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Screening selectively harnessed environmental microbial communities for biodegradation of polycyclic aromatic hydrocarbons in moving bed biofilm reactors

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HIGHLIGHTS

- Hydrocarbon contaminated soil and water microbes grown as biofilms.
- Biofilms used in scale reactors to treat 16 polycyclic aromatic hydrocarbons (PAHs).
- PAH degradation ability varies by biofilm inoculum source.
- Supplemental nutrients can increase prevalence of PAH degradation genes.

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ABSTRACT

Bacteria are often found tolerating polluted environments. Such bacteria may be exploited to bioremediate contaminants in controlled *ex situ* reactor systems. One potential strategic goal of such systems is to harness microbes directly from the environment such that they exhibit the capacity to markedly degrade organic pollutants of interest. Here, the use of biofilm cultivation techniques to inoculate and activate moving bed biofilm reactor (MBBR) systems for the degradation of polycyclic aromatic hydrocarbons (PAHs) was explored. Biofilms were cultivated from 4 different hydrocarbon contaminated sites using a minimal medium spiked with the 16 EPA identified PAHs. Overall, all 4 inoculant sources resulted in biofilm communities capable of tolerating the presence of PAHs, but only 2 of these exhibited enhanced PAH catabolic gene prevalence coupled with significant degradation of select PAH compounds. Comparisons between inoculant sources highlighted the dependence of this method on appropriate inoculant screening and biostimulation efforts.

1. Introduction

Defined as aromatic hydrocarbons with two or more fused benzene rings, polycyclic aromatic hydrocarbons (PAHs) are found ubiquitously in the environment. They are produced from fossil fuel combustion, waste incineration and petroleum refining (Bamforth and Singleton, 2005; Fernández-Luqueño et al., 2011; Haritash and Kaushik, 2009; Lu et al., 2011). Due to their toxic, mutagenic and carcinogenic nature, agencies including Environment Canada, the U.S. EPA and the European Union have identified PAHs as priority pollutants (Lu et al., 2011). PAHs accumulate in the environment and exhibit limited bioavailability for biodegradation due to their low solubility in water, high hydrophobicity, and complex chemical structure (Bamforth and Singleton, 2005; Lu et al., 2011; Peng et al., 2008). PAHs are generally categorized as either low or high molecular weight compounds. As molecular weight of a PAH increases, hydrophobicity increases and solubility decreases; therefore high molecular weight PAHs (4+ rings) are more persistent in the environment (Bamforth and Singleton, 2005; Peng et al., 2008). Despite their overall recalcitrant nature, PAHs are still subject to biodegradation by a variety of bacteria and fungi.

The biochemical pathway for aerobic biodegradation of both low and high molecular weight PAHs have been well studied and reviewed in detail by Peng et al. (2008). The principal mechanism for the activation of PAHs in aerobic degradation is the initial oxidation of an aromatic ring by a dioxygenase enzyme to form a dihydrodiol. The diol then undergoes subsequent dehydrogenation,

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typically generating a catechol-like intermediate. Cleavage of the aromatic ring is then achieved by a second dioxygenase enzyme (ortho or meta cleavage), upon which the product is readily degraded into TCA cycle intermediates. Examples of microorganisms capable of at least partial aerobic degradation of PAHs include *Pseudomonas*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium* spp. (Bamforth and Singleton, 2005; Fernández-Luqueño et al., 2011; Haritash and Kaushik, 2009).

The genetic determinants for naphthalene degradation in *Pseudomonas putida* G7 have long been resolved, and are widely considered the archetypal system (Lu et al., 2011). In short, *P. putida* G7 naphthalene catabolic genes (*nah* genes) are found on the transmissible, 83 kb NAH7 plasmid (Dunn and Gunsalus, 1973). The plasmid is split into two operons, an upper pathway which includes the degradation activating naphthalene dioxygenase (*nahA*), and the lower pathway which contains genes for the conversion of salicylate to catechol and subsequently to TCA cycle intermediates (Bamforth and Singleton, 2005; Peng et al., 2008). Degenerate primers encoding for naphthalene dioxygenase (NAH) and catechol 2,3-dioxygenase (C23O) are commonly used in molecular experiments (such as qPCR) to assess the bioremediation potential of a bacterial community (Baldwin et al., 2009, 2003; Isaac et al., 2013; Meyer et al., 1999; Salminen et al., 2008). The enzyme systems involved in degrading high molecular weight PAH systems are different, far less common, and often require co-metabolism with more amenable substrates as the mass transfer rates (and thus bioavailability) of high molecular weight PAHs are often too low to support cell growth alone (Fernández-Luqueño et al., 2011; Peng et al., 2008).

Biodegradation of low molecular weight PAHs has been studied extensively (Peng et al., 2008). The difficulties associated with degradation of high molecular weight PAHs, along with the variable extent and rate of microbial PAH degradation in general has thus far however prevented bacterial-mediated PAH bioremediation from developing into a mature biotechnology (Bamforth and Singleton, 2005; Lu et al., 2011). Factors that affect the rate and extent of PAH bioremediation include nutrient accessibility, PAH bioavailability and microbial composition (Bamforth and Singleton, 2005). Many current research foci are attempting to address these issues by examining the optimization of factors including the application of surfactants (to increase bioavailability) (Hickey et al., 2006) and co-substrates (van Herwijnen et al., 2003), as well as the development/selection of effective PAH degrading cultures (Boonchan et al., 2000; Mishra et al., 2001). In terms of microbial composition, many studies have indicated that a microbial consortium is preferential over single isolates, as the presence of a microbial community may stimulate coordinate metabolic activities resulting in enhanced PAH degradation not demonstrated by pure cultures (Obuekwe and Al-Muttawa, 2001; Peng et al., 2008; van Herwijnen et al., 2003; Wu et al., 2013).

One method to obtain a microbial consortium is to cultivate microbes as mixed species biofilms. A biofilm is a surface bound aggregation of microbial cells encapsulated in an extracellular matrix. Biofilms are renowned for their ability to tolerate environmental toxicants (Demeter et al., 2016), and offer a mode-of-life that promotes high diversity and interspecies interactions that may lead to enhanced xenobiotic degradation characteristics as a result of metabolic coordination and division of labor (Edwards and Kjellerup, 2013). As such, the overarching hypothesis of this research program has been that harnessing and tailoring environmental microbial communities as mixed-species biofilms is a choice method for acquiring microbes for application in aerobic hydrocarbon pollutant bioremediation. The studies described thus far have focused on the applicability of communities harnessed using in-house Calgary Biofilm Device (CBD) based technology for removal of naphthenic acids from oil sands process water (OSPW), and have demonstrated that mixed species communities are more effective for hydrocarbon degradation than single species isolates, and that supplementation with co-substrates affects both the community composition and the ability of the community to degrade the target pollutant (Demeter et al., 2014, 2015; Frankel et al., 2016; Golby et al., 2012). Moreover, evidence was supplied supporting the scalability and adaptability of the mixed species biofilm approach to current wastewater treatment industry practices. Lemire et al. (2015) demonstrated that targeted environmental microbial communities could be harnessed on moving bed biofilm reactor (MBBR) carriers, achieving a fully functional bioreactor in as little as 48 h.

In this study, it was hypothesized that novel methodologies described above could be used to culture environmental biofilms from site-samples contaminated with PAHs and subject them to various nutrients, and PAH-stress conditions in order to promote and direct the growth of a microbial biofilm with enhanced PAH degradation capacity in an aerobic *ex-situ* MBBR system. To this end, microbial communities were successfully cultivated as biofilms from PAH contaminated environments in the presence of 16 different PAHs using moving bed biofilm reactor (MBBR) carriers as surface substrates. Successful biofilm cultivation from OSPW and three different PAH-contaminated soil samples was followed up with an examination (by use of GC-FID) of the ability of these biofilms to aerobically degrade PAHs, and an analysis of their genetic potential to degrade PAHs by means of NAH and C23O qPCR gene analysis. In general, the bioreactor application of the microbes from both the well pad and downstream communities enhanced the prevalence of PAH degradation catabolic genes, which also correlated to an observed ability to degrade select PAH compounds. Comparatively, flare pit and OSPW communities exhibited poor genetic enhancement and ability to degrade PAHs despite growth in MBBR systems.

2. Materials and methods

2.1. PAH mixture

The U.S. EPA has identified 16 PAHs as priority pollutants (Keith, 2014). All 16 of these compounds (listed in Table 1) are hereafter referred to as the 16 EPA PAHs, and are included in the assay mixture. Working assay concentrations of each individual PAH (Table 1) were selectively chosen to model previously reported average concentrations found within gasoline (Marr et al., 1999). Modeling the synthetic 16 EPA PAH mixture after gasoline provides

Table 1
Abbreviations and working assay concentrations of PAHs used in in this study.

PAH	Abbrev.	Working concentration (μg/mL)	Approx. number of rings
Naphthalene	NAP	1500	2
Acenaphthene	ACE	0.3	3
Acenaphthylene	ACY	5	3
Phenanthrene	PHE	6	3
Fluorene	FLU	15	3
Anthracene	ANT	10	3
Benz[*a*]anthracene	BAA	2	4
Chrysene	CRY	3	4
Pyrene	PYR	1	4
Fluoranthene	FLT	1	4
Benzo[*b*]fluoranthene	BBF	0.3	5
Benzo[*k*]fluoranthene	BKF	0.2	5
Benzo[*a*]pyrene	BAP	0.5	5
Indeno[1,2,3-*cd*]pyrene	IND	0.5	6
Benzo[*ghi*]perylene	BGP	0.1	6
Dibenz[*a,h*]anthracene	DBA	0.01	5