination.

Environmental sustainability and bioremediation foot prints

The technological strategies for efficient bioremediation and biotransformation of toxic contaminants have continuously been enhanced the last decades with improved understanding of the microorganisms and their activities being involved in the biofilm processes thus these strategies have become more commonly applied. Bioremediation solutions are often more cost effective than conventional remediation efforts such as dredging, capping, incineration or extraction given that the end product is non-polluting and these strategies might also cause a smaller environmental foot print (Hashim et al. 2011). Unfortunately, at some contaminated sites more toxic and/or dead end products might be the result of bioremediation depending on the biodegradation pathways occurring at the specific site (Fagervold et al. 2007). Furthermore, implementation of some bioremediation solutions might cause broader environmental issues as a result of the implementation in situ, which has been the case for some sediment bioremediation strategies. Resuspension of sediment and thus the contaminant present in the sediment increased the aqueous concentration to detrimental levels for the residing microbiota (Cho et al. 2009; Hashim et al. 2011). Therefore, the approach: life cycle assessment (LCA) has been developed to evaluate quantitatively and qualitatively the overall environmental impact and sustainability of the potential bioremediation solutions (Lemming et al. 2010; Cui et al. 2012). In the LCA, known environmental impacts from "cradle to grave" can be included for the potential solutions such as change in contaminant toxicity, exposure to benthic organisms in case of dredging or biofilm barrier implementation, increased burden of greenhouse gas emissions, impact on biodiversity in case of bioaugmentation or biostimulation, impact on recreational areas, and many others because of the implementation of potential bioremediation solutions. Based on evaluation of this information, the bioremediation strategy with the lowest overall environmental impact can be selected. Currently, only a few studies have been performed within the field of bioremediation such as

evaluation of remediation solutions for a trichloroethene-contaminated site (Lemming et al. 2010) and comparisons of electron donors for reductive dechlorination of hexachlorocyclohexane (Cui et al. 2012). However, it is anticipated that this approach will result in enhanced sustainability and thus a smaller environmental footprint for implementation of bioremediation solutions in the future.

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