ULTRASONICALLY ENHANCED MASS TRANSPORT AND DEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SOLID-LIQUID TWO PHASE PARTITIONING SYSTEMS

by

Pedro Alejandro Isaza

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Abstract

The remediation of soil contaminated with polycyclic aromatic hydrocarbons (PAHs) is endorsed by environmental protection agencies worldwide. Recent studies demonstrated the removal of these contaminants from soil utilizing polymer beads, with subsequent PAH release and degradation in solid-liquid two phase partitioning bioreactors (TPPBs). Although such a process was successful, significant mass transport limitations involving PAH release from the polymers hampered productivity. The current work examined the possibility of applying sonication in solid-liquid partitioning systems to enhance delivery and degradation of PAHs.

Small scale physical testing revealed delivery rates of PAHs from Desmopan, increased by 5 fold under intermittent sonication relative to non-sonicated conditions. Enhancements were also displayed as shifts to higher release equilibria under sonicated conditions, agreeing with sonochemistry concepts. Improvements were demonstrated across a range of polymers, suggesting that sonication could enhance PAH release with any polymers deemed feasible for environmental applications. A PAH-degrading microbial consortium was enriched, and it was demonstrated that sonication also improved the rate of phenanthrene degradation delivered from Desmopan by four times, confirming transport improvements while minimizing cellular inactivation effects.

A mass transport analysis showed that without sonication, delivery of PAHs was restricted by the external resistance at the solid-liquid interface. Ultrasound was shown to enhance both external and internal transport properties, allowing rates not achievable through increased liquid mixing. Diffusivities quantified with and without ultrasound decreased as a function of permeant molecular size. Additionally, partitioning coefficients under sonicated and non-sonicated conditions decreased with PAH molecular size. Finally, an examination of

permeant property data demonstrated that polarizability was the best descriptor of thermodynamic and transport behaviour in solid-liquid systems.

The possibility of inducing equivalent improvements was investigated in a bench scale TPPB, in which sonic exposure improved degradation rates of phenanthrene by 2.7 fold when delivered from Desmopan. A window of on/off operation for ultrasonic cycling was also demonstrated, providing potential for optimizing sonication via rational selection of exposure times. DNA analysis also revealed that the consortium composition was maintained in the presence of sonication and also demonstrated that the consortium was comprised of bacteria belonging to the *Pandoraea*, *Sphingobium*, and *Pseudoxanthomonas* genera.

Co-Authorship

Chapters 3, 4 and 5 have been accepted or submitted to refereed journals and were co-authored with Dr. Andrew J. Daugulis, who provided editorial and technical advice. Chapter 4 was co-authored with Dr. Kunal Karan, who provided editorial and technical modeling advice.

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Chapter 1
Introduction

1.1 Background

The removal of deleterious compounds from the environment is an area of focus at the forefront of environmental protection agencies worldwide. Polycyclic aromatic hydrocarbons (PAHs) are a group of such pollutants and are naturally present at low levels in the environment. However, fossil fuel combustions, industrial processing, and burning of organic substances (Lijinsky 1991) has increased their presence to levels that require human attention. Exposure to PAHs poses a significant human health risk in industrialized areas due to the carcinogenic character possessed by some of these aromatics (Cerniglia 1992). However, although carcinogenicity has driven their introduction into the US Environmental Protection Agency priority pollutant list (Keith and Telliard 1979), these organics can also interfere with endocrine and immune systems (Lyons 1997).

In the environment, PAHs tend to accumulate in the vadose zone due to their hydrophobic nature and affinity for soil organic matter, which makes removal and destruction difficult. Degradation has been demonstrated using technologies such as incineration, chemical pre-oxidation, composting, land farming, and phytoremediation (Antizar-Ladislao, et al. 2006; Denys, et al. 2006; Kulik, et al. 2006; Onwudili and Williams 2006); however, the large costs and/or insufficient removal efficiencies associated with these techniques can make them unfavorable for environmental purposes. Microbial degradation presents an alternative cost-effective treatment constrained only by the low solubility of these organic moieties (Cerniglia 1992; Juhasz and Naidu 2000). Methods for improving such limitations include the use of specially designed reactors, which can enhance bioavailability of poorly soluble substrates and increase rates of microbial degradation (MacLeod and Daugulis 2003).

Two phase partitioning bioreactors (TPPBs) are such systems and are comprised of an aqueous phase containing degrading organisms, which coexists with an immiscible second phase loaded with high concentrations of organic pollutants (Daugulis 2001). In the case of PAHs, partitioning into the aqueous phase is based on mass transport, thermodynamics, and biological demand. Recent studies have shown that polymers can be used as the second phase and are not subject to bioavailability issues, allowing for microbial consortia to be used (Amsden, et al. 2003). Additionally, as shown by Rehmann et al. (2008), the possibility of using polymers allows for the sequestering phase to be contacted directly with contaminated soil sources for PAH uptake, with degradation following in a TPPB. Although such a remediation train has been demonstrated to be successful, degradation in solid-liquid two phase systems is subject to significant mass transport constraints (Rehmann, et al. 2008). Potential solutions have arisen in the field of biomedical engineering by means of ultrasonic exposure (Kost and Langer 2001), in which it has been shown that sonic irradiation can enhance release rates of compounds with different molecular sizes, chemical compositions, and hydrophobic characteristics from a variety of polymers, both *in vivo* and *in vitro*. It is therefore possible to anticipate that sonication could enhance delivery of PAHs in polymer-liquid systems, allowing previously proposed remediation trains to be improved without major adjustments.

1.2 Objectives

This research was focused on examining the possibility of applying ultrasound in solid-liquid two phase partitioning systems and evaluating its ability to improve degradation of PAHs. To accomplish this, a consortium of bacteria capable of degrading PAHs first needed to be enriched, and subsequently, effects of sonication on delivery and degradation of PAHs needed to be examined in solid-liquid systems at a flask scale.

The intent of the second part of this work was to develop a model capable of describing mass transport of PAHs from polymers with and without sonication. The aim was to gain better understanding of ultrasonic effects on internal and external mass transport as well as thermodynamics.

The final piece of this work attempted to examine the possibility of enhancing phenanthrene delivery and degradation in a bench scale solid-liquid TPPB via sonication. Additionally, effects of exposure on consortium composition needed to be evaluated through molecular techniques in order to address bacterial robustness and inactivation effects.

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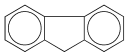
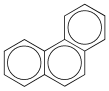
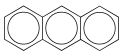
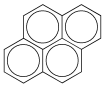
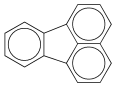
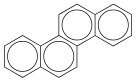
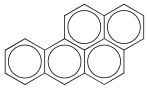
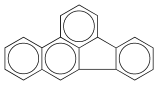
Chapter 2
Literature Review

2.1 Introduction

The removal of organic contaminants from the environment has been strongly endorsed by environmental protection agencies worldwide. Polycyclic aromatic hydrocarbons (PAHs) are a group of such polluting compounds, and their degradation has been actively studied using a number of technologies (Antizar-Ladislao, et al. 2006; Denys, et al. 2006; Kulik, et al. 2006; Onwudili and Williams 2006). These compounds are comprised of two or more benzene rings linked in a linear, angular, or cluster arrangement. They are omnipresent in the environment but are most abundantly generated in industrial processes during the burning of gas, oil, coal, wood, garbage, and other organic substances (Lyons 1997). Naturally occurring PAHs are generated during forest fires, but in the last century, the production of PAHs has steadily grown in developed nations due to increasing numbers of anthropogenic sources. Overall, low concentrations of polycyclic aromatic hydrocarbons are now dispersed world wide due to atmospheric emissions (Piskonen and Itaevaara 2004).

PAHs are typically categorized based on size. Small molecular weight PAHs are comprised of three benzene rings or fewer, while large molecular weights contain four or more aromatics. In the environment, these cyclic contaminants tend to accumulate in the vadose zone due to their hydrophobic nature and affinity for soil organic matter. Additionally, their environmental persistence has been found to be a strong function of the number of rings present in the molecule and linkage pattern (Kanaly and Harayama 2000). For the most part, increasing the number of aromatics enhances their hydrophobic character and chemical stability, and leads to higher resistance to biological degradations and increased binding to soil organic matter. Table 2-1 depicts the structural and chemical properties of various PAHs.

Table 2-1: Structural and Chemical Properties of Various PAHs

PAH	Structure	Molecular Weight (g/mol)	Water Solubility at 25°C (mg/L)[a]	Log $K_{O/W}$ [b]	EPA Carcinogen Class[c]
Naphthalene		128.2	31.7	3.37	C
Fluorene		166.2	1.98	4.18	D
Phenanthrene		178.2	1.29	4.57	D
Anthracene		178.2	0.045	4.54	D
Pyrene		202.3	0.135	5.18	D
Fluoranthene		202.3	0.26	5.22	D
Chrysene		228.3	0.002	5.86	B
Benzo[*a*]pyrene		252.3	0.0038	6.04	B
Benzo[*b*]fluoranthene		252.3	0.014	5.80	B

[a] (Vandermeer 2005);
[b]Logarithm of the octanol-water partition coefficient (Alves de Lima Ribeiro and Ferreira 2003);
[c]Under EPA's classification of carcinogenicity, class A compounds are "known human carcinogens", class B are "probable human carcinogens", class C are "possible human carcinogens", class D are "not classifiable as to human carcinogenicity" and class E are "non-carcinogenic" (EPA 2009)

The removal of these compounds from the environment has been motivated by their carcinogenic character. If present in populated areas, these compounds impose a serious human health risk. Studies have shown that small molecular weight PAHs, composed of two or three rings are acutely toxic (Heitkamp, et al. 1988). However, PAHs of high molecular weights have been found to induce genotoxic effects, making their removal from the environment necessary

(Heitkamp, et al. 1988; Juhasz and Naidu 2000) (as observed in Table 2-1). Additional studies have also shown that PAHs can interfere with endocrine and immune systems, further driving the need for their remediation and treatment (Collins, et al. 1998; Lyons 1997). Currently, 16 PAHs are present in the US Environmental Protection Agency priority pollutant list (Kanaly and Harayama 2000; Shuttleworth and Cerniglia 1995), which has challenged researchers to find innovative ways to eliminate these organic contaminants from the environment.

In particular Benzo[*a*]pyrene (BaP), a large molecular weight PAH, has presented a challenge to the field of bioremediation for many years. Its ability to alkylate DNA makes it a strong cancer causing agent and a prime candidate for investigation. Furthermore, its carcinogenic potency has been demonstrated in multiple animal studies (Collins, et al. 1991) as well as in prokaryotic and mammalian cells (Juhasz and Naidu 2000). However, its molecular stability limits biological treatment and complete removal from the environment has yet to be demonstrated.

2.2 Remediation

The fate of these organic contaminants in the environment is highly influenced by their hydrophobic nature. In soil, PAHs tend to sorb to organic matter, limiting their ability to be treated through biotic and abiotic means. Additionally, the efficiency of biological treatment is further limited by the inhibitory and toxic nature of these cyclic compounds. Two remediation strategies currently exist for the treatment of most organic contaminants: *in situ* and *ex situ*. *In situ* treatments typically attempt to enhance microbial flora in contaminated soils by means of nutrient addition and/or introduction of degrading microorganisms. *Ex situ* remediation requires the extraction of contaminated soils to different locations for treatment. Most physical, chemical, and biological modes of PAH removal belong to this latter category. The following techniques are typical remediation strategies.

2.2.1 Physio-Chemical Remediation Strategies

Developments in incineration technology have made it extremely attractive for *ex situ* remediation due to its ability to completely destroy PAHs as well as other organic compounds (Onwudili and Williams 2006). The oxidation process, termed flameless supercritical water incineration, exploits an aqueous fluid phase under conditions that exceed the critical point of water (T_c = 374 ^oC, P_c = 21.9 MPa). Both gases and PAHs are soluble and completely miscible at these high temperatures and pressures, resulting in significantly increased rates of reaction. In fact, it has been found that up to 99.9 wt% PAH destruction can be achieved under these conditions irrespective of molecular weight (Onwudili and Williams 2006). This mechanism of removal, although efficient, has inherently large costs associated with it, making it unattractive for environmental purposes.

Volatilization is a secondary method commonly used for removal of organic materials in soil. This strategy belongs in the *ex situ* category and was presented by Ashok and Saxena (1995) for the removal of PAHs. Essentially, organic compounds dissolved in soil are volatilized as a means of elimination. However, removal efficiencies for PAHs are limited by their low vapour pressures and hydrophobic characteristics. Naphthalene (containing two fused benzene molecules) is the only exception. With a vapour pressure of 6.25 Pa (at 20^{o}C), it significantly volatilizes both from the soil-water interface and solid state directly (Ashok and Saxena 1995). Therefore, as a treatment strategy, volatilization is typically limited to low molecular weight PAHs.

Thermal desorption is an additional strategy commonly investigated for removal of PAHs. This process applies increased heat (66-127^{o}C) for volatilization and removal of organic contaminants from soil (Timberlake and Garbaciak 1995). The resulting gases are then condensed and collected as an oil residue, which is later disposed. Another similar method used for removal of PAHs is solvent extraction. This particular treatment places contaminated soils in intimate

contact with solvents that strip PAHs based on affinity (Timberlake and Garbaciak 1995). These two strategies, although efficient, only relocate the problem and are therefore not applied commercially.

Chemical oxidation is an additional *ex situ* remediation strategy that uses high temperatures and pressures to induce oxidation of organics in soil. On its own, it lacks cost effectiveness and public acceptance for environmental purposes (Vandermeer 2005). However, combined with biological treatment, enhanced degradations of PAHs have been demonstrated (Kulik, et al. 2006). As a whole, this technology strongly depends on soil type and moisture content. Oxidations with hydrogen peroxide followed by biological degradation have been shown to achieve up to 94% PAH degradation, while with ozonation followed by biodegradation removals of 75% have been reported (Kulik, et al. 2006). Although efficient, chemical oxidation alone is constrained by molecular weight. Lighter PAHs have been found to be more rapidly removed, and to larger extents, than heavier PAHs (Kulik, et al. 2006).

A final physio-chemical treatment used for PAH degradation is photolysis. Research in this area has been focused on natural degradation occurring in water via solar radiation. Photodegradation in aqueous media involves a series of complicated mechanisms requiring ubiquitous oxygen in solution (Miller and Olejnik 2001), with successful degradation of high molecular weight PAHs reported (Fasnacht and Blough 2003; Lehto, et al. 2000; Miller and Olejnik 2001). Specifically, 50% degradation of benzo[*a*] pyrene (BaP) was achieved in an aqueous solution within 20 seconds of irradiation by Miller and Olejnik (2001). Important photo-degradation by-products of BaP include 3,6- and 1,6-quinones and traces of 6,12-quinones as reported by Mill et al. (1981). Additionally, 50% degradations of fluoranthene and chrysene were reported in a matter of minutes (Miller and Olejnik 2001). These techniques are not directly applicable to soil degradation. However, technologies capable of transferring aromatics from soil to solution could potentially take advantage of the high molecular weight PAH degradation

associated with photolysis. Such a combination was recently reported by Guieysse and Viklund (2005).

2.2.2 Biological Remediation Strategies

A number of biological treatment strategies are available for removal and degradation of PAHs present in soil. Research in this field has progressed over the past few decades due to the economical benefits inherent to these technologies. The term 'bioremediation' is commonly used to describe processes which use microbial organisms to remove or degrade deleterious components from the environment (Vandermeer 2005).

Composting belongs to this family of remediation strategies. Studies have shown up to 82% removal of PAHs from soil at optimal operating conditions after 98 days (Antizar-Ladislao, et al. 2006). However, a strong dependence on PAH molecular weight was also shown. Low molecular weight PAHs were significantly removed while heavier PAHs remained undegraded in composted soil (Antizar-Ladislao, et al. 2006).

Alternative techniques have also been shown to be major contributors for biodegradation in soil (Kästner and Mahro 1996; Shuttleworth and Cerniglia 1995). Mineralization (complete destruction) and partial degradation of PAHs have been demonstrated through the use of plants in many studies (Denys, et al. 2006; Paquin, et al. 2002; Parrish, et al. 2004; Saison, et al. 2004). This process is referred to as phytoremediation and has successfully removed PAHs both from soil (Denys, et al. 2006) and marine sediments (Paquin, et al. 2002). Additional bioremediation studies have focused on degradation through the use of fungi (Cerniglia 1997) and demonstrated that PAHs with up to 6 fused rings could be eliminated. The use of algae as a means of removing PAHs in aquatic environments has also been investigated (Kirso and Irha 1998). However, results revealed that treatments occurred through bioaccumulation of aromatics within the biomass. This essentially relocates the problem and requires further treatment if such a strategy were applied industrially.

For the most part, bioremediation studies have focused on the use of bacteria for degradation of PAHs. Additional investigations using groups of microbial species have also been carried out, and results have varied depending on conditions.

2.3 Aerobic Degradations of PAHs

The aerobic degradation of multiple PAHs has been well documented for a wide variety of microbial species. Particular importance has been placed on bacteria, which have shown increased versatility for degradation of PAHs with varying sizes (Cerniglia 1992).

Many aspects play a significant role in the efficiency of aerobic processes. These include inhibition, co-metabolism and augmentation. Inhibition is a reduction of rates and extents of metabolism of a particular compound due to high concentrations of the target molecule or presence of secondary inhibitory compounds (Vandermeer 2005). Conditions of this nature are often encountered during aerobic biodegradation of PAH mixtures (Hughes, et al. 1997). Creosote contaminated soils, containing numerous PAHs, commonly exhibit inhibitory conditions as demonstrated by Lotfabad and Gray (2002). Under such conditions, it was reported that the presence of phenanthrene introduced a significant lag phase in the degradation of fluoranthene and chrysene in soil. Additional studies by Bouchez et al. (1995a) further demonstrated that the presence of naphthalene, fluorene, phenanthrene, and anthracene had an inhibitory effect on the degradation of other PAHs. It was also found that such effects were more pronounced when the secondary molecules, inducing inhibition, had a lower molecular weight and higher solubility than the target compounds. Similar results by Stringfellow and Aitken (1995) reported that phenanthrene degradation was inhibited by the presence of lower molecular weight naphthalene and fluorene. This effect was attributed to the inhibition of a common enzymatic pathway (Stringfellow and Aitken 1995). Additional reports by Trzesicka-Mlynarz and Ward (1995) showed similar degradation results. It was found that degradation of high molecular weight PAHs was delayed by the presence of smaller aromatics. However, such effects are not always the case.

Studies by Guha et al. (1999) reported that the presence of naphthalene had no impact on the rates of phenanthrene or pyrene degradation. In fact, it was found that the rates of removal of naphthalene decreased under the presence of the heavier aromatics.

Co-metabolism is a second phenomenon that commonly arises in PAH degradation studies. It is defined as the transformation of non-growth compounds in the obligate presence of a growth substrate or additional transformable compounds (Hughes, et al. 1997). Numerous studies have reported this effect specifically for fluorene degradation in the presence of PAH mixtures (Bouchez, et al. 1995a). Additional studies have also reported bacterial co-metabolic degradation of pyrene and BaP in the presence of fluoranthene (Kanaly and Harayama 2000). Similar results obtained by Ye et al. (1996) showed that the presence of fluoranthene induced the production of co-metabolic enzyme(s) capable of degrading BaP and other high molecular weight PAHs in *Sphingomonas paucimobilis* EPA 505. In another study, it was reported that fluorene served as a source of carbon and energy for *Sphingomonas* LB126, while phenanthrene and fluoranthene were co-metabolised (van Herwijnen, et al. 2003). Additional results by Stringfellow and Aitken (1995) showed that *Pseudomonas saccharophila* could not degrade fluorene as a sole carbon source, however, under the presence of phenanthrene co-metabolism of fluorene readily occurred. More recent studies by Kanaly et al. (2000) demonstrated co-metabolic benzo[*a*]pyrene degradation in the presence of diesel fuel acting as the primary substrate. Unlike the above examples, an undefined microbial consortium displayed such metabolic characteristics, and degradative extents and rates were a function of primary substrate and benzo[*a*]pyrene concentrations, as well as microbial densities in the inoculum. Overall, it can be concluded that co-metabolism is an important factor that can be exploited for the degradation of high molecular weight PAHs.

Augmentation is a final method commonly involved in the degradation of PAHs. It is defined as the process by which the degradation of one compound is enhanced by the presence of

another (Hughes, et al. 1997). Examples of this effect have been reported for the degradation of pyrene when phenanthrene is present (Tiehm and Fritzsche 1995). Such a situation resulted from increased biomass arising from phenanthrene metabolism, which augmented pyrene degradation (Hughes, et al. 1997). This process of enhancing degradation differs from co-metabolism in that the aromatics of interest are also degraded as growth substrates (Vandermeer 2005). However, these effects are not common and are difficult to predict in the presence of a mixture of PAHs (Guha, et al. 1999). Additionally, mixed bacterial populations present in soil employ complex pathways for PAH degradation, which differ from the single strain studies presented above (Guha, et al. 1999). Furthermore, the potential for synergistic and inhibitory effects, related to the presence of different microorganisms, could lead to a better understanding of natural degradation in soil and laboratory.

2.4 Degradation via Microbial Consortia

The degradation of PAH mixtures using individual microbial strains is often subject to numerous effects leading to enhanced or inhibited degradations. However, the use of mixed microbial populations, referred to as consortia, has been previously used to alleviate such detrimental effects (Bouchez, et al. 1999). These mixed populations can either be defined or undefined. Defined consortia consist of known microbial organisms. Undefined consortia are usually enriched through selection pressures for degradative capabilities and consist of species that are unknown (Vandermeer 2005). As mentioned previously, the use of consortia arises from their potential ability to degrade a wider range of PAHs. Commonly, individual microbial strains have the enzymatic capacity to degrade only a limited number of PAHs. However, in the presence of different microorganisms, the number of PAHs that can be metabolized increases significantly. The overall degradative ability of a consortium is not necessarily the result of adding together the capacities of the strains. However, individual members in the group may play significant roles and lead to faster rates, and wider degradations, than those achieved by the individual

microorganisms (Ghazali, et al. 2004). This effect was demonstrated by Bouchez et al. (1999), where a microbial consortium degraded PAHs faster than any combination of the isolated strains. Additionally, the use of mixed microbial populations can mitigate inhibitory situations. As demonstrated by Hughes et al. (1997), consortia are more resilient to toxic concentrations/products and can degrade metabolites that normally halt microbial activity. These advantages have lead to the use of consortia in more advanced degradation technologies such as two-liquid phase bioreactors (Vandermeer and Daugulis 2007; Villemur, et al. 2000).

2.5 Molecular Techniques

Based on the benefits discussed for microbial consortia, an important development has recently become the ability to identify individual consortium members. This is particularly important in the case of undefined consortia enriched from contaminated soil sources. Plating techniques have previously been used to culture and isolate microbial organisms. However, as summarized by Theron and Cloete (2000) only a minor fraction (0.1-10%) of bacteria can be cultivated by standard techniques. Additionally, as discussed by Vandermeer (2005), culture-dependent methods do not necessarily reflect bacterial consortium make-ups, but rather the selectivity of certain growth media for bacterial cell lines capable of thriving in the given conditions. As such, recombinant DNA techniques utilizing rRNA have been developed for circumventing the above limitations and efficiently identifying bacteria. Specifically, 16S rRNA gene sequences have been utilized as their lengths of approximately 1500 nucleotides (Theron and Cloete 2000) contain considerable information. This ribosomal RNA contains base pair regions that are highly conserved across kingdoms *Archea* and *Bacteria* (Theron and Cloete 2000), while significant variability is present in other sections allowing for strain level identification. These universal regions provide binding sites for oligonucleotide probes, or complementary base sequences, which when combined with polymerase chain reaction (PCR)

amplification, generate an efficient tool for studying and identifying members in microbial consortia.

A particular method for isolating DNA across a consortium is denaturing gradient gel electrophoresis (DGGE). This method utilizes amplified fragments of nucleic acids (16S rRNA in many cases) of nearly identical length, and resolves them electrophoretically based on different base pair compositions (Theron and Cloete 2000). In such an analysis, the DNA fragments are separated based on their ability to migrate across a vertical polyacrylamide gel containing increasing concentrations of DNA denaturants. As the DNA migrates, partial melting of the double stranded DNA occurs at discrete regions of the gel. These regions are specific to the nucleotide sequence, and melting retards any additional migration, generating individual bands of identical DNA sequences (Theron and Cloete 2000). As a secondary analysis, the separated bands can then be excised, amplified (for a second time) and sequenced (Theron and Cloete 2000). Comparison in genetic banks then allows for identification of consortium members at least to the genus level.

2.6 Degradation Strategies for PAH Removal

PAHs in the environment, especially in soil, behave differently than in laboratory settings. Their physio-chemical properties, low aqueous solubility and increased hydrophobicity, lead to sequestration in soil organic matter or accumulation in non-aqueous phase liquids (NAPLs). Additional interactions of PAHs with soil clay particles can lead to oligomerizations, which can render them unextractable for degradation or treatment purposes (Karimi-Lotfabad, et al. 1996). Furthermore, the contact time between PAHs and soil can have a strong impact on their availability for microbial degradation (Karimi-Lotfabad, et al. 1996). Concerns about this weathering effect are present in soils contaminated for extended periods of time. Under such conditions, irreversible binding to clay particles occurs and diffusion of PAHs into sediment micro-channels complicates microbial degradation (Karimi-Lotfabad, et al. 1996). Additionally,

predation in these pores reduces the number of degraders and further restricts microbial degradation (Johnsen, et al. 2005). Other factors influencing PAH degradation in soil include: nutrient concentrations, pH, moisture content, temperature, presence of inducers, concentrations of microbial degraders, oxygen and PAH concentrations, and the presence of toxic compounds such as heavy metals (Juhasz and Naidu 2000). All of the above effects combined lead to naturally slow degradations in soil.

As such a series of anthropogenic strategies have been implemented in recent years to overcome the above effects and enhance extents and rates of PAH biodegradation.

2.6.1 Surfactant Enhanced Biodegradations

Surfactants are amphipathic compounds, possessing both hydrophobic and hydrophilic groups in their structure. They are therefore soluble in both organic solvents and water, and as such, have been used to try to enhance the bioavailability of hydrophobic PAHs during microbial degradation. Surfactants decrease capillary forces in sediment matrices, allowing PAHs to desorb and induce apparent aqueous concentration at levels higher than normal solubilities (Hughes, et al. 1997). Above the critical micelle concentration (CMC), surfactant molecules cluster together and arrange themselves as micelles. These molecular aggregates consisting of a hydrophobic core and a hydrophilic shell can envelop organic particles, such as PAHs, allowing for efficient transfer from a solid or liquid phase, to a water phase (Vandermeer 2005). This increased aqueous concentration was previously believed to induce faster microbial degradation. However, contradictory results have been reported.

For example, the presence of surfactants was shown to be toxic for phenanthrene degradation by Birman and Alexander (1996). Similar results were presented by Bramwell and Laha (2000), Jimenez and Bartha (1996) and Wong et al. (2004), who showed that at low surfactant concentrations, no positive effects on PAH degradation rates and extents were observed. However, at concentrations higher than the CMC, inhibition was observed,

demonstrating that increased micellar concentrations did not translate into increased bioavailability. These effects were hypothesized to arise from: Toxic effects due to the presence of surfactants and/or PAHs at high concentrations, preferential use of surfactants as growth substrates, and/or interference of the surfactant micelles with metabolic processes (Bramwell and Laha 2000; Hickey, et al. 2007). Similar effects were also reported in several studies (Efroymson and Alexander 1991; Jimenez and Bartha 1996; Laha and Luthy 1991; Piskonen and Itaevaara 2004; Tiehm 1994; Willumsen and Karlson 1997; Wong, et al. 2004; Woo, et al. 2004; Ye, et al. 1996). On the other hand, opposite effects have also been observed by Jimenez and Bartha (1996), as well as Volkering et al. (1995), demonstrating enhanced PAH degradation at surfactants levels below CMC. It has also been reported that enhanced rates of PAH degradation could also be achieved with surfactant concentrations higher than the CMC (Efroymson and Alexander 1991; Hickey, et al. 2007).

Overall, effects of surfactants on PAH degradations still remain unclear. They are a function of concentrations, microbial species and the additional effects mentioned previously. This lack of control has limited their applicability in field studies.

2.6.2 Inducers

Metabolic inducers can also be added to stimulate both selection of degraders and induction of PAH metabolism (Vandermeer 2005). It has been found that the presence of one PAH can activate the enzymatic pathway required to degrade a series of aromatic compounds (Chen and Aitken 1999). It has therefore been concluded that inducers which activate the degradation of one PAH can potentially co-induce the degradation of a series of PAHs (Chen and Aitken 1999). Potential inducers are typically intermediates in the degradation pathway, and for PAHs, include salicylate, phthalate, gentisate, and cinnamate (Woo, et al. 2004). However, inducer effects vary depending on the microbial culture (Woo and Park 2004). As a specific example, Chen and Aitken (1999) found that salicylate induced the degradation of benzo[*a*]

anthracene, chrysene and benzo[*a*] pyrene in a *Pseudomonas saccharophila* P15 strain. This was evidence that a link in the degradation pathway was present between high and low molecular weight PAHs. Additional studies by Ogunseitan and Olson (1993) and Woo et al. (2004) showed that salicylate also enhanced the degradation of naphthalene and phenanthrene.

2.6.3 Bioaugmentation and Genetically Engineered Microorganisms

Bioaugmentation is the process by which microorganisms are selected and grown for their ability to metabolize contaminants and are then introduced into the environmental to carry out such degrading functions (Hughes, et al. 1997; Woo and Park 2004). Several studies have investigated the potential for bioaugmentation in order to promote degradation of PAHs in soil. Results achieved showed enhanced degradation in lab scale settings (Grosser, et al. 1991); however, such effects cannot be easily translated to field studies since additional factors, such as nutrient and inducer presence, normally limit degradations in the environment (Woo and Park 2004).

Genetically engineered microbes have shown potential for bioremediation but only in laboratory studies. Additionally, this strategy has yet to be widely accepted and is costly for environmental applications (Woo and Park 2004). The idea behind this technique is to introduce the desirable enzymatic capacities, present in different microbial species, into a single host capable of carrying out all degrading functions (Vandermeer 2005). Although, only a few studies have investigated the potential for using genetically engineered microbes in bioremediation settings (Pieper and Reineke 2000), results show significant promise.

2.6.4 Two Phase Partitioning Bioreactors

Two phase partitioning bioreactors (TPPBs) are a technology platform actively explored over the last two decades. More recently, these bioreactors have been implemented in environmental applications because of their ability to eliminate delivery limitations often

observed in the degradation of toxic and/or hydrophobic substrates. As such, they are well suited for the degradation of PAHs. Their principles of operation arise from biological considerations, as well as mass transport and thermodynamic theory. In a typical system, an aqueous phase containing substrate degrading organisms is allowed to coexist with an immiscible second phase acting as a reservoir for high concentrations of hydrophobic substrates (Daugulis 2001). Low levels of organics are then partitioned into the aqueous phase due to equilibrium (Daugulis 2001). Although the name implies that only two phases are present, the reactor actually contains four phases: Hydrophobic, aqueous, gas, and cellular phases (van der Meer, et al. 1992).

The secondary phase (hydrophobic phase) in TPPBs has multiple applications. If the cells are the product of interest, the second phase can act as a growth substrate for the microorganisms (Déziel, et al. 1999). Additionally, it can act as a receiving phase for valuable compounds manufactured through microbial biosynthesis in the aqueous phase (Déziel, et al. 1999). Finally, as in the case of PAHs, the second phase can act as a non-biodegradable, biocompatible reservoir for nonpolar substrates, which are then delivered into the aqueous phase for degradation (Déziel, et al. 1999).

The transfer of organic contaminants from the secondary phase is typically measured by means of a partitioning coefficient. This parameter is specific to the compound in question and highly influenced by aqueous and organic phase solubilities. Once in the aqueous phase, hydrophobic substrates, such as PAHs, are degraded by the microorganisms, causing a disruption in the equilibrium condition of the reactor. As a result, more substrate partitions into the aqueous phase and the process continues until all the substrate is consumed (Daugulis 2001; Vandermeer 2005).

Microbial access to poorly soluble substrates, such as PAHs, occurs via three modes. In the first mode, only the organic substrate dissolved in the aqueous phase is bioavailable for degradation. This condition makes the process dependent on mass transfer between phases and is

generally accepted as necessary during degradations of low molecular weight PAHs (Déziel, et al. 1999).

In the second mode of transfer, cells come into direct contact with the second phase. A biofilm develops at the interface and microbes acquire the substrate by transfer through their membrane (Déziel, et al. 1999). This mechanism is believed to be of significant importance for the degradation of hydrophobic substrates in TPPBs of two liquid phases. As demonstrated by Jimenez and Bartha (1996), bacteria isolated from interfaces can sometimes degrade PAHs approximately 8.5 times faster than cells present in aqueous suspensions.

In the final mode of transfer, microorganisms produce surface active compounds (biosurfactants) that induce formation of micelles utilized for solubilization of hydrophobic substrates (Déziel, et al. 1999). This mechanism is poorly understood, however emulsifications of non-aqueous phases in TPPBs confirm that such conditions may actually be present and enhance degradation of organic substrates (Desai and Banat 1997; Hommel 1990; Vandermeer 2005).

All three modes of transfer may be active (either consecutively or simultaneously) during typical TPPB operations. However, the dominant mechanism is normally dependent on microbial culture properties and growth stage, reactor operational conditions, and type substrate being investigated (Déziel, et al. 1999).

2.7 Two-Liquid-Phase Partitioning Bioreactors for Degradation of PAHs

As mentioned previously, TPPBs are an attractive alternative for bioremediating polyaromatic hydrocarbons. Consequently, the use of bioreactors with two liquid phases has been extensively employed for the degradation of these aromatic contaminants and a summary table of degradations has been compiled and presented by Vandermeer (2005).

The first degradation of PAHs delivered from a secondary phase was demonstrated by Wodzinski and Larocca (1977). Their results showed that naphthalene was degraded when

delivered from heptamethylnonane (HMN). Furthermore, growth rates indicated that the *Pseudomonas* sp. utilized acquired naphthalene at the aqueous-hydrocarbon interface. Similar studies carried out by Efroymson and Alexander (1991) demonstrated the delivery of naphthalene from HMN at increased rates. Additional investigations have addressed the degradation of phenanthrene using silicone oil, HMN and other organic solvents as delivery phases (Birman and Alexander 1996; Bouchez, et al. 1995; Guieysse, et al. 2001; Muñoz, et al. 2003). Pyrene has also been delivered individually from a number of secondary phases including paraffin oil, squalene, tridecylcyclohexane, *cis*-9-tricosene, HMN and silicone oil (Bouchez, et al. 1997; Guieysse, et al. 2001; Jimenez and Bartha 1996).

The above studies all demonstrated the delivery of individual or pairs of PAHs from secondary phases. However, in the environment, PAHs are commonly present as mixtures, and as previously mentioned, interactions can have significant effects on their respective degradation. Efforts have therefore been made to expand the concept of TPPBs to address mixtures of PAHs. Vanneck et al. (1995) reported the first mixture degradation in a TPPB where the secondary phase was silicone oil and the PAHs delivered were naphthalene, phenanthrene, fluoranthene, and benzo[*a*]pyrene. An undefined microbial consortium was used in the aqueous phase to carry out the remediation, and rates of degradation for naphthalene, phenanthrene, fluoranthene and pyrene of 1.9 mg $L^{-1}d^{-1}$ were reported, while benzo[*a*] pyrene degradation was found to be 1.8 mg $L^{-1}d^{-1}$. In another study conducted by Villemur et al. (2000), rates of degradation for pyrene, chrysene and benzo[*a*]pyrene in a TPPB, with a silicone oil secondary phase, were found to be 19, 3.5, and 0.94 mg $L^{-1}d^{-1}$ respectively.

More recent studies have achieved higher rates of degradation. Janikowski et al. (2002) reported a total degradation rate 2160 mg $L^{-1}d^{-1}$ for naphthalene, phenanthrene, acenaphthene and anthracene by a *Sphingomonas aromaticivorans* B0695 strain in a TPPB using dodecane as the delivery phase. In a similar study by MacLeod and Daugulis (2003), the degradation rates of

pyrene and phenanthrene using a *Mycobacterium* strain were found to be 138 and 168 mg $L^{-1}d^{-1}$ respectively, using bis(ethylhexyl) sebacate as the second phase. Additional studies used a combination of strains in TPPBs that demonstrated synergistic interactions achievable for PAH degradations (Daugulis and McCracken 2003). The study reported a complete degradation of naphthalene, phenanthrene and fluoranthene at a total rate of 1452 mg $L^{-1}d^{-1}$. Pyrene, chrysene and benzo[*a*]pyrene were degraded subsequently by 93, 99 and 64% with respective rates of 16, 17 and 11 mg $L^{-1}d^{-1}$. The strains utilized were *Sphingomonas aromaticivorans* B0695 and *Sphingomonas paucimobilis* EPA505 with a dodecane secondary phase. A final study by Vandermeer and Daugulis (2007) demonstrated complete naphthalene, phenanthrene and fluorene degradations at rates of 400, 250 and 200 mg $L^{-1}d^{-1}$. This study also utilized a defined microbial consortium consisting of *Sphingomonas aromaticivorans* B0695 and *Sphingomonas paucimobilis* EPA505. Pyrene and benzo[*a*]pyrene on the other hand, were degraded by 64% and 11%, at rates of 42.9 and 7.5 mg $L^{-1}d^{-1}$ respectively. The delivery phase in this study was silicone oil.

The above investigations demonstrated the potential of TPPBs for the degradation of PAH mixtures by either individual microorganisms or consortia. The highest rates of biodegradation of PAHs to date have actually been achieved in TPPBs (Vandermeer 2005). However, the need for secondary liquid solvents limits their environmental applicability. In an overall process scheme, PAHs in soil would have to be transferred to the secondary phase in order to be degraded in TPPBs, and contacting soils with organic solvents for pollutant extraction further contaminates the matrix. As a result, two-liquid-phase bioreactors do not provide the possibility for completely treating PAHs, if removal from contaminated sources is necessary.

2.8 Solid–Liquid Two Phase Partitioning Bioreactors

Recently, a study by Amsden et al. (2003) demonstrated that liquid secondary phases could be replaced by solid polymeric compounds serving the same purpose in TPPBs. This was a breakthrough because polymers could be introduced into soil for uptake of deleterious molecules,

without inducing further contamination. Subsequent treatments could then be carried out in TPPBs. Furthermore, it was demonstrated that polymers were non-bioavailable, allowing undefined microbial consortia to be potentially employed in TPPBs, and were also reusable without any loss in performance (Amsden, et al. 2003). In the case of hydrophobic substrates, polymers act as a reservoir for high concentrations of contaminants, which are then delivered based on mass transport, thermodynamic equilibrium and metabolic demand. In practice, polymers have been shown to extract phenol, as well as polychlorinated biphenyls, from soil with a subsequent degradation in TPPBs (Prpich and Daugulis 2006; Rehmann and Daugulis 2008). An additional study by Rehmann and Daugulis (2007) also demonstrated the biodegradation of biphenyl in a solid-liquid two phase partitioning bioreactor. Like PAHs, biphenyl is hydrophobic in nature and the second phase increases bioavailability in the reactor.

An important parameter in these solid-liquid bioreactors is mass transfer. As commented by Déziel et al. (1999), one of the modes of transport present for microbial uptake is the dissolution of organic substrates from the secondary to the aqueous phase prior to degradation. If this mode is prominent in the degradation of hydrophobic substrates, it is possible that overall rates of degradation will be mass transfer limited. Rehmann and Daugulis (2007) demonstrated such conditions for biphenyl. The study used Hytrel^{TM} polymers (thermoplastic polyester) for delivery of biphenyl in a TPPB. The area available for transfer was varied while degradation rates were monitored, and results demonstrated a clear mass transfer limitation. A similar situation was shown for the delivery of phenanthrene, pyrene, and fluoranthene by Rehmann et al. (2008), from Desmopan (polyurethane) in a solid-liquid two phase reactor. Based on such results, solutions to these constraints need to be found on order to improve the rates of PAH degradation in these novel reactors.

2.8.1 Polymer Properties Influencing Mass Transfer in Solid-Liquid TPPBs

Molecular transport within polymers is also an important aspect that needs to be addressed to enhance degradation rates of hydrophobic substrates in solid-liquid TPPBs. This transfer issue has been extensively analyzed in applications ranging from reverse osmosis and pervaporation to drug delivery. Additionally, the transport of aromatic molecules, similar to PAHs, has been extensively studied in the film industry and effects of polymer properties as a function of transport have been reported (Slark and O'kane 1997).

As demonstrated by Slark and O'kane (1997) the most influential property affecting molecular transport in polymers is the glass transition temperature (T_g). Transfers occurring below this temperature were significantly retarded due to poor chain segmental motions required to accommodate diffusing organic molecules (George and Thomas 2001). This effect arises from free volume availability, which has a strong effect on polymer diffusive and permeation properties (Nagel, et al. 2002). The creation of void spaces is necessary for efficient exchange in positions between polymer chains and diffusing solutes. Temperatures below T_g inhibit such exchanges, and thus molecular transport (Slark and O'kane 1997). Therefore, polymers with low glass transition temperatures would be better suited for delivery of PAHs in TPPBs.

A secondary factor affecting transport of organic compounds, in semi-crystalline polymers, is degree of crystallinity. Lützow et al. (1999) showed that transport of solutes in such matrices was inversely correlated to increasing degree of crystallinity. Polymers of this nature have compact structures with low porosities preventing diffusion of penetrant molecules. Possible methods for decreasing crystallinity include crosslinking, which generates disorder in the polymer matrix (Kumar, et al. 1997). However, careful consideration must be paid when balancing crystallinity with crosslinking. Extensive vulcanizations result in polymer membranes with decreased free volume and poor transport properties. Overall, however, polymers with low degrees of crystallinity would aid in the diffusion of hydrophobic substrates in TPPBs.

Crosslinking density has been found to be another crucial polymer property having a strong effect on transport. Kumar et al. (1997) and Harogoppad and Aminabhavi (1991) demonstrated that diffusion of organic solutes decreased with increasing crosslinking due to inhibited chain mobility in the polymer segments. Theoretically, vulcanization reduces the free volume available for transport and restricts movement of solutes in the membrane (Harogoppad and Aminabhavi 1991). Compounds of decreased molecular size are less influenced by this effect. However, for TPPBs, polymers with low crosslinking densities would enhance PAH delivery.

Temperature of transport is an additional parameter influencing rates and extents of transfer in polymer membranes. Slark and O'kane (1997) found that temperatures above T_g led to faster deliveries of organics due to the increased energy present for chain rearrangements. George et al. (1996) also showed that increased temperatures enhanced the rates of transport in styrene-butadiene rubber (SBR) membranes, while Kumar et al. (1997) and Harogoppad and Aminabhavi (1991) demonstrated the same trends in another wide variety of polymers. Additionally, adsorption, which can be an intermediate step in the transport process of organic solutes in polymers, as stated by Rzeszutek and Chow (1998), has also been found to be a function of temperature (Lin and Huang 1999). Therefore, to enhance molecular delivery of aromatic contaminants in TPPBs, reactor operating temperatures must be significantly above polymer T_gs.

Furthermore, the chemical composition of the polymer has been found to enhance the transfer of solutes. Aromatics examined by Harogoppad and Aminabhavi (1991) displayed enhanced uptakes in SBR polymers. This was due to a structural resemblance between the penetrant and the polymer. Extractions of phenols using polyurethane membranes have also shown chemical composition effects (Rzeszutek and Chow 1998). Strong intermolecular interactions (hydrogen bonding) reduced phenol transfer and resulted in slower deliveries from the polyurethane matrix (Rzeszutek and Chow 1998). This effect was also noted by Prpich and Daugulis (2006) and yielded slower phenol degradations in TPPBs. Therefore, polymer-solute

interactions play an important role in mass transfer and as such must be addressed. In the case of PAHs, polymers with similar structures will likely enhance uptakes from soil but retard deliveries in TPPBs due to their increased affinities for these aromatic moieties.

Special block copolymers could also be utilized to improve transport of hydrophobic solutes. These matrices can trigger specific properties depending on surrounding conditions (Gadelle, et al. 1995). They are surfactant analogues capable of producing micelles, which can extensively solubilize organic compounds in aqueous solutions. This effect can be tailored by varying the composition of hydrophobic and hydrophilic blocks in the polymer (Gadelle, et al. 1995). Additionally, such copolymers would be expected to be non-bioavailable, potentially eradicating toxic effects exhibited by solution surfactants, opening the door for solubilized degradations.

Overall, polymers with low glass transition temperatures, degree of crystallinity, crosslinking density, increased surface area and selective chemical interactions with PAHs will likely improve mass transport in TPPBs. Complementary factors to be examined include temperature of transfer and degradation and the use of block copolymers.

2.8.2 Additional Methods for Improving Polymer Delivery

Additional solutions to mass transfer limitations may also be present in the field of responsive drug delivery. Novel techniques improving delivery from polymers by means of different stimuli have been summarized by Kost and Langer (2001) and could be applied in environmental settings. In the case of TPPBs, it is likely that several of the techniques could play a role in improving mass transport of hydrophobic substrates.

As summarized by Kost and Langer (2001) polymer deliveries can be activated through numerous external stimuli, including ultrasound (also referred to as sonication), magnetic and electrical fields as well as different types of irradiation. In ultrasonic studies, compounds

delivered include p-nitroaniline, p-aminohippuric acid, bovine serum albumin and insulin (Kost, et al. 1988; Kost, et al. 1989), while polymeric delivery phases include biodegradable polyglycolide and polylactide, as well as non-erodible ethylene vinyl acetate copolymer (EVAc). The use of biodegradable polymers in TPPBs has yet to be investigated and thus, effects of using such polymers still remain unknown. Kost et al. (1989) demonstrated that ultrasonically enhanced drug release could be induced from erodible polymers loaded with p-nitroaniline and p-aminohippuric acid, *in vitro* and *in vivo* respectively. This latter compound is used in the measurement of renal blood flow and has a similar backbone aromatic structure to PAHs (Phillips and Hamilton 1948), possibly leading to analogous findings. The study monitored the concentration of p-aminohippuric acid in urine, delivered from a bioerodible polymer matrix, after ultrasonic treatment on rats and findings showed that concentrations rose as a function of exposure. Additional studies have also shown enhanced delivery of aromatic 5-fluorouracil (5-FU) (Miyazaki, et al. 1985) and bovine insulin (Miyazaki, et al. 1988) from ethylene vinyl alcohol copolymers implanted in diabetic rats after ultrasonic treatment. The results presented, therefore, demonstrate that ultrasonic irradiation can enhance release rates of compounds with different molecular sizes, chemical compositions and hydrophobic characteristics from a variety of polymers both *in vivo* and *in vitro*. However, the applicability for enhanced transport via sonication of PAHs in solid-liquid bioreactors remains to be demonstrated. It is important to note that while biomedical studies demonstrated improved deliveries in the presence of sonication, cell damage to mammalian cells during *in vivo* studies was typically minimal or negligible (Kost and Langer 2001). This is a necessary characteristic for successful application of ultrasound in TPPB degradations.

Magnetism is a secondary external stimulus that has been commonly utilized for enhancing delivery rates in polymeric systems. A series of studies has shown improved release rates of different proteins from EVAc, *in vivo*, via external oscillating magnetic fields (Edelman,

et al. 1985; Kost, et al. 1985; Kost, et al. 1987). More recently, magnetic fields were also used to trigger insulin release from EVAc polymers in diabetic rats (Kost, et al. 1987). Additional studies have also found similar effects using polymeric alginate gels (Saslawski, et al. 1988). Furthermore, rates have been found to be a function of magnetic field characteristics, in which higher amplitudes increased extents of release observed (Edelman, et al. 1985). Not only is there potential to employ magnetic fields for enhanced delivery of PAHs in solid-liquid TPPBs but also amplitude variations provide an inherent control point for such delivery schemes.

Another way to achieve improved deliveries is by means of an electric field modulating solute release from a polymeric membrane (Kost and Langer 2001). Effects enhancing transport are induced by changes in electrical and chemical properties of the polymeric membranes. Firstly, electrical fields generate polymer swelling leading to larger pore sizes and solute permeability (Grimshaw, et al. 1989). Secondly, electric fields can augment solute fluxes within the polymer membrane, inherently improving transport (Grimshaw, et al. 1989). Finally, electrostatically enhanced partitioning of charged solutes can occur in the matrices (Grimshaw, et al. 1989). Studies on organic solutes by Bhaskar et al. (1985) demonstrated such effects, showing that permeability in crystalline membranes was altered through the use of electric fields. The electric current induced a phase change in the matrix by aligning the polymeric molecules. Additional studies by Weiss et al. (1986) demonstrated that a 16 fold increase in permeability of dextran, a cyclic polysaccharide, in poly(methyl methacrylate) matrices could be achieved through the use of electric fields. As a whole, electric fields provide an additional mechanism for enhancing mass transfer of solutes, and it is possible that such a stimulus could be applied for PAHs delivered from polymers as a pre-treatment stage to the two phase degradation.

Various types of irradiation are the final external stimuli demonstrated to enhance transfer of solutes from polymer matrices. Studies by Miyazaki et al. (1989) showed that microwave irradiation increased the release rates of 5-fluorouacil from EVAc polymers. It was

also found that upon discontinuation of microwave exposure, release rates were deactivated and returned to baseline levels. In another study by Mathiowitz and Cohen (1989), azobenzene transfer from polyamide microcapsules was improved by photolysis. It was seen that exposure to light caused a rupture in the polymeric membrane, which increased the release rate of solutes. Azobenzene is a hydrophobic molecule with a similar structure to polyaromatic hydrocarbons and it is therefore likely that this stimuli type could potentially be an important method for enhancing PAH delivery in TPPBs. Furthermore, it must be recalled that photolysis is a physio-chemical treatment process that can be combined with biological degradation schemes, such as TPPBs, to enhance rates of degradation of high molecular weight PAHs.

Overall, the four alternatives presented possess a significant amount of promise in TPPB environmental applications. For the most part, they are capable of inducing enhanced deliveries in the presence of animal cells without giving rise to harmful side effects, when used appropriately. Therefore, it can be hypothesized that if applied alongside microbial consortia, which are typically more resilient cellular entities than animal cells, improved deliveries and degradations of hydrophobic solutes, such as PAHs, can be achieved in polymer-liquid TPPBs.

2.9 Ultrasonic Delivery and Contributing Factors

2.9.1 Physical Insight

As presented previously, sonication enhancements have been demonstrated for numerous solute-polymer combinations. The versatility of this technique is evident as improvements in delivery of both hydrophilic and hydrophobic drugs have been demonstrated from various resins (Levy, et al. 1989). Additionally, sonication has shown the capability of "on-demand" improved delivery as a function of application presence, power levels and/or intensity, while being reversible in most cases (Kost, et al. 1989; Levy, et al. 1989). Therefore, it seems possible to

expect that if applied appropriately, enhanced solid-liquid TPPB biodegradation of PAHs could be obtained.

The main factor leading to such improvements has been attributed to cavitation (Kost, et al. 1989). During sonication, formation and collapse of gas cavities in the liquid phase induce conditions of several thousand Kelvin and few hundred bar (Breitbach and Bathen 2001). Such a condition generates high speed liquid streams, termed "microjets", which at a polymer surface level induce increased release of components within matrix pores (Breitbach and Bathen 2001; Ji, et al. 2006; Li, et al. 2002). On a molecular level, it has also been proposed that such conditions induce severe polymer strain, generating rupture in attractions between sorbed molecules and the matrix itself, resulting in more rapid release of solutes (Kost, et al. 1989; Li, et al. 2002). Additionally, it has been proposed that shockwaves generated during sonication induce microscopic turbulence and can enhance both internal and external mass transfer from polymer matrices (Ji, et al. 2006).

Several ultrasonic wave properties have been shown to affect the extent of enhancements obtained. Frequency is one such property and low ranges (ie. 20kHz) induce larger cavitation bubbles, which upon collapse result in the most powerful hydro-mechanical shear forces (Mitragotri and Kost 2004; Tiehm 2001), and thus improved deliveries (Skauen and Zentner 1984). Ultrasonic intensity is another property having profound effects on delivery enhancements. As shown by Skauen and Zentner (1984), the degree of solute migration via ultrasound was proportional to the intensity of sonication. Kost et al. (1989) further demonstrated such conditions for p-nitroaniline release, where higher intensities resulted in improved release rates.

A final beneficial aspect of sonication is its ability to induce the above effects while maintaining structural and physio-chemical properties of the solutes in question. Skauen and Zentner (1984) demonstrated unchanging properties for numerous drugs under exposure to ultrasound, while Kost et al. (1988) showed similar effects for insulin, a structure-sensitive

compound. It can therefore be proposed that if sonication does not induce molecular changes, even on "sensitive" molecules such as insulin, then effects on electronically stable PAHs will likely be negligible.

2.9.2 Biological Insight

Important aspects to be balanced with sonically enhanced deliveries are effects on cell viability. The damaging effects of sonication were first noticed by Harvey and Loomis (1929), who reported cell disruption/destruction of luminous bacteria at ultrasonic frequencies. The following decades focused on studying the effects of sonication predominantly in the context of cellular inactivation (Dahi 1976; Harvey and Loomis 1929; Raso, et al. 1998) and/or extraction of intracellular contents (Neppiras and Hughes 1964; Wang and Sakakibara 1997). However, more recently it has been shown that cyclic applications of sonication can enhance biological activities. Wood et al. (1997) demonstrated such conditions for bio-production of ethanol by showing that enzymatic activity was actually enhanced or remained constant in the presence of ultrasound. Additionally, a similar study by Wang et al. (1996) also demonstrated enhanced lactose hydrolysis by *Lactobacillus bulgaricus* under cyclic application of sonication.

Ultrasonic frequency, intensity, and period of exposure have profound effects on the extent of cellular inactivation. As demonstrated by Tiehm (2001), increasing ultrasonic frequency has an inverse effect on cellular inactivation in activated sludge. That is, as the frequency of sonication increases, the extent of cellular damage decreases. Additionally, increasing intensity and sonication periods increase microbial damage (Tiehm 2001) with results being demonstrated for bacteria (Jacobs and Thornley 1954; Raso, et al. 1998; Scherba, et al. 1991), yeasts (James, et al. 1972; Neppiras and Hughes 1964) and fungi (Scherba, et al. 1991).

Overall, it can be concluded that under appropriate sonication conditions, including suitable sonication frequency, intensity, and cycling periods, it is possible to improve delivery of

solutes from polymers while maintaining cellular activity in solid-liquid two phase partitioning bioreactors.

2.10 Scope of Thesis

The above literature all points towards the potential of enhancing PAH degradation in TPPBs by means of ultrasonication, and the possibility of applying such a strategy could solve the limiting step of a previously proposed technology for soil remediation. However, to date, such an approach for improving delivery of PAHs in two phase systems has yet to be explored. Maintaining biological activity through appropriate sonication cycling would also need to be confirmed to ensure improved degradation rates. Therefore, this work examined the potential for improving delivery and degradation of PAHs, through ultrasonic exposure, in polymer-liquid two phase partitioning systems.

The objectives of the current work were to examine physical improvements of sonication on PAH delivery and thermodynamics in polymer-liquid partitioning systems. Subsequently, improvements in overall degradation of PAHs, delivered from polymers, were evaluated in the presence of sonic cycling utilizing a consortium of bacteria capable of degrading a wide variety of PAHs. A model describing delivery of PAHs from polymers, both with and without sonication, was then developed to gain a better mechanistic understanding of ultrasonic effects on internal and external mass transport as well as thermodynamics. Finally, effects of sonic exposure on the biological consortium were evaluated at a molecular level and the possibility of up scaling results to bench scale TPPBs were studied in order to asses the feasibility of sonication in soil remediation schemes proposed by Rehmann et al. (2008).

2.11 References

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Chapter 3

Ultrasonically Enhanced Delivery and Degradation of PAHs in a Polymer-Liquid Partitioning System by a Microbial Consortium

Pedro A. Isaza and Andrew J. Daugulis

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3.1 Preface

As noted in Chapter 2, the possibility of utilizing ultrasonic exposure to enhance the delivery of PAHs in two phase partitioning systems is proposed in this work. These systems are designed to allow high concentrations PAHs to be sequestered in the polymer phase with delivery into the aqueous phase for degradation, based on mass transport and thermodynamic considerations, as well biological demand. Although the field of biomedical engineering has demonstrated that drug delivery from polymeric matrices can be significantly aided, both *in vivo* and *in vitro* through ultrasonic application, cellular inactivation effects associated with such exposure in two phase systems, have yet to be examined.

As mentioned in Chapter 2, sonication effects on delivery and thermodynamics have been demonstrated in a variety of systems. However, the possibility of inducing such "on demand" and "on time" improvements in polymer-aqueous PAH deliveries has yet to be explored. Examining potential effects of sonication on solid-liquid mass transport and overall degradation of PAHs will help alleviate limiting steps of remediation processes proposed in previous literature. Additionally, it will provide a means for triggering enhanced delivery in other biodegradation and bio-production two phase schemes facing similar limitations.

The current chapter quantified effects of sonication at the flask level. Specifically, ultrasonic effects on delivery rates of PAHs in polymer-liquid systems were examined along with associated effects on thermodynamic equilibrium positions. Additionally, effects of sonication on biology were investigated and finally, effects of exposure on solid-liquid two phase degradations of PAHs were evaluated.

3.2 Abstract

The current study examined the effects of ultrasonic irradiation on mass transfer and degradation of PAHs, by an enriched consortium, when delivered from polymeric matrices. Rates of release into methanol under sonicated conditions, relative to unmixed cases, for phenanthrene,

fluoranthene, pyrene and benzo[*a*]pyrene were increased approximately 5 fold, when delivered from Desmopan 9370A (polyurethane). Similar effects were observed in Hytrel® 8206 and Kraton® D4150K polymers as well as recycled Bridgestone tires. Enhancements were also displayed as shifts to higher release equilibria under sonicated conditions, relative to non-sonicated cases, agreeing with current knowledge in sonochemistry and attributed to cavitation. Ultrasonic effects on microbial activity were also investigated and cell damage was found to be non-permanent with consortium re-growth being observed after sonic deactivation. Finally, the lumped effect of sonication on degradation of phenanthrene delivered from Desmopan was examined under the absence and presence of sonication. Rates of degradation were found to be increased by a factor of four demonstrating the possibility of using ultrasonic irradiation for improved mass transport in solid-liquid systems. Cellular inactivation effects were not evident, and this was attributed to the attenuation of sonic energy arising from the presence of solid polymer materials in the medium. The findings of the study demonstrate that sonication can be used to improve mass transport of poorly soluble compounds in microbial degradations, and alleviate limiting steps of soil remediation processes proposed in previous research.

Key Words: Ultrasound, Mass Transfer, Solid-Liquid Partitioning Systems, PAHs, Consortium, Biodegradation

3.3 Introduction

Polyaromatic hydrocarbons (PAHs) are toxic organic contaminants arising from fossil fuel combustion and industrial processing (Cerniglia 1997) and the need to remove these aromatic compounds is driven by their carcinogenic nature, environmental persistence and chronic toxicity (Cerniglia 1992; Cerniglia 1997; Juhasz and Naidu 2000). In soil, PAHs accumulate in the vadose zone due to their hydrophobic nature and remediation has been achieved via incineration, chemical oxidation, composting, land farming, and phytoremediation (Antizar-Ladislao, et al. 2006; Denys, et al. 2006; Kulik, et al. 2006; Onwudili and Williams 2006). Controlled microbial degradations present an alternative cost-effective treatment for PAH contaminated soils, limited only by the poor aqueous solubility of PAHs. Recently, Rehmann et al. (2008), proposed a treatment process to overcome this limitation, in which inert polymeric materials were used to recover PAHs from soil, followed by biotreatment in a solid-liquid two phase partitioning bioreactor (TPPB).

TPPBs consist of a microorganism-containing aqueous phase, and an immiscible phase serving delivery or recovery purposes of target compounds (Daugulis 2001). Such a second phase acts as a reservoir for high concentrations of PAHs, which partition into the aqueous phase based on thermodynamic equilibrium and metabolic demand. These systems originally used immiscible organic solvents as the partitioning phase (Birman and Alexander 1996; Bouchez, et al. 1995; Muñoz, et al. 2003; Vandermeer and Daugulis 2007; Daugulis and McCracken 2003; Janikowski, et al. 2002; Villemur, et al. 2000), however were limited to pure microbial strains or well defined consortia due to the possible degradation of the immiscible solvent itself. Furthermore, PAH extractions from soil by means of organic solvents are difficult and limit the applicability of liquid-liquid TPPBs for environmental purposes. Recently, Amsden et al. (2003) demonstrated that solid polymeric materials could act as the second phase, while allowing for direct contact with a contaminated soil/water source.

A critical factor to be optimized is substrate delivery to cells. In liquid-liquid systems, agitation rates enhance interfacial areas (Chatzi, et al. 1989) and exponential rates of PAH degradation, under fast mixing, have been demonstrated by Köhler et al. (1994). In solid-liquid systems polymer delivery areas are constant and may limit PAH transport as demonstrated for biphenyl (Rehmann and Daugulis 2007), although improved biodegradation rates have been achieved through augmented interfacial areas. Alternatives can be found in the field of biomedical engineering and as summarized by Kost and Langer (2001), delivery from polymers can be enhanced by external stimuli, including ultrasound, magnetic, and electrical fields and microwave irradiation. For solid-liquid TPPBs, sonication would provide an ideal solution for improved delivery, as numerous studies have shown reversible and instantaneous enhanced transport of compounds having both lipophilic and hydrophilic properties (Kost, et al. 1988; Levy, et al. 1989; Miyazaki, et al. 1985; Miyazaki, et al. 1988), providing a true on-time and on-demand control variable (Levy, et al. 1989; Miyazaki, et al. 1988). Although sonication has been used for cell disruption purposes, under appropriate cyclic conditions, rates of microbial growth and ultrasonic inactivation can be offset to result in enhanced biological activities (Wood, et al. 1997; Wang, et al. 1996).

The objectives of the present work were: To obtain a microbial consortium capable of degrading PAHs; to assess the physical effects of sonication on rates of PAH delivery and equilibrium; to determine the effects of sonication on consortium growth; and finally to determine the lumped effects of sonication on mass transport and rates of phenanthrene degradation in a polymer-liquid two phase partitioning system.

3.4 Materials and Methods

3.4.1 Chemicals and Polymers

All salts and spectrophotometric grade methanol (95+%) were purchased from Fisher Scientific (Guelph, Canada). Phenanthrene (98+%), fluoranthene (98%) were purchased from Alfa Aesar (Ward Hills, MA) while pyrene (95%), and benzo[*a*]pyrene (BaP, 99+%, scintillation grade) were purchased from Sigma-Aldrich (Oakville, Canada). Silicone oil, Poly(dimethylsiloxane) 200 fluid at viscosity of 5cSt, was purchased from Sigma-Aldrich. Polymer samples were all of commercial grade: Desmopan 9370A (polyurethane) obtained from Bayer Material Science (Leverkusen, Germany), Hytrel® 8206 supplied by DuPont (Kingston, ON, Canada), Kraton® D4150K obtained from Kraton (Pernis, The Netherlands). Recycled tires were ground and sieved Bridgestone tires. Additional polymer properties can be found in Table 3-1.

Table 3-1: Basic Polymer Properties

Polymer		Source	Glass Transition Temperature (T_g, ^oC)	Density (g/mL)	Particle Size Distribution (Shape)
Commercial or Common Name	Chemical Name				
Kraton® D4150K	Styrene/butadiene linear triblock copolymer	Kraton	Styrene: 90; butadiene: -90	0.920	4.6±0.6mm by 3.8±0.85mm (Rice)
Hytrel® 8206	Polyether-ester	DuPont	-59	1.170	3.3±0.17mm by 3.8±0.1mm (Rice)
Desmopan 9370A	Polyurethane elastomer	Bayer Material Science	-70	1.060	2.8±0.15mm by 2.4±0.15mm (Rice)
Bridgestone Tires	Polybutadiene rubber or styrene-butadiene rubber	Bridgestone	N/A (Proprietary and product dependant)	0.44 (Typical Recycled Tires)	<4.76mm by 4.76mm (Square)

3.4.2 PAH Analytical Procedures

Analytical procedures were adapted from those described by Vandermeer and Daugulis (2007). PAH concentrations in methanol were quantified via fluorescence spectroscopy using a QuantaMaster QM-2000-6 fluorescence spectrometer (Photon Technology International, London, Ontario, Canada). All samples were diluted in methanol to within the linear range of detection (0-0.1mg/L) as described by Rehmann et al. (2008). Silicone oil concentrations were determined by diluting oil samples in methanol to within detection range, and analyzing via fluorescent spectrophotometry. Polymeric concentrations of PAHs were obtained by desorbing a small quantity of polymers (circa 0.1g) in 5mL of methanol as detailed by Rehmann et al. (2008). Extracts were then analyzed via fluorescence spectrophotometry, and equilibrium polymer concentrations were determined using partitioning coefficient curves, detailed subsequently.

3.4.3 Partitioning Coefficients

Partitioning coefficient procedures were adapted from those described by Prpich and Daugulis (2006), as well as Rehmann et al. (2008). Methanol stock solutions containing all four PAHs at 500, 250, 125 and 50mg/L concentrations were generated and distributed in 20mL scintillation vials. Varying masses of Desmopan beads (1 to 3g) were then introduced and vessels were sealed and agitated for 24h at 180rpm and 20^{o}C. Equilibrium concentrations of PAHs in methanol were determined via spectrophotometry and polymer uptakes were calculated via mass balance.

3.4.4 PAH Release Tests

Polymer pellets (6 grams equivalent to about 280 polymer beads), or used tires, were loaded by equilibrating a stock methanol solution containing all PAHs at a concentration of 500mg/L for 24 hours at 180rpm and 20^{o}C. Uptake of PAHs was then determined via mass balance by analyzing methanol, analogous to partitioning coefficient procedure. After uptake, methanol was decanted, and polymer beads were washed with water for 3 minutes and allowed to

air dry for 24 hours to volatilize residual solvent. Loaded polymers were then separated into two sections and transferred into new vials. Fresh methanol was introduced into the scintillation vials, containing the loaded polymers, and concentrations in methanol were monitored as a function of time. Release conditions examined were natural convection (no external mixing-control) and sonication at 20^{o}C (see Figure 3-1).

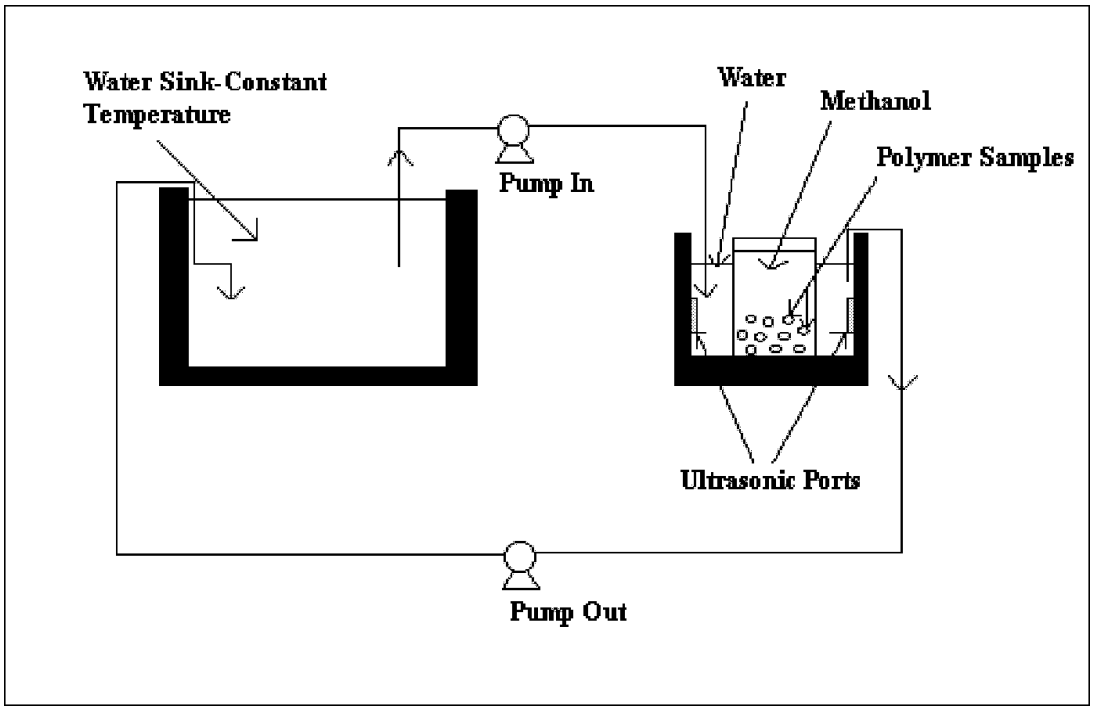

Figure 3-1: Schematic diagram of experimental setup used to determine the effect of ultrasound on release and degradation rates of PAHs. Note that methanol was replaced with medium during degradation experiments.

3.4.5 PAH Equilibrium Tests

3.0g of Desmopan were loaded to 4.0 and 5.5mg/g of phenanthrene and pyrene respectively and allowed to equilibrate with 15mL of methanol for 24 hours at 180rpm and 20^{o}C. Concentrations were monitored for 30 minutes to establish equilibrium positions without sonication. After such a time, the vial was sonicated for 30 minutes, and PAH concentrations were measured. At 60 minutes, sonication was deactivated and measurements were obtained for

an additional 30 minutes. At 90 minutes, sonication was re-activated and readings were obtained until time 120 minutes.

3.4.6 Selective Enrichment, Medium and Culture Conditions

A microbial consortium was obtained via selective enrichment in a 5L Bioflo III reactor (New Brunswick Scientific, Edison, NJ, USA). Initial seed samples included PAH contaminated soil, biosolids from a plastics plant wastewater treatment facility, soil from Sydney tar ponds in Nova Scotia, Canada (Courtesy of Sydney Tar Ponds Agency) and pure strains of PAH degraders (Vandermeer and Daugulis 2007). The enrichment was carried out using maintenance medium (Vandermeer and Daugulis 2007), without tryptone, yeast extract and glucose. Equal masses of all four PAHs, at 0.25g/L, were added as crystals and PAHs were added on a weekly basis to ensure selection pressure. After 2 weeks, 10mL of liquid culture were filtered through glass wool, to remove PAH crystals, and added to a 250mL flask containing 50mL of medium and 10mL of silicone oil with phenanthrene and fluoranthene dissolved at 500mg/L, while pyrene and BaP were at 250 and 100mg/L respectively (referred to as standard concentrations). Unequal concentrations were used due to solubility limitations for pyrene and BaP. The flask was then agitated for 24h at 180rpm and 20^{o}C, and samples were removed daily and centrifuged at 3500rpm for 15 minutes. Separated silicone oil layer samples were then assayed via spectrophotometry for PAH concentrations and returned to maintain approximately constant aqueous and organic phase volumes.

3.4.7 Consortium Growth on Glucose in the Presence and Absence of Sonication

A stock sample of the enriched consortium was grown in 40mL of medium with the inclusion of glucose (5g/L) for 48 hours. The cell culture was centrifuged, rinsed twice and re-suspended in fresh growth medium with glucose (1g/L) to yield an optical density of OD_{600}=0.6. The culture suspension was then divided into two 100mL samples. The first solution was

maintained as a control (no sonication) and OD was obtained for 300 minutes of growth at 20^{o}C. In parallel, the second culture was exposed to continuous sonication at 20^{o}C for 280min while obtaining OD readings. After 280min, sonication was switched off and cell re-growth was monitored for an additional 30 minutes, and after 24 hours.

3.4.8 Degradation of Phenanthrene Delivered from Silicone Oil in the Absence of Sonication

50mL of medium and 10mL of silicone oil, loaded with PAHs at standard concentrations, along with a frozen sample of stock culture, preserved in DMSO (10v/v%) at –80^{o}C, were added to a 250mL flask. The vessel was agitated for 96h at 180rpm and 20^{o}C, sampled on a daily basis and the silicone oil phase was assayed for PAHs. After 4 days the entire solution was centrifuged and the silicone oil was aspirated and cells were re-suspended and added to a new 250mL flask. In parallel, phenanthrene was loaded into silicone oil to a concentration of 500mg/L and 10mL of the resulting solution was introduced in the flask containing the re-suspended culture. Mixing at 600rpm was applied at all times. 5mL were removed at various times, centrifuged and silicone oil samples assayed for PAHs. After analytics, samples were returned to the original vessel to prevent silicone oil losses.

3.4.9 Degradation of Phenanthrene Delivered from Silicone Oil in the Presence of Sonication

The above experiment was repeated with the introduction of a sonication cycle subsequent to the re-suspension of cells, following an identical 96 hour growth period and introduction of phenanthrene loaded silicone oil. The cycle consisted of 25 minutes on and 3 hours off, with the first cycle beginning at 5.5 hours. This cycle was adapted from Wood et al. (1997), who demonstrated enhanced microbial ethanol production (with similar ultrasound conditions) without cell damage. Note that the culture was mixed at 600rpm except if/when being ultrasonically irradiated.

3.4.10 Degradation of PAHs Delivered from Desmopan under Biotic and Control Conditions in the Absence of Sonication

Identical growth conditions and analytical procedures to those detailed for silicone oil degradation experiments were used to generate re-suspended cultures. In parallel, 20g of Desmopan were loaded with phenanthrene (1mg/g). Five grams of the loaded polymers were then added to the flask and degradation was allowed to proceed for 144 hours at 600rpm and 20^{o}C. Small quantities of polymers (approximately 0.1g) were periodically removed, in triplicate, desorbed in methanol, and assayed for PAHs. For the sterilized control, identical conditions were provided except for the inclusion of an autoclave sterilization cycle prior to the addition of polymers.

3.4.11 Degradation of PAHs Delivered from Desmopan under Biotic and Control Conditions in the Presence of Sonication

The above experiment was repeated with a sonication cycle (and stirring at 600rpm), commencing after the addition of Desmopan polymers, which consisted of 25 minutes on and 3 hours off with the first cycle beginning at time 0. In the case of the sterilized sonicated control, identical experimental conditions were used except for the inclusion of a sterilization cycle prior to the addition of polymers.

3.4.12 Ultrasonic Treatment and Stirring

Sonication was provided by an ultrasonic bath (Fisher FS20) with an output frequency and intensity of 42kHz and 70W respectively, and maintained at 20^{o}C as shown in Figure 3-1. Flasks were placed in the bath and sonication was triggered in cycles of 25 minutes on and 3 hours off. Stirred release conditions were obtained using a Fisher Scientific Thermolyne Cimarec 3 stir plate.

3.5 Results and Discussion

3.5.1 Enriched Consortium

Figure 3-2 shows a characteristic degradation curve using the enriched consortium for PAHs delivered from silicone oil. Optical density was not monitored due to microbial adhesion at the aqueous/organic interface. Figure 3-2 conclusively demonstrates the degradation of all four PAHs, including BaP, a difficult to degrade aromatic with increased chemical stability, poor aqueous solubility and genotoxic properties. Additionally, phenanthrene seemed to be degraded exponentially, supporting the conclusion of Köhler et al. (1994) of its biologically limited consumption when delivered from an organic solvent under strong agitation. It can be proposed that liquid-liquid systems provided a metabolically controlled degradation profile of PAHs that contrasts to mass transfer limited degradations of phenanthrene in solid-liquid systems as shown by Rehmann et al. (2008). Further experiments are in progress to determine co-metabolic and/or diauxic behavior of the consortium, as well denaturing gradient gel electrophoresis (DGGE).

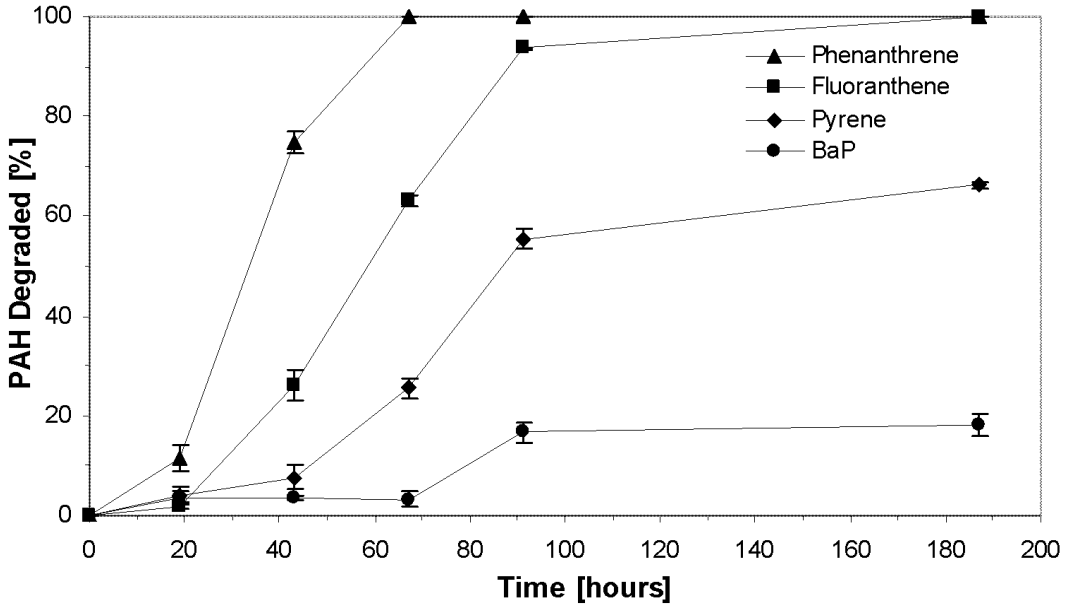

Figure 3-2: Degradation of PAHs delivered from silicone oil at 600rpm by enriched microbial consortium. Triplicate measurements taken at each time point were used as a standard deviation.

3.5.2 PAH Release Tests

A series of release experiments were subsequently conducted to characterize sonication effects on transport of PAHs from a number of polymers. Figure 3-3 shows the results obtained for Desmopan (polyurethane). It is clear that initial rates of release of all PAHs were enhanced through continuous sonication, irrespective of the PAH type. Such findings support improved mass transfer results by Kost et al. (1988), Levy et al. (1989), Miyazaki et al. (1985) and Miyazaki et al. (1988), where sonication was used to demonstrate improved transport, regardless of permeant hydrophobicity from a number of polymer matrices.

Because the control case was unmixed, a true measure of the external limitation in PAH transfer could not be quantified. The presence of mixing could result in different releases profiles, relative to the unmixed case, if transport were externally controlled. This would arise from shearing of the external film resistance controlling delivery under such conditions. Such data are still currently unavailable and it is not possible to assess the extent of such a resistance. Therefore, sonication results in Figure 3-3 do not differentiate between internal or external resistance improvements. A full mass transfer analysis would be required and is currently underway in our group. Enhancements in rates were more apparent for BaP relative to phenanthrene. Under the presence of sonication 10 minutes were required for BaP to achieve its equilibrium concentration or final percent release position, compared to 60 minutes needed for phenanthrene. Similar effects were found by Lavon and Kost (1998) and attributed to the fact that sonication enhancements were more pronounced for more transfer limited compounds. Therefore, BaP being the most hydrophobic PAH studied was more resistant to transport into methanol. Furthermore, BaP's increased molecular weight likely made it subject to higher transport resistances within the polymer matrix.

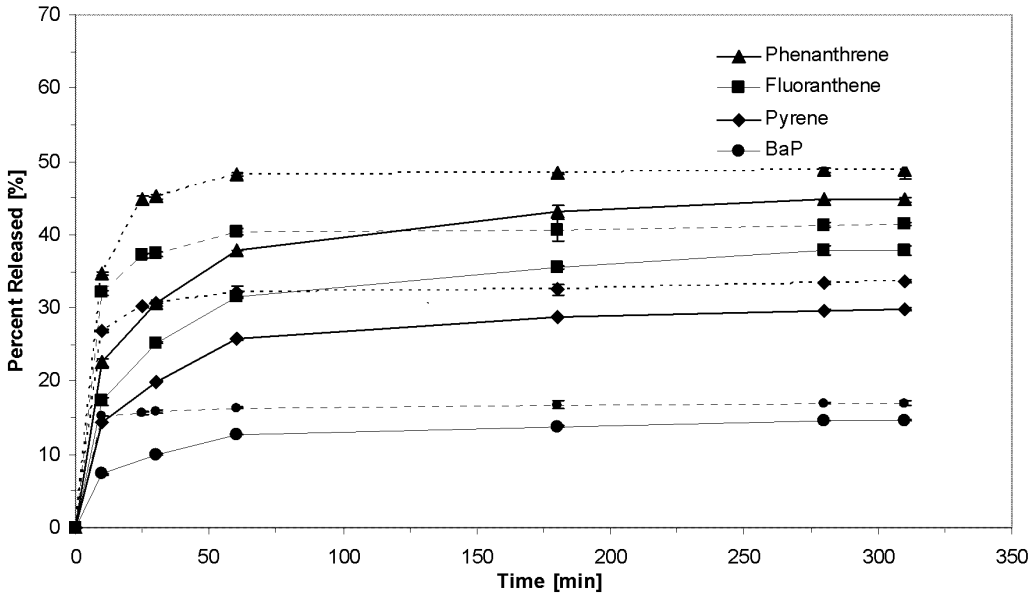

Figure 3-3: Release of PAHs from Desmopan polymer into methanol in the presence and absence of continuous sonication. Solid lines represent control/non-sonicated data while dashed lines represent sonicated results. Triplicate measurements taken at each time point were used as a standard deviation.

Figure 3-3 also shows that final release positions were reached at least 5 times faster in the presence of ultrasound for all PAHs. At most, only 60 minutes were required to reach the ensuing equilibria compared to 300 minutes necessary for the non-sonicated control. Similar results have been noted by Kost et al. (1988) for various molecules and as described by Breitbach and Bathen (2001) such improvements likely arise due to cavitation. During sonic exposure, bubbles are generated and subsequently collapse in a process termed cavitation, inducing conditions of several thousand Kelvin and a few hundred bar. Near a polymer surface asymmetric collapse forms "microjets" reaching speeds up to 500m/s, which can enter polymer pores and induce increased desorption and turbulence both at a surface and pore level (Breitbach and Bathen 2001). It seems plausible that improvements observed occurred due to such a process irrespective of whether PAH transport was internally or externally controlled. A secondary explanation can be drawn from a more energetic standpoint. As mentioned by Harogoppad and

Aminabhavi (1991), transport in polymers requires energy for exchange in positions between solutes and polymer chains. It can therefore be hypothesized that sonication provided surplus energy for position exchanges and led to the enhanced transfer seen in Figure 3-3.

A shift in equilibrium position was also induced by sonication and is displayed as the change in the final extent of release seen between sonicated and control cases shown in Figure 3-3. For phenanthrene, approximately 50% was present in methanol at equilibrium under sonicated conditions, compared to 40% in the control release. Such thermodynamic changes were observed for all PAHs. Similar effects have been observed and analyzed by Breitbach and Bathen (2001), as well as by Li et al. (2002), and attributed to the generation of "microjets". These high pressure streams could have induced tearing of sorbed molecules at a surface level. Although absorption is the primary method of uptake of PAHs in polymers, adsorption has been shown to be an important step in the transport of organic compounds in polyurethane matrices (Rzeszutek and Chow 1998), a family to which Desmopan belongs. It could, therefore, be hypothesized that PAH stripping at the surface resulted in the observed equilibrium shifts. Furthermore, the polymer samples utilized had a large surface area to volume ratio (16:1) and therefore any area related effects could be possible causes for the thermodynamic changes observed. From an energetic standpoint, it could also be proposed that additional energy introduced via sonication affected PAH-polymer interactions and thus equilibrium positions in a manner that is not surface restrictive. From Figure 3-3 it also appears as if the shifts in equilibrium were a function of the permeant character. Higher shifts were seen for less hydrophobic phenanthrene relative to BaP. However, conclusive trends would require a detailed mass transport analysis. Finally, trends of release under both sonicated and control conditions were in close agreement with partition coefficients reported by Rehmann et al. (2008). The highest coefficient was found for BaP, followed by pyrene, fluoranthene and phenanthrene under the presence and absence of sonication. For this study, a larger coefficient represented a higher affinity for the polymeric phase relative to

methanol. This is consistent with the fact that phenanthrene, having the lowest polymer affinity, would have the smallest coefficient and the highest extent of release. Analogous conclusions can be drawn for other PAHs.

3.5.3 Enhanced Release Results for Additional Polymers Examined

Equivalent release experiments were also conducted using various polymers, and effects of sonication on initial rates (taken between 0 and 10 minutes) are summarized in Table 3-2. It is clear that sonic exposure enhanced initial rates of release in all matrices examined, validating earlier conclusions. For instance, un-mixed rates of release (control) of phenanthrene from Hytrel® 8206 increased from 4.16 to 4.6mg/L.min in the presence of sonication, representing a 10% increase in rates. Similarly, a 19% improvement for phenanthrene was observed in Kraton® D4150K. Pyrene on the other hand, demonstrated a 13% increase in Hytrel® 8206 and 28% in Kraton® D4150K. Similar assessments could be made for the remaining data presented in Table 3-2. It is difficult to quantify effects as a function of chemical and structural polymer properties. However, effects were present in all cases examined, opening the door for improved delivery of other poorly soluble compounds in solid-liquid degradation schemes. Furthermore, effects were present even in recycled tires, demonstrating that sonication enhancements could be induced in polymers deemed to be economically favorable for large scale environmental applications.

Table 3-2: Initial rates of release for three additional polymers examined, under the presence (labelled Ultra) and absence of sonication (labelled Control) [(mg/L min)]

PAHs	Hytrel Polymer		Kraton Polymer		Tires	
	Control	***Ultra***	***Control***	***Ultra***	***Control***	***Ultra***
Phenanthrene	4.157±0.018	4.601±0.017	4.545±0.019	5.414±0.020	1.164±0.005	1.169±0.006
Fluoranthene	N/A	N/A	4.341±0.013	5.597±0.018	N/A	N/A
Pyrene	4.199±0.009	4.767±0.015	4.886±0.010	6.261±0.0020	1.210±0.003	1.746±0.006
BaP	N/A	N/A	4.147±0.007	5.286±0.010	N/A	N/A

3.5.4 Equilibrium Shifts

To demonstrate the true "on-line" and "on-demand" effects induced by sonication, release extents were examined as a function of the incidence of irradiation. A Desmopan sample containing phenanthrene and pyrene was allowed to equilibrate in methanol for 24 hours and the final equilibrium position was monitored for 30 minutes (corresponding to the initial 30 minutes in Figure 3-4), followed by the initiation of sonication. From Figure 3-4 at least a 5 minute lag period was required to induce a shift in equilibrium for both phenanthrene and pyrene. This was clear as no changes in release percentage were detected from 30 to 35 minutes. The triplicate measurements demonstrated that shifts were outside the range of error in analytic detection. It is possible that the observed lag period in the first sonication cycle was required for the polymer to achieve a new conformational state necessary for the equilibrium shift. It could, therefore, be proposed that such a time of exposure was necessary for sufficient energy to be introduced in the system allowing for such a change to occur. After 30 minutes of exposure the ultrasound was switched off and the equilibrium position returned to original levels. A second irradiation cycle was then commenced at 90 minutes, however, no lag period was observed. By definition, sonic waves induce cyclic contraction and expansion in materials (Sprawls 1987) and resemble sequential shape memory tests used for characterizing polymer hysteresis. It therefore seems possible that sonic strain may have induced breakage of attractions within the polymer matrix, during the first period of exposure, which did not regenerate during subsequent control periods and resulted in the disappearance of the lag phases discussed. This effect is polymer dependent; however polyurethane resins have been studied and demonstrated by Kim et al. (2000) as well as Gorce et al. (1993) to display hysteresis at a wide range of temperatures.

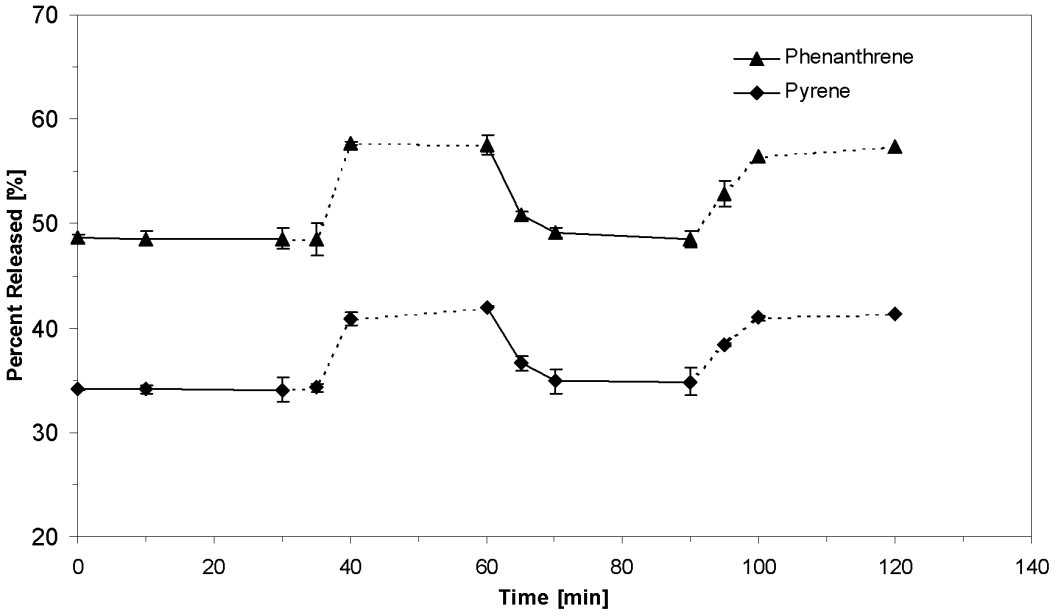

Figure 3-4: Equilibrium concentration of phenanthrene and pyrene, between Desmopan polymer pellets and methanol, in the presence and absence of sonication. Solid and dashed lines represent data obtained for non-sonicated (control) and sonicated periods respectively. Triplicate measurements taken at each time point were used as a standard deviation.

Furthermore, it is important to note that irrespective of the presence or absence of ultrasonic exposure, equilibrium positions were achieved. Since positions were maintained for the duration of sonication, effects did not degenerate or lose effectiveness over time. Original equilibria were also re-established after sonic deactivation, demonstrating the true "on-off" nature of the effect. Similar "on time" observations for rates have been made by Kost et al. (1988). Effects were found to be reproducible and independent of permeant molecule, as shown for phenanthrene and pyrene in Figure 3-4. Equivalent experiments were also conducted using Kraton® D4150K, Hytrel® 8206 and recycled tires and shifts were observed in all systems (results can be found in Appendix A).

3.5.5 Ultrasonic Effects on Consortium Growth Supported on Glucose

It was also important to examine the effects of sonic exposure on microbial viability. As a first measure, optical density readings for growth on glucose were taken in the presence and absence of sonication. As shown in Figure 3-5, rates of OD increased in both the control and continuously sonicated consortia and were identical for the initial 90 minutes, suggesting that induced microbial inactivation was not evident over such a time period. It is possible that glucose, a readily utilizable substrate, brought about large rates of cell growth and made it difficult to differentiate effects arising from ultrasonic inactivation. Therefore, effects on cell viability were indiscernible for 90 minutes of continuous exposure. However, further irradiation began to decrease OD readings until a plateau was reached and sustained up to 280 minutes when the ultrasound was deactivated. Cell numbers did not further increase until sonication was disabled. It could be hypothesized that rates of cell growth were offset by ultrasonic inactivation.

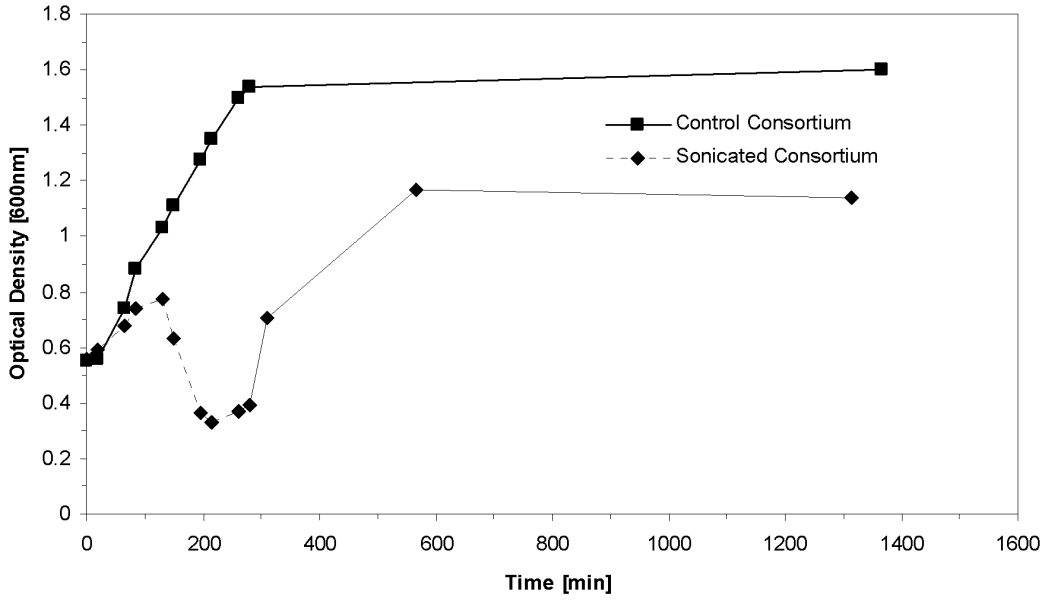

Figure 3-5: Optical density values for growth on glucose under presence and absence of continuous sonication. Solid and dashed lines represent non-sonicated and sonicated periods respectively.

A secondary aspect investigated was the extent of the cell damage and consortium ability for re-growth following irradiation. After 280 minutes, sonication was terminated and optical density changes were measured for an additional 1200 minutes. From Figure 3-5, it is apparent that after 280 minutes, OD began to increase demonstrating non-permanent cell damage and efficient consortium re-growth following irradiation. It would be interesting to investigate if the cell population was equivalent following sonic exposure; however, this is unlikely, as Tiehm (2001) has detailed that microbial properties, such as cell size, shape, cell-wall composition and physiological state, make certain cell lines more resistant to sonication damage. Overall results shown in Figure 3-5, demonstrated that continuous sonication effects for up to 100 minutes induced only temporary cell damage to the consortium. It therefore appears feasible to balance sonically enhanced mass transport and inactivation effects.

3.5.6 Ultrasonic Effects on Consortium Growth on Phenanthrene Delivered from Silicone Oil

A subsequent experiment examined growth on PAHs, delivered from silicone oil, in the presence and absence of sonication. The purpose of this experiment was to characterize effects of sonication on more difficult to degrade aromatics, and phenanthrene was utilized as a model PAH, as it was not subject to mass transport limitations, as shown in Figure 3-2, and discussed by Köhler et al. (1994) under conditions of intensive agitation in two-liquid phase systems. It was therefore expected that for the mixing conditions utilized (600rpm) accurate depictions of nearly biologically controlled degradations resulted. Furthermore, such profiles would provide a benchmark for solid-liquid systems. Sonication effects on degradation are displayed in Figure 3-6. In contrast to the glucose experiments, exposures were cyclic and were not initiated for the sonic consortium until after 5 hours of controlled growth. Note that the hydrophobic nature of the population induced cell growth at the silicone oil-water interface and it was difficult to obtain representative samples for OD measurements. Therefore, in order to provide equivalent biological

start-up conditions, a stringent inoculum procedure was utilized for the control and sonicated cases and the 5 hours of unexposed initial growth, allowed in the case of the ultrasonicated culture, provided an indication of microbial consistency with respect to the control case. The first sonication cycle was initiated after 5.5 hours, lasted 25 minutes and was followed by 3 hours of non-sonicated mixing, a cycle adapted from Wood et al. (1997). From Figure 3-6, it is inconclusive whether sonication enhanced rates of phenanthrene degradation after the first cycle (finishing at 9 hours). The rates of PAH disappearance for the sonicated culture, between times 0 and 5 hours, were within error of being equivalent to those between times 5.5 and 9 hours (post first cycle). The second sonication cycle started at 9 hours, and from Figure 3-6 it is clear that rates of phenanthrene disappearance were subsequently altered, seen as a change in the slope of the line of sonicated degradation after 9 hours. Unlike growth on glucose, it appears as though the period of re-growth provided (3 hours), after a couple of sonic cycles, was insufficient to recover the metabolic activity necessary to maintain initial rates of phenanthrene consumption. It is likely that inactivation effects could not be offset due to the slow growth rates resulting from the metabolism of this more difficult to degrade aromatic. After the 3rd, 4th and 5th cycles at times 12.5, 16 and 19.5 hours respectively, it was clear that a new rate of degradation was established. This could be the result of a new cell number equilibrium, arising from an increased presence of more resilient consortium members capable of offsetting rates of inactivation and re-growth based on advantageous cellular properties, as detailed by Tiehm (2001). A DGGE analysis as a function of sonication cycles would be of interest to address such a point.

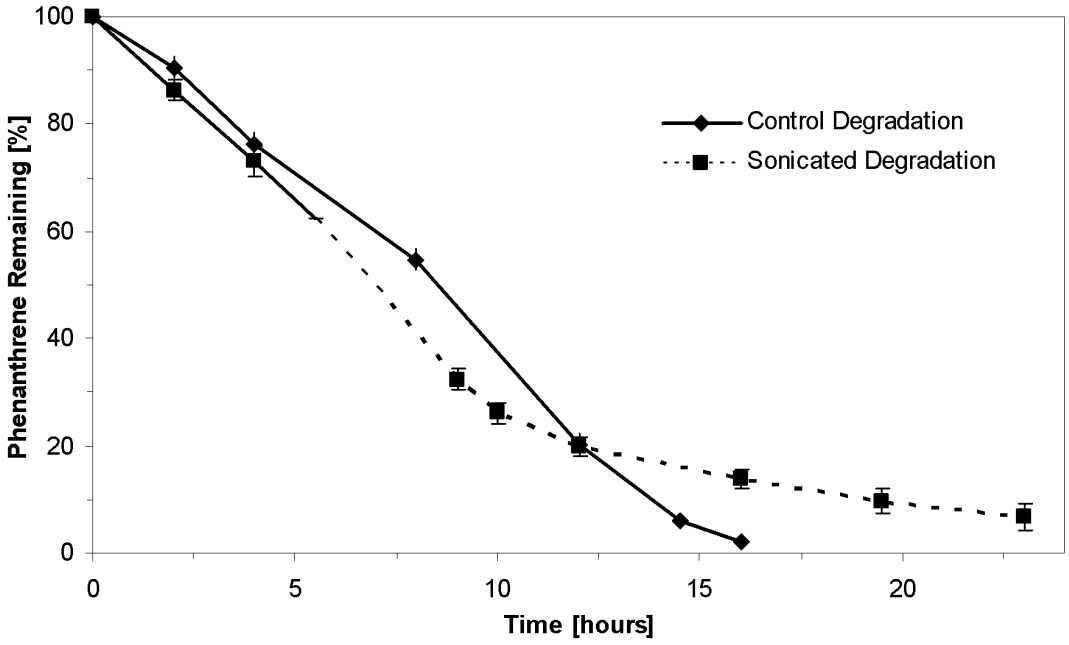

Figure 3-6: Phenanthrene degradation delivered from silicone oil in the presence and absence of sonication. Solid and dashed lines represent non-sonicated and sonicated periods respectively. Note that sonication was applied cyclically (25 min on followed by 3 hours off) and began after 5.5 hours as adapted from Wood et al. (1997). Triplicate measurements taken at each time point were used as a standard deviation. Both sonicated and control cultures were mixed (600rpm) at all times except if/when being ultrasonically irradiated.

3.5.7 Ultrasonic Effects on Phenanthrene Growth Delivered from Solid Desmopan Polymer Beads

In a final experiment, the effect of sonication on phenanthrene degradation delivered from Desmopan was examined. Analogous to the two-liquid phase experiment, cycles of sonication of 25 minutes on and 3 hours off were applied. From Figure 3-7, it can be seen that both abiotic degradations examined did not show disappearance of phenanthrene. Furthermore, such a lack of disappearance, in sonicated controls, demonstrated that irradiation did not induce molecular changes, also seen by Kost et al. (1988) for ultrasonically delivered insulin, a structurally sensitive protein. It is also clear that sonication enhanced transport and degradation rates of phenanthrene. It could be speculated that sonication addressed both internal and external

transport resistances; however, a full transport analysis would be required to validate such effects. The time required to degrade 70% of the phenanthrene was decreased approximately 4 fold, since 144 hours were required to reach the 30% remaining mark in the control degradation, compared to approximately 36 hours in the sonicated case. It is therefore clear that irrespective of whether transport was internally or externally controlled, sonication decreased the limiting resistance and accelerated rates of phenanthrene delivery. Interestingly both sonicated and control cultures seemed to plateau at approximately 30% substrate remaining. This was likely due to an experimentally induced mass transfer limitation. Although sonication likely enhanced parameters such as diffusivity and/or external resistances, transport of phenanthrene into the medium was still a function of a concentration driving force. Therefore, as the concentration in the polymer decreased, so did the gradient driving phenanthrene into the aqueous phase. The 30% remaining mark may be the critical concentration at which point the driving force is insufficient to support biological activity. It is also possible that at such a concentration, the partitioned phenanthrene did not support metabolic activity. Such a condition is likely since partitioning coefficients of PAHs between Desmopan and water heavily favor the polymer side as demonstrated by Rehmann et al. (2008).

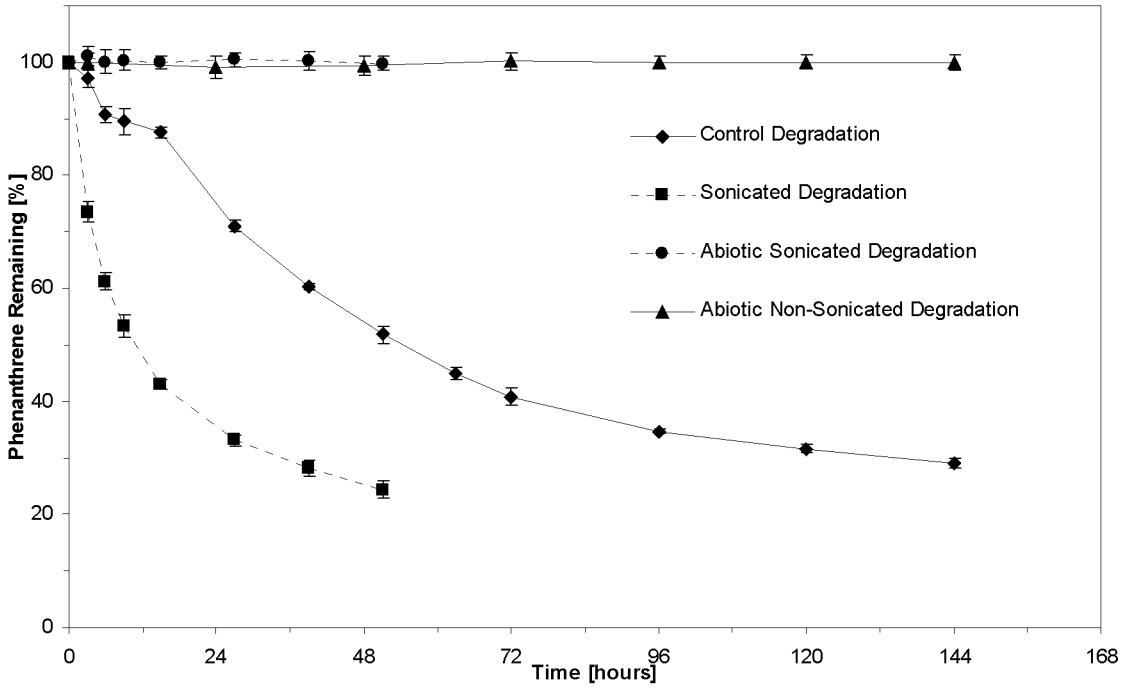

Figure 3-7: Biotic and abiotic phenanthrene degradation delivered from Desmopan in the presence and absence of sonication. Solid and dashed lines represent non-sonicated and sonicated periods respectively. Sonication was applied cyclically (25 min on followed by 3 hours off) as adapted from Wood et al. (1997). All sonicated and control cultures were mixed (600rpm) at all times except if/when being ultrasonically irradiated.

It is also important to note that inactivation effects arising from sonication in the liquid-liquid system (using identical sonication cycles), discussed for Figure 3-6, were not apparent in the solid-liquid set-up. Degradation profiles for both sonicated and control cases, seen in Figure 3-7, seemed to be the same, suggesting no sudden shifts in microbial activity after numerous sonication cycles. It is likely that phenanthrene delivery was exceedingly slow, even under the presence of sonication, and transport limitations masked inactivation effects. It also seems plausible that the polymers acted as a sonic barrier providing protection for degrading microbes and reduced inactivation effects. Sonic wave energetics are known to be medium dependent, and transfers through composites, such as those present (water/polymer/water) here, would induce significant attenuation effects. This is the process by which sonic energy is lost as a wave pulse

moves through matter (Sprawls 1987) and is a strong function of the transport material. Strong attenuation effects have been clearly demonstrated for composite systems (water/bone/water) relative to homogenous liquid media by Hosokawa and Otani (1997). Although the system examined here has a different attenuation potential, due to property differences between bone and polymers, sonic energy losses in solid matter are still significantly larger than those of liquid media. As such, similar energy reductive effects to those reported by Hosokawa and Otani (1997) would be expected in the solid-liquid system presented here. It can be proposed that the constant changes in material of transport as well as absorption of energy by matter may have reduced sonic energy in certain parts of the medium. Thus, it can be hypothesized that much of the energy was absorbed by the polymers, resulting in improved transport, while providing aqueous zones with diminished inactivation effects. As a final note, preliminary calculations of power requirements for reactor mechanical stirring in the presence and absence of sonication demonstrated potential for reduced overall energy requirements, for ultrasonic cases, by virtue of reduced degradation time periods.

3.6 Conclusion

In the current study, a microbial consortium capable of degrading phenanthrene, fluoranthene, pyrene and BaP was enriched using a two phase partitioning bioreactor. Additionally, the possibility of using ultrasound to enhance transport of PAHs from polyurethane matrices was examined. Rates were found to improve by approximately 5 fold relative to unmixed control cases when sonication was applied. This effect was also investigated and observed in Kraton® D4150K and Hytrel® 8206 polymers as well as recycled tires demonstrating its wide ranging application. Enhancements were also shown as shifts in thermodynamic positions, agreeing with current sonochemistry knowledge and attributed to the presence of cavitation. Effects of sonication on consortium growth were also examined with glucose and phenanthrene as carbon sources. It was found that cell damage was non-permanent, and efficient

consortium re-growth following irradiation could be achieved. Finally, the lumped effect of ultrasound on degradation of phenanthrene delivered from Desmopan (polyurethane) was examined. As a crude estimate, rates were increased by a factor of 4 demonstrating the possibility of using sonication as a means to improve mass transport in solid-liquid systems. Cellular inactivation effects were not observed and this was attributed to the increased attenuation of sonic waves by polymer solids present in the medium. Overall, it was shown that ultrasonic irradiation could help overcome the mass transport limitation currently limiting certain solid-liquid two phase degradations. A full mass transport analysis is currently being undertaken to isolate and quantify sonication effects on transfer of PAHs. Additionally, work is underway for evaluating enhanced PAH delivery in a bench scale solid-liquid bioreactor by means of an ultrasonic probe.

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Chapter 4

Mass Transport and Thermodynamic Analysis of PAHs in Partitioning Systems in the Presence and Absence of Ultrasonication

Pedro A. Isaza, Andrew J. Daugulis and Kunal Karan

With minor editorial changes to fulfill formatting requirements, this chapter is substantially as it has been submitted to: *AIChE Journal* (2009)

4.1 Preface

The work in Chapter 3 provided motivation for conducting a full transport analysis which would allow for sonication effects to be characterized in terms of delivery and thermodynamics. Additionally results presented in Chapter 3, showed that sonication induced a significant enhancement on the rate of PAH delivery. However, effects of exposure could not differentiate whether transport was being improved internally or externally and therefore further research was necessary. Furthermore, previous results also demonstrated that thermodynamic positions were shifted in the direction of transport (ie. into the liquid phase) under sonic exposure which further aided in the delivery of PAHs. However, shifts and trends could not be conclusively ascertained due to lack of data. As a final side note, although Rehmann et al. (2008) suggested a trend between polymer-aqueous and octanol-water (see Table 2-1) partitioning coefficients, inconsistencies in such a correlation still exist to date.

The current chapter allowed for PAH deliveries from polymers to be analyzed. Specifically ultrasonic effects on internal and external resistances were quantified along with shifts in thermodynamic equilibrium positions. Finally, the study attempted to re-examine correlations present for estimating polymer-water partitioning coefficients and expand knowledge of solid-liquid two phase thermodynamics in order to provide better coefficient estimations.

4.2 Abstract

Transport of PAHs from Desmopan polymers to methanol under various mixing conditions and in the presence of ultrasound was analyzed. PAH transport was influenced by external transport resistances; however, agitation greater than 800 rpm yielded PAH transport completely limited by internal resistances. Delivery rates of phenanthrene, fluoranthene, and pyrene with ultrasonication were faster than that under any mixing condition, suggesting enhanced internal transport properties. Ultrasound also induced increased concentrations of PAHs in solution at equilibrium. A model developed described PAH delivery under sonicated/non-

sonicated conditions, while quantifying diffusive and thermodynamic properties. Diffusivities with and without ultrasound decreased with permeant molecular size agreeing with coefficients determined for similar aromatic compounds in polymers. Partitioning coefficients under sonicated and non-sonicated conditions conclusively differed from each other and decreased as a function of PAH molecular size. Quantitative structure-property relationship data of PAHs yielded factors predicting thermodynamic and transport behaviors, with polarizability being the best descriptor.

Key Words: Ultrasound, Mass Transfer, Equilibrium, Solid-Liquid Partitioning Systems, PAHs

4.3 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are comprised of two or more benzene rings fused in a linear, angular or cluster arrangement and are naturally present at low levels in the environment. Industrial processes have resulted in an increased presence through the burning of gas, oil, coal, wood, garbage and other organic substances (Juhasz and Naidu 2000). The need to remove PAHs from the environment is driven by their carcinogenic nature which imposes a human health risk in populated areas. Studies have shown that although lower molecular weight PAHs are not acutely toxic, increasing molecular sizes correlate well with carcinogenicity, environmental persistence, and chronic toxicity (Cerniglia 1992; Cerniglia 1997; Juhasz and Naidu 2000). Currently, 16 PAHs are present in the US Environmental Protection Agency priority pollutant list (Cerniglia 1997).

The removal and destruction of PAHs has been studied through numerous technologies (Antizar-Ladislao, et al. 2006; Denys, et al. 2006; Kulik, et al. 2006; Onwudili and Williams 2006). Two phase partitioning bioreactors (TPPBs) are a technology platform recently explored for biological degradation of PAHs by Birman and Alexander (1996), Bouchez et al. (1995) Janikowski et al. (2002), Muñoz et al. (2003), Rehmann et al. (2008), Vandermeer and Daugulis (2007) and Villemur et al. (2000), as well as the study presented in Chapter 3. In TPPB setups, an aqueous phase containing degrading micro-organisms co-exists with an immiscible second phase acting as a reservoir for high concentrations of hydrophobic pollutants (Daugulis 2001). Low levels of contaminants are then transported and partitioned into the aqueous phase, where microbes degrade these toxic substrates. TPPB fundamental principles of operation are governed by mass transport, thermodynamic and biological considerations.

A recent study demonstrated that the sequestering phase of TPPBs was not restricted to immiscible organic solvents but could consist of solid polymeric compounds (Amsden, et al. 2003). This provided an opportunity for removal of hydrophobic contaminants directly from soil,

water and air, as polymers have the ability to sorb organic compounds without inducing further environmental contamination. Once loaded, the polymers could then be introduced into a TPPB where pollutants would be microbially degraded. Such a remediation strategy was proposed and demonstrated for treating PAH contaminated soil (Rehmann, et al. 2008).

An important aspect of these solid-liquid bioreactors is inter-phase mass transfer which has been demonstrated to restrict rates of degradation in these novel reactors for poorly water soluble substrates (Rehmann, et al. 2008). As noted, microbial uptake in TPPBs requires transport and partitioning of organic substrates into the aqueous phase prior to degradation (Déziel, et al. 1999). Since polymer-aqueous systems introduce fixed interfacial areas for transport, it is not surprising that they may be subject to delivery limitations. Such constraints have been demonstrated for biphenyl as well as for PAHs (Rehmann and Daugulis 2007; Rehmann, et al. 2008).

Potential solutions are present in the field of responsive drug delivery which uses a variety of external stimuli to improve transport within and from polymeric matrices. These include ultrasonication as well as magnetic and electrical irradiation (Kost and Langer 2001). Sonication presents an interesting option as it has been shown to improve delivery of compounds having both lipophilic and hydrophilic properties (Kost, et al. 1988; Levy, et al. 1989; Miyazaki, et al. 1985; Miyazaki, et al. 1988). The possibility of using ultrasound for improved PAH transport in polymer-liquid systems was examined in Chapter 3 and results showed significant enhancements in substrate delivery and degradation. Although previous results and conclusions (Breitbach and Bathen 2001; Ji, et al. 2006; Kost, et al. 1989; Li, et al. 2002) suggest that sonication enhances internal and external delivery, as well as partitioning coefficients, results presented in Chapter 3 cannot be adequately interpreted to identify specific effects in two phase systems. A mass transport analysis is therefore required to provide such insight.

The objective of the present study was to examine the transport of PAHs from a polymer to a surrounding liquid phase (methanol) in the presence and absence of ultrasonication. To

quantify the influence of ultrasonication on mass transport, a mathematical model was developed accounting for the inter- and intra-phase mass transfers and the interfacial chemical equilibrium of PAHs between the polymer and liquid phase. The model equation was solved numerically, and the unknown transport and thermodynamic parameters were obtained by minimization of least squares differences between model calculations and experimental data. Finally, predictive knowledge in solid-liquid partitioning systems was expanded using collected transport and thermodynamic properties.

4.4 Model Development

4.4.1 Thermo-physical Processes Considered

The model developed considered two separate phases – a polymer phase and a surrounding liquid methanol phase. Methanol was the chosen solvent based on its increased solubility for the target PAHs relative to aqueous media. Initially, PAHs were considered to be present in spherical polymer beads. Upon exposure of the beads to methanol, transport of PAHs from the polymeric phase to the methanol phase occurred until chemical equilibrium was established. The transport of PAHs within the polymer was described by diffusion while transport into the bulk liquid phase, considered to be well-mixed, was described in terms of classical interfacial mass transfer coefficients. PAH concentrations in the bulk liquid phase were considered to change with time and were obtained from a PAH mass balance. Concentrations of PAHs in the polymer phase varied both spatially and temporally and the pertinent partial differential equation was solved subject to appropriate initial and boundary conditions.

4.4.2 Model Assumptions

The following assumptions were made during model development:

1. Fickian diffusion appropriately described transport within the polymer (dilute solution assumption). This was a reasonable approximation since the system temperature, at which transport occurred, was higher than the polymer glass transition temperature and thereby

the rates of polymer chain relaxation could be assumed to be much faster than permeant movement, allowing for delivery to progress in a Fickian manner (Harogoppad and Aminabhavi 1991).

2. The sole mechanism of transport within the polymer matrix was diffusion.
3. The system considered had constant thermophysical properties. That is, the diffusivity was independent of concentration, direction (isotropic) and presence of other PAH species.
4. Spatial distribution of PAHs was symmetric and initially uniform across the polymer.
5. Partitioning of PAHs between the polymer and methanol phases could be described by Henry's Law-type relationships. Dilute conditions allowed solid–liquid equilibria to maintain constant proportionalities.
6. There was negligible change in polymer volume due to PAH transport or polymer-solvent interactions. The polarity differences between methanol (relatively polar) and the polyurethane matrix (hydrophobic) discouraged solvent penetration and thus volume changes.
7. The rate of PAH partitioning was faster than Fickian transport and assumed to be spontaneous under conditions examined (Cesário, et al. 1997).
8. Constant temperature was maintained throughout the transport process.
9. Polymers pellets were approximately spherical and PAH transport was unidirectional (ie. radial).

4.4.3 Mass Transport Equation and Boundary Conditions

The mathematical equation governing Fickian transport in the polymer phase was given by the following equation in one-dimensional spherical coordinates:

$$\frac{1}{D}\frac{\partial C}{\partial t} = \frac{\partial^2 C}{\partial r^2} + \frac{2}{r}\frac{\partial C}{\partial r} \qquad (1)$$

where, C was the permeant concentration in the polymer phase, and D was the diffusive coefficient of the permeant in the polymer matrix.

The initial condition for polymer phase concentration was given by:

$$\text{At } t = 0; \qquad C(r,0) = C_o \tag{2}$$

Boundary conditions are described below via equations (3) and (4). Symmetry boundary condition (equation 3) was applied at the centre of the spherical beads. At the polymer bead surface, the mass flux out of the polymer sphere was coupled to the mass flux into the liquid phase (equation 4). The liquid phase flux was described by the product of the liquid mass transfer coefficient and the difference in PAH concentrations at the polymer-liquid interface and in the well-mixed, bulk liquid phase.

$$\text{At } r = 0; \qquad \frac{\partial C(0,t)}{\partial r} = 0 \tag{3}$$

$$\text{At } r = r_p; \qquad -D\frac{\partial C(r_p,t)}{\partial r} = k_l(C_l - C_b) \tag{4}$$

where, C_o was the initial concentration of solutes of interest in the polymer matrix, k_l was the convective mass transfer coefficient in the solvent (adjacent to the polymer surface, where the polymer radius is r_p), C_l was the concentration in the solvent at equilibrium with that at the polymer surface, and C_b was the solute concentration in the bulk liquid phase. By definition $K_{s/l} = C_l / C_s$, where $K_{s/l}$ described the partitioning or equilibrium behavior of solutes at the polymer-liquid interface and C_s was the concentration of solute at the polymer surface. Additionally, note that k_l was imperative for model completeness and parameter estimation purposes in further research.

4.4.4 Liquid Phase Concentration

Liquid concentrations were determined from the following material balance.

$$C_b(t) = \frac{\left[V_p C_o - \left(\int_0^{Vp} C(t) \cdot dV\right)\right] n_p}{V_l} \tag{5}$$

where V_p was the volume of a single polymer pellet, n_p the number of polymer bead pellets present in the liquid phase, V_l the volume of liquid, and C_b the concentration in the solvent.

4.4.5 Internal and External Mass Transfer Control

The model consisted of three unknown parameters – polymer phase diffusivity (D), polymer-liquid partitioning coefficient ($K_{s/l}$) and liquid-phase convective mass transfer coefficient (k_l). Depending on the material properties and operating conditions, the rate of PAH release may be either dominated by internal mass transport (i.e. low D) or external mass transfer (k_l), or a combination of the two. The external mass transfer coefficient can be manipulated by various methods, including mixing of the liquid phase, e.g. by use of a magnetic mixer. Thus, with increasing mixing, and thereby increasing k_l, the net rate of PAH release from the polymer phase will become increasingly dominated by the internal mass transport resistance. If the external mass transport becomes sufficiently high, the internal mass transport will be the rate controlling step in the overall PAH release. It is important to indicate that for such a situation, an exact value of the k_l is not required in the mathematical model to capture the temporal behavior of the system/process. Experimentally, the condition of internal mass transfer control could be determined by increasing the mixing characteristics (e.g. rpm) until no changes in the temporal release profiles are observed with further increases in mixing speed.

In the present study, the model was applied for the condition of internal mass transport control, which was experimentally verified by confirming the similarity in the PAH release profile at two high mixing speeds.

4.4.6 Solution Method

The partial differential equation (PDE) subject to initial (equation 2) and boundary conditions (equations 3 and 4) must be solved to obtain the solution. It must be noted that Crank (1979) has provided an analytical solution for similar systems, wherein the surrounding fluid is of finite volume (thus, solute concentrations change with time) resulting in time-varying flux and/or concentration at the spherical surface. However, the solution to the equation requires determination of non-zero roots of a non-linear equation, i.e. an iterative solution is required.

In this study, the PDE was discretized both in time and space using an implicit finite difference scheme. A linear algebraic equation for each discretized point was obtained. Thus, for a given time step, a set of linear algebraic equations representing the system of PDE for each discrete point in the computation domain was obtained. The solution to this set of equations was obtained by matrix inversion using MATLAB®.

For the computation, the model domain was divided into 100 grid points. Doubling the number of grid points resulted in less than 0.2% change in the solution. Although the implicit method was inherently stable, a time step of 1 minute ensured accuracy of results (measured via regression) while reducing computational requirements.

4.4.7 Parameters for numerical solution

The model parameters - diffusivities (D) and partitioning coefficients ($K_{s/l}$) - were estimated using the built in MATLAB® least squares regression function, *lsqcurvefit*. This algorithm used an interior-reflective Newton method to determine function parameters which best fit a series of data. Iterations involved the approximate solution to large linear systems using the

method of preconditioned conjugate gradients (PCG), as described in MATLAB®. Additional polymer properties used in model developed can be found in Table 4-1.

Table 4-1: Polymer properties (obtained through measurements and/or manufacturer information)

Approximate Radius per bead, r_p, (cm)	0.181
Number of Polymer Beads, n_p	140
Desmopan 9370A Density (g/L)	1060

4.5 Materials and Methods

4.5.1 Materials

Spectrophotometric grade methanol (95+%) was purchased from Fisher Scientific (Guelph, Canada). Phenanthrene (98+%) and fluoranthene (98%) were purchased from Alfa Aesar (Ward Hill, MA) while pyrene (95%), and benzo[*a*]pyrene (BaP, 99+%, scintillation grade) were purchased from Sigma-Aldrich (Oakville, Canada). Desmopan 9370A (polyurethane elastomer beads) was graciously donated by Bayer Material Science (Leverkusen, Germany).

4.5.2 Analytical procedures

Analytical methods were adapted from those described in Chapter 3. PAH concentrations in methanol were quantified via fluorescence spectroscopy using a QuantaMaster QM- 2000-6 fluorescence spectrometer (Photon Technology International, London, Canada). All samples were diluted in methanol to within the linear range of detection (0-0.1mg/L) (Rehmann, et al. 2008).

4.5.3 PAH Loading in Polymer Beads

Fifteen grams of Desmopan beads were equilibrated with a stock methanol solution containing all PAHs, for 24 hours at 20^{o}C, to achieve loadings of approximately 1.6, 1.6, 1.8 and 1mg/g of phenanthrene, fluoranthene, pyrene and benzo[*a*]pyrene, respectively. Uptake of PAHs

was determined through the mass balance detailed in Chapter 3. After uptake, methanol was decanted, and polymer beads were washed with water for 3 minutes and allowed to air dry for 24 hours to volatilize residual solvent (Rehmann, et al. 2008). Loaded polymer beads were then divided equally into 5 (ie. 3 grams of polymer each) vials and stored in the dark until further use.

4.5.4 PAH Release Tests

Four different release conditions were examined to address the nature of PAH transport. 15 mL of fresh methanol were introduced into the scintillation vials containing the loaded polymers, and concentrations were monitored as a function of time. At most, approximately 400 minutes were required to reach equilibrium under all conditions, and duplicate samples were obtained to address variability. Release conditions examined were natural convection (no external mixing control), 800 and 1000 rpm mixing, and sonication without additional mixing. Release tests were all carried out at 20^{o}C and mixed conditions were achieved as detailed below.

Stirred release conditions were achieved using a Fisher Scientific Thermolyne Cimarec 3 stir plate. A Fisher Scientific ultrasonic bath (model FS20) with an output frequency and intensity of 42 kHz and 70 W was used for sonication experiments. The sample vial (containing the loaded polymer beads and methanol) was placed in the bath and continuous sonication was triggered while PAH concentrations, in methanol, were monitored with time. Temperature was maintained at 20^{o}C via a recirculation loop detailed in Figure 3-1.

4.5.5 PAH partitioning coefficients from Desmopan 9370A and Methanol

Partitioning coefficient procedures were equivalent to those described in Chapter 3. Equilibrium concentrations of PAHs in methanol were determined via spectrophotometry and polymer uptakes were calculated via a mass balance. Partitioning coefficients and confidence intervals, for each PAH, were determined through a least squares regression.

4.6 Results and Discussion

4.6.1 Release of PAHs from Desmopan 9370A into Methanol

The concentration of phenanthrene, fluoranthene, pyrene and benzo[*a*]pyrene in the liquid phase (methanol) as a function of time is presented in Figure 4-1 under the different mixing conditions examined. Each plot represents the release profiles of the individual PAHs. It must be recalled that the four data sets for each PAH correspond to three sets in the absence of sonication - no mixing, mixing at 800 rpm, and mixing at 1000 rpm – and one set in the presence of sonication but without any additional or external mixing. The model predictions for the cases of external mixing and sonication are also presented as line plots.

Several noteworthy features can be observed in Figure 4-1. In all tests, an equilibrium-like condition was achieved at longer times. It must also be noted that the release rate was rapid at early times, when the driving force was large, and nearly 80 percent of the equilibrium concentration was attained in approximately 50 minutes. It can also be observed that the release rates for 800 and 1000 rpm mixing conditions were similar and can be considered to be the same. Additionally, for each system (PAH) examined, the three non-sonicated experiments resulted in the same final liquid phase concentration, which provided further confidence that a true equilibrium condition had been attained. One of the most interesting features of the plots was that the equilibrium concentration levels observed under sonication conditions, for all PAHs examined, was higher than those found under non-sonicated conditions.

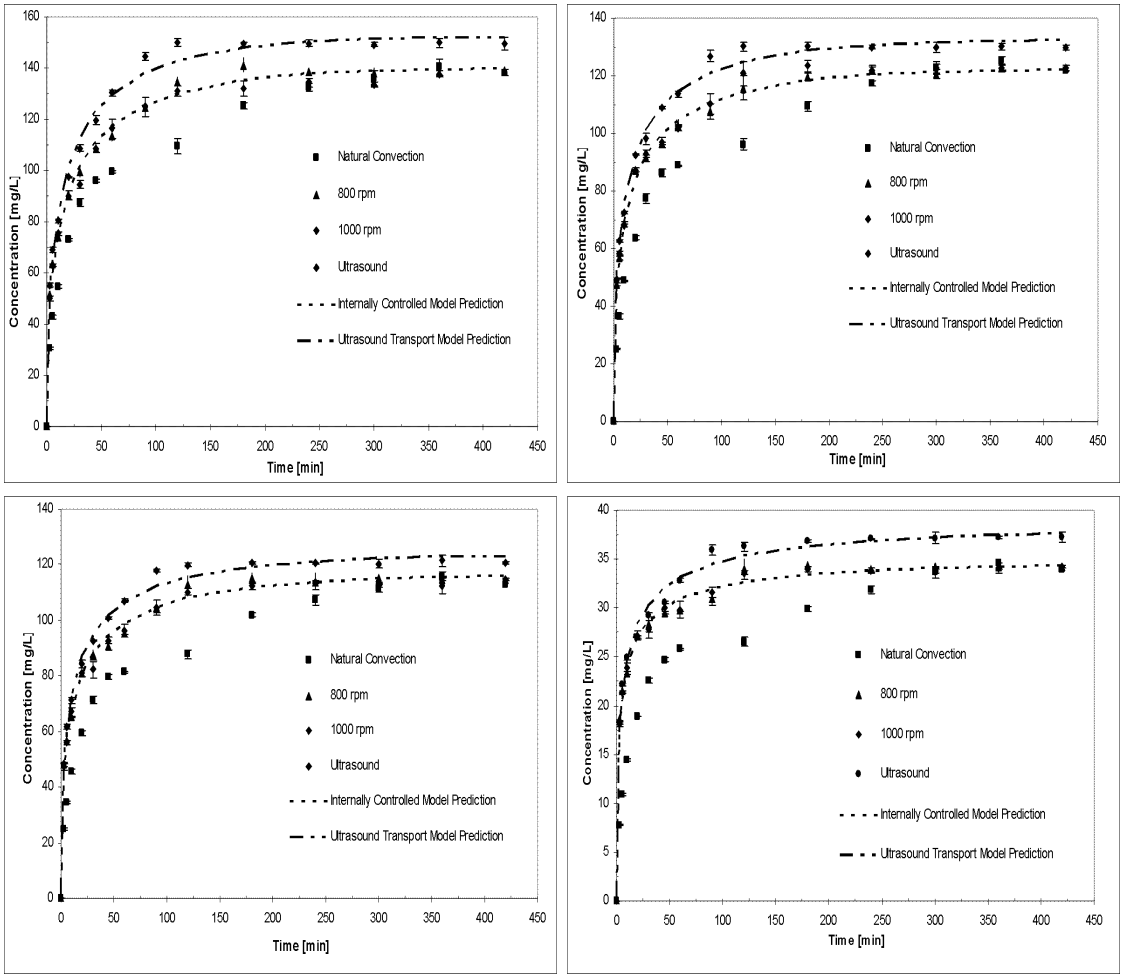

Figure 4-1: Delivery data of phenanthrene (Top Left), fluoranthene (Top Right), pyrene (Bottom Left) and benzo[*a*]pyrene (Bottom Right) under non sonicated (natural convection, 800 and 1000 rpm) and sonicated conditions. Dashed lines represent data obtained for non-sonicated and sonicated model predictions of all PAHs.

4.6.2 PAH Release Under Non-Sonicated Conditions

As discussed above, the PAH release rate for the no mixing (natural convection) condition, displayed for all PAHs in Figure 4-1, was slower than that found under any mixed condition, suggesting that delivery was at least partially externally controlled (at the polymer-methanol boundary layer). This is not surprising as PAHs are hydrophobic aromatics and transport into methanol, the more polar phase relative to Desmopan, would likely be restricted at the polymer-solvent boundary. Also, since the release profiles for 800 rpm and 1000 rpm tests

were essentially the same, it could be concluded that under mixing conditions greater than 800 rpm the transport of PAHs was controlled by the internal mass transport resistance. Thus, such experimental data could be fitted without a need for estimating convective mass transfer coefficients, k_l. As an aside, it could be hypothesized that delivery of PAHs into more polar solvents, such as aqueous media, would also be externally restricted. Therefore, if sonication could yield internally controlled deliveries, significant improvements could result. Such a possibility elucidates a potential reason for the enhancements demonstrated in Chapter 3.

4.6.3 Influence of Sonication on PAH Release Rate and Equilibrium

Data presented in Figure 4-1 also show that the rates of release under sonication, for all PAHs, are at least as great as those obtained for internally controlled conditions (>800 rpm). Therefore, ultrasonic irradiation appeared to remove the external mass transfer resistance. The role of sonication can be understood by recognizing that it produces "microjets," (Breitbach and Bathen 2001) which generate turbulence at the polymer surface and in turn, reduce external resistances to the point where internal mass transport completely dominates PAH release into the surrounding liquid phase. A closer look at Figure 4-1 also shows that rates of release under ultrasonic exposure, for phenanthrene, fluoranthene, and pyrene were slightly faster than those obtained under internally controlled conditions, thus hinting at the possibility of improved diffusive properties. Enhancements of this nature (ie. beyond internally controlled deliveries) would only arise from changes in internal properties and could be attributed to sonic shockwaves generating microscopic turbulence within polymer pores (Ji, et al. 2006). Similar results have been observed for delivery of various drugs from a number of polymers, in which transport increased as a function of agitation to a certain point, after which rates became independent of mixing (Kost, et al. 1989). However, as also seen in this study sonicated deliveries were faster than those observed under any mixing conditions examined, suggesting that sonic improvements were not solely external (Kost, et al. 1989).

Sonication effects on PAH transport were also found to be a function of structural and chemical solute properties. Based on trends displayed in Figure 4-1, enhancements in transport via sonication decreased with increasing PAH molecular weight/volume (see Table 2-1 or 4-2). This was evident as the presence of sonication induced a more apparent enhancement in the rate of phenanthrene delivery followed by fluoranthene, pyrene and BaP. Transport in polymers requires energy for exchange in positions between solutes and polymer chains (Harogoppad and Aminabhavi 1991). Therefore, higher energy requirements for delivery of larger solutes, such as BaP, would lead to slower position exchanges and more restricted transport. Furthermore BaP, being the most hydrophobic PAH, likely had the strongest interactions with Desmopan resulting in reduced internal movement. Finally, based also on hydrophobicity, BaP was likely subject to the most restrictive external resistance due to its delivery into more polar methanol.

Figure 4-1 also established that all non-sonicated release conditions (natural convection, 800 and 1000 rpm) reached the same equilibrium position as dictated by thermodynamics. However, it is apparent that sonication induced higher PAH concentrations in methanol at equilibrium with similar effects reported in previous studies (Breitbach and Bathen 2001; Ji, et al. 2006; Li, et al. 2002). During sonication, bubbles are generated and subsequently collapse, in a process termed cavitation, inducing conditions of several thousand Kelvin and a few hundred bar. Near a solid surface, such as a polymeric matrix, asymmetric bubble collapse forms “microjets” reaching speeds up to 500 m/s, which along with sonic shockwaves can break up of interactions between sorbed molecules and sorbent surfaces (Li, et al. 2002). This condition leads to increased desorption and higher solute concentrations in the solvent, providing one plausible explanation for the phenomenon seen in Figure 4-1. Additionally, ultrasonic waves diffusing through particles or polymers increase the energy of sorbed molecules at balance sites causing them to vibrate more violently and desorb (Ji, et al. 2006), thus providing an additional explanation for the increased endpoint concentrations observed in Figure 4-1.

It could also be speculated that local temperature variations, resulting from bubble collapse near polymer-PAH balance sites, may have induced part of the shifts in thermodynamic position observed. Li et al. (2002) examined and found such thermal effects to be non-dominant and thus incapable of accounting for the totality of the effects observed. Nevertheless, temperature effects induced by sonication at a local level should be regarded as a potential (partial) reason for shifts in thermodynamic positions observed.

4.6.4 Quantification of Diffusive and Equilibrium Properties for Sonicated and Non-Sonicated Systems

The diffusivity and partitioning coefficients of all PAHs were estimated for internally controlled conditions for systems not subjected to sonication (i.e. mixing >800 rpm). Experimental data collected under ultrasonic irradiation were also fitted assuming internally controlled conditions. Table 4-2 summarizes diffusive and thermodynamic parameters of best fit (determined through the minimization of least squares detailed in the materials and methods).

Table 4-2: Transport and thermodynamic properties estimated in the absence and presence of sonication. Note that 95% confidence regions are provided for all parameter estimates.

Phenanthrene (Molecular Weight =178.24 g/mol**)**			
	Non-Sonicated	**Sonicated**	**Percent Change* (%)**
Diffusion Coefficient, *D*, (cm^2/s, $\times 10^{-7}$)	4.115 ± 0.521	5.012 ± 0.674	21.8
Partitioning Coefficient, $K_{s/p}$ (unitless)	0.134 ± 0.005	0.153 ± 0.006	14.0

Fluoranthene (Molecular Weight =202.26 g/mol**)**			
	Non-Sonicated	**Sonicated**	**Percent Change* (%)**
Diffusion Coefficient, *D*, (cm^2/s, $\times 10^{-7}$)	3.747 ± 0.347	4.502 ± 0.563	20.2
Partitioning Coefficient, $K_{s/p}$ (unitless)	0.105 ± 0.002	0.117 ± 0.004	11.4

Pyrene (Molecular Weight =202.26 g/mol**)**			
	Non-Sonicated	**Sonicated**	**Percent Change* (%)**
Diffusion Coefficient, *D*, (cm^2/s, $\times 10^{-7}$)	2.912 ± 0.400	3.471 ± 0.464	19.2
Partitioning Coefficient, $K_{s/p}$ (unitless)	0.081 ± 0.002	0.087 ± 0.003	7.4

BaP (Molecular Weight =252.32 g/mol**)**			
	Non-Sonicated	**Sonicated**	**Percent Change* (%)**
Diffusion Coefficient, *D*, (cm^2/s, $\times 10^{-7}$)	1.979 ± 0.338	1.722 ± 0.306	-14.9
Partitioning Coefficient, $K_{s/p}$ (unitless)	0.036 ± 0.001	0.040± 0.001	11.1

* Percentage change calculated for sonicated case with respect to non-sonicated case.

By examining Table 4-2, it is evident that for the non-sonicated case, diffusion coefficients decreased as a function of PAH molecular size. Such a trend agrees with previous notions that consider the movement within a polymer matrix to be more restricted for solutes of increased volume. Diffusivities decreased from smaller sized phenanthrene, followed by fluoranthene and pyrene, and finally BaP. It is useful to note that fluoranthene and pyrene have equivalent molecular weight and, accordingly, reduction in coefficients is further affected by more subtle property differences. However, it is clear that a trend was present between diffusivities and penetrant sizes. Similar correlations have been shown in a variety of polymers

for similar aromatic compounds (benzene, toluene, p-xylene and mesitylene) with diffusivities ranging in the order of 10^{-7} cm^2/s (Harogoppad and Aminabhavi 1991), which validated the range of parameters estimated in the current study. Additional information by Kumar et al. (1997) also supported the trend of decreased diffusivity as a function of solute size, with findings showing decreasing coefficients for benzene, toluene, and xylene respectively, in poly(ethylene-co-vinyl acetate) (EVA) matrices, ranging in the order of 10^{-7} cm^2/s (Kumar, et al. 1997). In another study, benzene diffusivities in various polyurethane membranes were reported to range between 10^{-5} and 10^{-7} cm^2/s (Ponangi, et al. 2000), comparable to the order of magnitude of diffusivities presented in Table 4-2. It can be hypothesized that correlations between internal transport restrictions and permeant molecular sizes are for the most part matrix independent. Such a pattern agrees with free volume theory (FVT) (Kumar, et al. 1997).

As outlined in FVT, the mobility of polymer segments and diffusion coefficients of permeants are a function of the polymer matrix free volume (Ponangi, et al. 2000). Therefore, larger molecular size solutes require higher free volumes for efficient transport. Conversely, in the case of a matrix with an established free volume, the larger the solute the more restricted its pathway and thus lower diffusion coefficient. Diffusivities determined under the presence of sonication also decreased as a function of penetrant molecular size. It appears that even in the presence of sonication, providing increased energetics, transport of larger solutes was more restricted due to free volume requirements.

From Table 4-2, it is also evident that partitioning coefficients obtained under sonication were significantly larger than those in the absence of irradiation. Increasing coefficients represented augmented desorption or higher concentrations of PAHs in methanol at equilibrium (also seen in Figure 4-1). Sonically induced shifts of this nature (towards increased desorption) have been observed for a number of solute-polymer combinations (Breitbach and Bathen 2001; Ji, et al. 2006; Li, et al. 2002). With the exception of BaP, the percent increase in partitioning coefficient shifts induced via sonication decreased as a function of PAH hydrophobicity

(displayed in Table 4-2). This was not surprising as interactions between non-polar Desmopan (polyurethane) and PAHs, likely increased as a function of permeant hydrophobicity. Since the sonic energy provided was constant, the magnitude or extent of breakage of interactions decreased as function of attraction strength, which for the most part followed hydrophobic solute behaviors.

4.6.5 Direct Determination of Partitioning Coefficient Data from Batch Experiments

Independent equilibrium experiments were conducted without sonication to directly determine partition coefficients. The slope of a plot of equilibrium concentration in the liquid phase versus corresponding equilibrium concentration in the polymer phase yielded the partitioning coefficients shown in Figure 4-2. By comparing parameters estimated by the fit of the model (Table 4-2) with those presented in Figure 4-2, it is evident that the partition coefficients lie within each others' 95% confidence regions. As reported in a previous study, the partition coefficients decreased as a function of PAH hydrophobicity (Rehmann, et al. 2008). This trend was clearly observed in Figure 4-2, as most lipophilic BaP, had the highest concentration in the polymer at equilibrium, relative to methanol. Pyrene, fluoranthene and phenanthrene followed respectively with decreasing hydrophobicity and increasing methanol concentrations (representing higher partitioning coefficients). As an important aside, all partitioning coefficients estimated under sonicated delivery (Table 4-2) were also conclusively outside confidence regions presented in Figure 4-2, providing further evidence that ultrasonic exposure truly shifted equilibrium positions.

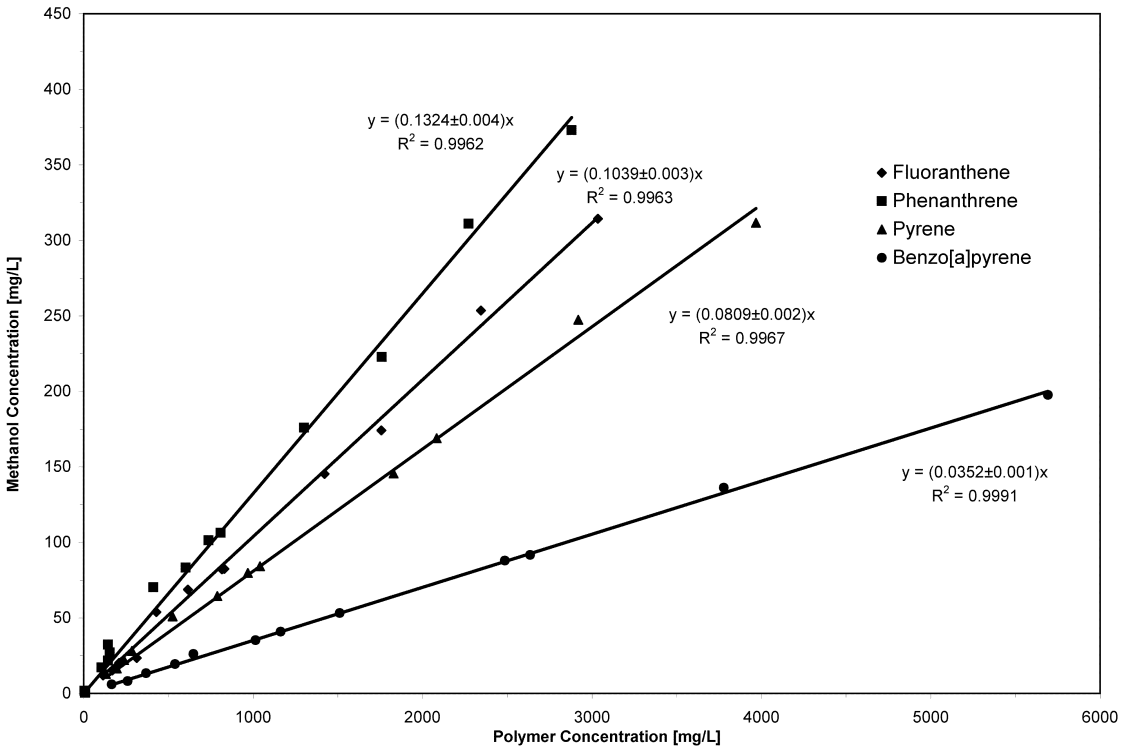

Figure 4-2: Experimentally determined partitioning coefficient in Desmopan 9370A and methanol of all PAHs.

4.6.6 Correlating Diffusion Coefficients and Partitioning Coefficients to Permeant Structural/Chemical Properties

It is intuitive, and has been previously discussed in some detail, that both transport and thermodynamic parameters are a function of permeant structural and chemical properties. Polymer characteristics may also play a role, however, in this study only a single type of matrix was examined (Desmopan). Previous research indicated that partitioning coefficients of PAHs in a Desmopan-aqueous system correlated well with solute hydrophobicity, measured through octanol-water partitioning coefficients (Rehmann, et al. 2008). For the most part, such a trend was correct in which pyrene (log $K_{O/W}$ = 5.18 (Means, et al. 1980)) and fluoranthene (log $K_{O/W}$ = 5.20 (Scheele 1980)), having higher octanol-water coefficients than phenanthrene (log $K_{O/W}$ = 4.46 (Hansch and Fujita 1964)), correlated well with polymer-aqueous thermodynamic parameters (Rehmann, et al. 2008). A similar assessment could be made for the coefficients determined in

this study. However, the relationship presented could not be extended to explain the behavior of pyrene and fluoranthene whose polymer-methanol partitioning coefficients differed, but log $K_{O/W}$ values were approximately equivalent. This demonstrated a limitation in the ability of log $K_{O/W}$ to predict PAH partitioning coefficients in polymer-methanol systems. Figure 4-3 demonstrates such a correlation for both partitioning and diffusive coefficients. Note that for consistency, all log $K_{O/W}$ values utilized were obtained from Alves de Lima Ribeiro and Ferreira (2003), as summarized in Table 2-1.

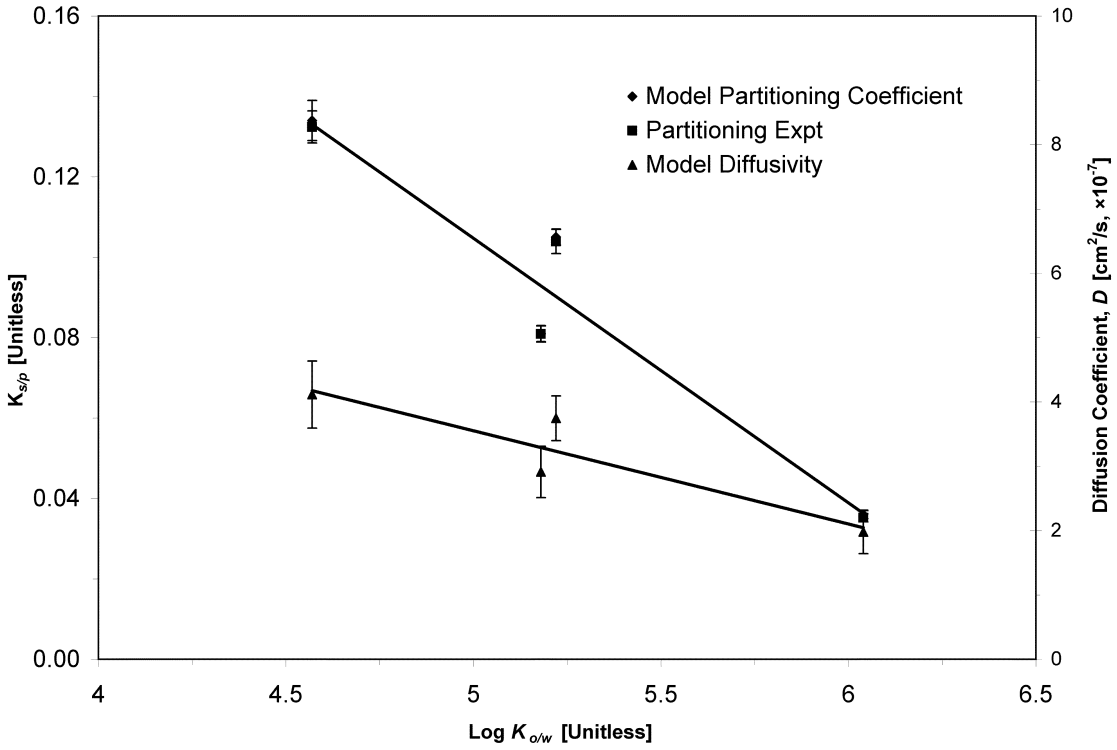

Figure 4-3: Transport and thermodynamic parameters as a function of PAH octanol-water partitioning coefficients.

It is evident from Figure 4-3 that the overall trend presented for log $K_{O/W}$ also correlated well with transport parameters. Since log $K_{O/W}$ is typically proportional to increasing PAH molecular sizes (Alves de Lima Ribeiro and Ferreira 2003), it is not surprising that the diffusion coefficient trend, discussed previously, could be extended to log $K_{O/W}$. Though an overall trend is clear, there is an apparent discontinuity in Figure 4-3 where log $K_{O/W}$ fails to describe differences

in thermodynamic and transport behaviors of pyrene and fluoranthene, which have equivalent molecular weights. This may be due to log $K_{O/W}$'s inability to account for certain chemical and/or attractive effects between penetrants and polymer-methanol environments.

Boiling point (BP) is another material property capturing structural and chemical permeant characteristics of a chemical species, which correlates with thermodynamic and transport parameters. The diffusion coefficient and partitioning coefficients were plotted as a function of the boiling point of the PAHs and the results are presented in Figure 4-4. From the figure, it is evident that diffusivities and partitioning coefficients estimated correlated well with boiling points (phenanthrene - 340^{o}C(Alves de Lima Ribeiro and Ferreira 2003), fluoranthene - 383^{o}C (Alves de Lima Ribeiro and Ferreira 2003), pyrene - 393^{o}C (Alves de Lima Ribeiro and Ferreira 2003) and benzo[*a*]pyrene - 496^{o}C (Alves de Lima Ribeiro and Ferreira 2003)). However, boiling point does not provide an indication as to the independent properties that induce such an accurate correlation. Differences in BP arise from structural and chemical properties and need to be dissected as such to provide further insight.

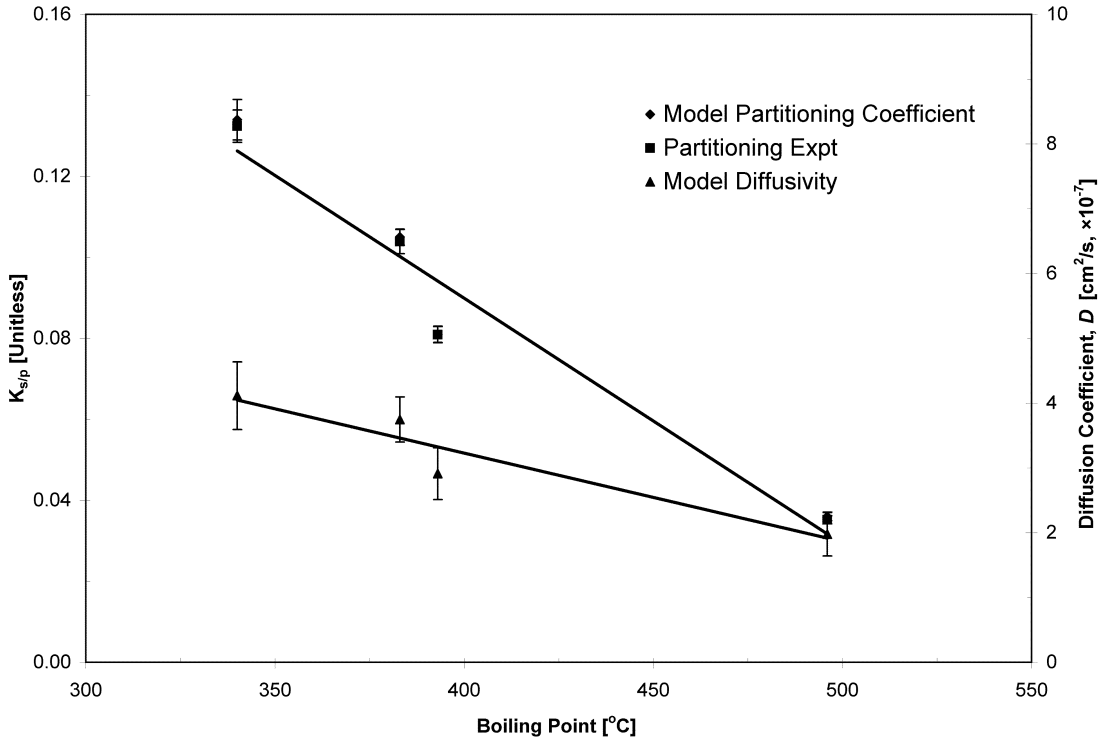

Figure 4-4: Transport and thermodynamic parameters correlated as a function of PAH boiling point.

Statistical studies have examined a series of thermodynamic, electronic, steric, and topological descriptors of PAHs to develop quantitative structure-property relationships (QSPR) with boiling points, log $K_{O/W}$ and others (Alves de Lima Ribeiro and Ferreira 2003). Figure 4-5 demonstrates the correlation between boiling point and molecular volume (MV) (Alves de Lima Ribeiro and Ferreira 2003) of PAHs examined in this study, which displayed the same inconsistency discussed for log $K_{O/W}$. This was due to the fact that MV is strictly a structural property incapable of accounting for chemical and/or attractive effects. The same outcome was observed for the correlation of BP and molecular weight (MW). As a final measure, a correlation between BP and Randic index was examined as discussed in a previous study (Alves de Lima Ribeiro and Ferreira 2003). The Randic connectivity index measures the sum of relative accessibility areas in the penetrant molecule (Estrada 2002). This area represents the total area accessible for molecular interactions with surroundings. However, based on such a definition, it is

evident that the Randic index is again a structural property and, therefore, unable to fully account for chemical/attractive interactions.

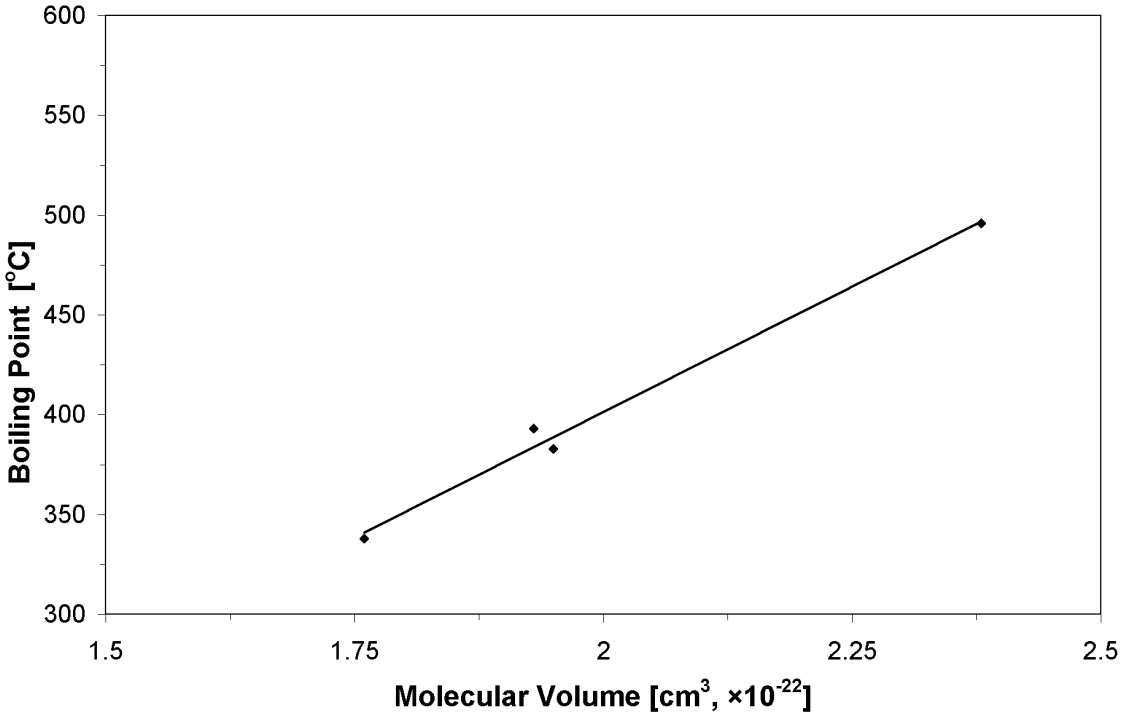

Figure 4-5: Boiling point of PAHs as a function of PAH molecular volume.

A closer examination revealed that polarizability (an additional descriptor reported by Alves de Lima Ribeiro and Ferreira (2003)) provided an accurate correlation for BP. Figure 4-6 displays such a relationship. It is likely that polarizability is one of the properties captured by boiling point, which allowed for its excellent description of transport and thermodynamic parameters of the PAHs examined.

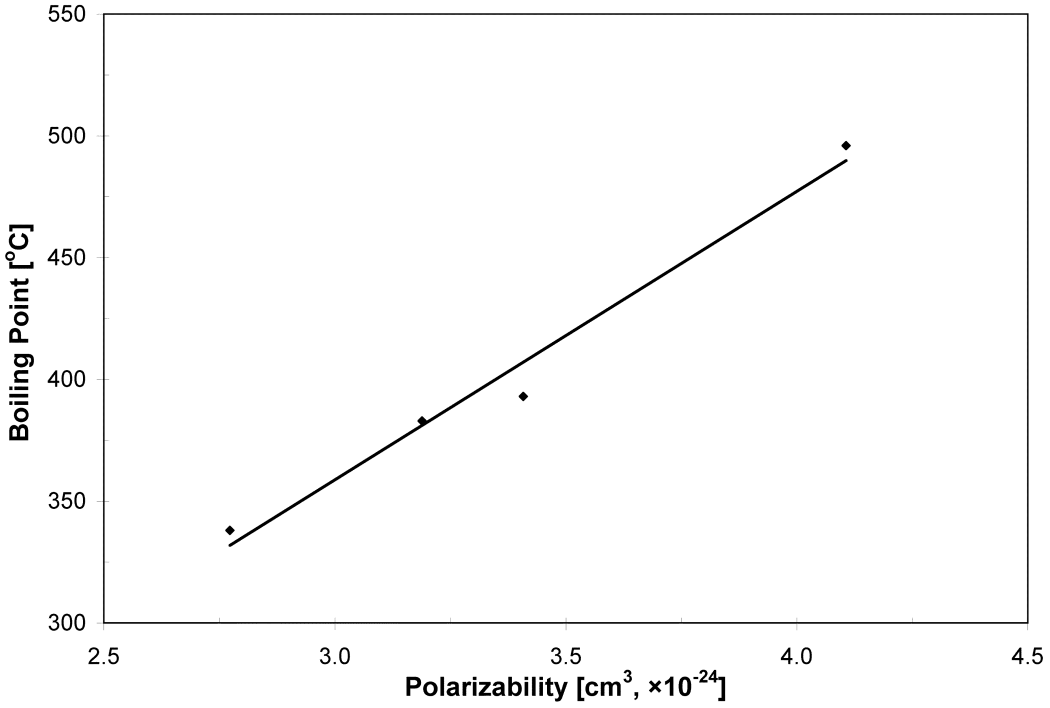

Figure 4-6: Boiling point of PAHs as a function of polarizability (converted to SI units from reported atomic units).

Plotting diffusivities and partitioning coefficients, in the absence of sonication, as a function of polarizability yielded the results shown in Figure 4-7.

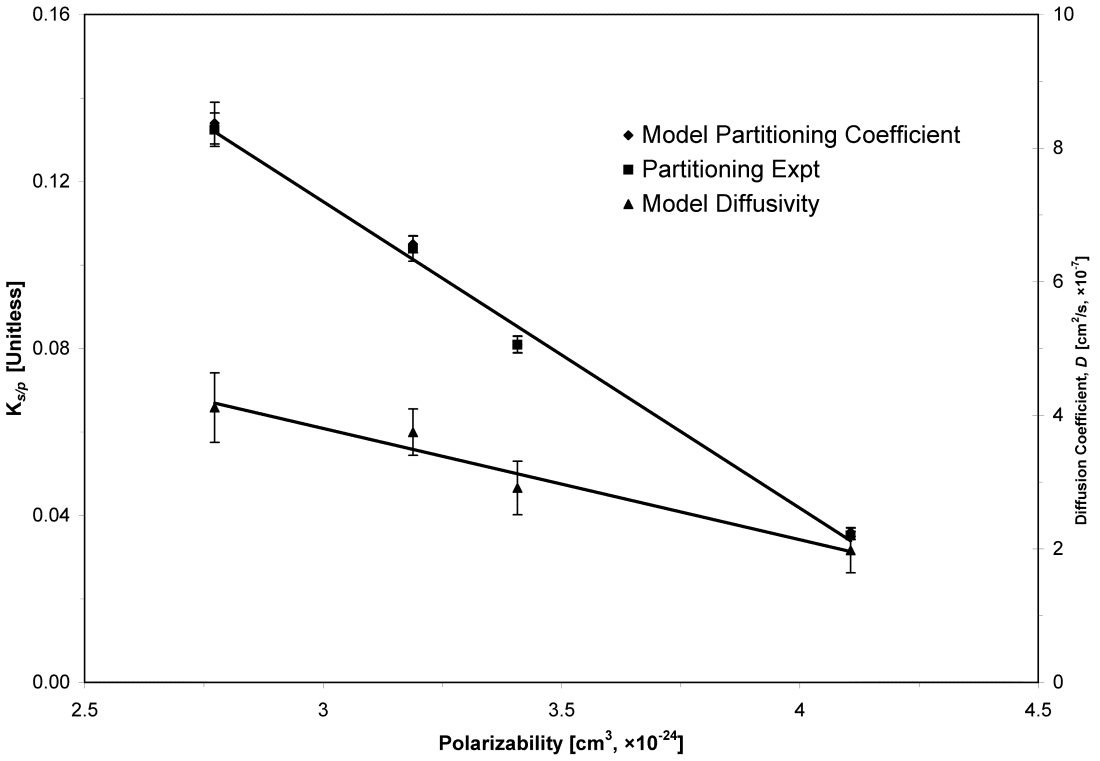

Figure 4-7: Transport and thermodynamic parameters correlated as a function of polarizability. Note that polarizability was converted to SI units from reported atomic units.

Polarizability indicates the ease with which a species can be deformed by an electric field (Alves de Lima Ribeiro and Ferreira 2003). Additionally, one of the most polarizing influences on a particular species is the presence of another species in the nearby surroundings. Therefore, it provides a measure of attraction between PAHs and their polymer-methanol environment. As discussed in previous studies (Valderrama, et al. 2007), interactions of PAHs and polymeric matrices arise from Van der Waals attractions. The magnitude of this attraction is dictated by the instantaneous and induced dipole moments, which depend on the polarizability of the molecule. Therefore, as polarizability increases, via increased size and/or molecular arrangement so do Van der Waals attractions, thus explaining the patterns observed for PAHs which were more efficiently sorbed onto Desmopan by virtue of their increased Van der Waals attraction for the polymeric surface. In terms of partitioning coefficient differences between fluoranthene and pyrene, it seems that polarizability captures attractive interactions. Pyrene being more polarizable,

relative to fluoranthene, has a stronger attraction for the more hydrophobic polymer resulting in its lower partitioning coefficient. As for transport properties, pyrene and fluoranthene diffusivities vary as a function of polarizability demonstrating that attractive interactions play a role in delivery. It can be suggested that stronger pyrene-polymer attractions significantly delayed its movement through the matrix and resulted in decreased diffusivities, relative to fluoranthene. Overall, it appears that polarizability adequately correlates linearly with transport and thermodynamic properties accounting for both molecular size and chemical/attractive properties.

4.7 Conclusions

In the current study, it was determined that the transport of PAHs from Desmopan into methanol was influenced by external mass transport resistance in the solvent phase. Enhanced mixing conditions yielded faster release rates of PAH to an extent, after which the overall transport was limited entirely by internal resistances at mixing speeds of 800 rpm or greater. Release rates for phenanthrene, fluoranthene, and pyrene in the presence of sonication were also slightly faster than those found under any mixing conditions examined, suggesting possible improvements in polymer transport properties. Additionally, the presence of ultrasonic exposure conclusively shifted thermodynamic positions inducing higher concentrations of PAHs in solution at equilibrium. Such an observation is well in accordance with current sonochemistry knowledge. Furthermore, the model developed was capable of describing the transport of all PAHs in the presence and absence of sonication, as well as quantifying both diffusive and thermodynamic properties. The diffusivities of phenanthrene, fluoranthene, pyrene, and BaP, with or without sonication decreased as a function of molecular size and were in the order of 10^{-7} cm^2/s. These were in agreement with coefficients of similar aromatic compounds in a variety of polymer matrices. Additionally, partitioning coefficients estimated with and without sonication conclusively differed from each other and a trend of decreased coefficients, and thus affinity for Desmopan, as a function of molecular size was demonstrated. Finally, thermodynamic and

transport parameters were correlated with a series of permeant properties yielding factors usable for predictive purposes in polymer-solvent systems. Polarizability was the most accurate descriptor found in the current study.

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Chapter 5

Enhanced Degradation of Phenanthrene in a Solid-Liquid Two Phase Partitioning Bioreactor via Sonication

Pedro A. Isaza and Andrew J. Daugulis

With minor editorial changes to fulfill formatting requirements, this chapter is substantially as it has been submitted to: *Biotechnology and Bioengineering* (2009)

5.1 Preface

The results presented in Chapters 3 and 4 demonstrated the possibility of utilizing sonication to enhance the delivery of PAHs from polymers and elucidated the mechanistic reasons as to why these improvements were observed. Additionally, the results obtained in Chapter 3 hinted towards the robustness of the microbial consortium, which under the cyclic sonication conditions could still thrive and not lead to biologically limited degradation. This knowledge was imperative to characterize the applicability of ultrasonic exposure both as a physical and biological phenomenon. However, all demonstrations of concept and effects were shown at the flask level.

A final study was conducted in a 5 litre TPPB to validate that equivalent results could be obtained at a bench level. In the study, it was imperative to both confirm that improved deliveries and degradations could be obtained at this larger scale and also that sonic energy could be introduced without extensive customizing of the vessel. These two issues are crucial to the feasibility of up scaling this technology and would provide an indication of whether equivalent results are to be expected at larger scales. Although effects of sonic energy on biology were briefly examined in Chapter 3, the work in the present chapter describes the use of molecular techniques to provide further insight as to effects at the microbial scale. Specifically, consortium compositions with and without irradiation were examined, and bacterial strains present in the consortium were identified both at the start of the experiment and after sonicated conditions were completed.

5.2 Abstract

The current article examined the feasibility of inducing improved delivery and degradation of phenanthrene in a solid-liquid partitioning bioreactor system at bench scale by means of ultrasonic energy input. Degradation rates of phenanthrene, delivered from Desmopan

(polyurethane), by a microbial consortium were improved 2.7 fold in the presence of sonication relative to un-sonicated controls. Results demonstrated that an operating window involving on/off sonication cycling improved substrate delivery and reduced microbial inactivation effects, and rational selection of ultrasound cycling profiles could lead to even further enhancements. Additionally, all results were obtained in a conventional bioreactor with commercial ultrasonic equipment and a commercially-available polymer. Subsequent DGGE analysis demonstrated that the sonication cycles selected maintained consortium compositions, relative to control cases, and suggest that exposure would not reduce degradative capabilities even after long periods of irradiation. Finally, consortium members were identified as belonging to the *Pandoraea*, *Sphingobium*, and *Pseudoxanthomonas* genera. Comparison of genetic sequences in the Ribosomal Database Project revealed that some of the bacterial members, identified at the strain level, had been previously observed in PAH degradations, while others have been reported only in the degradation of other aromatics, such as pesticides.

Key Words: Sonication, Solid-Liquid Two Phase Partitioning Bioreactors, Biodegradation, Phenanthrene, Enhanced Delivery

5.3 Introduction

The removal and treatment of anthropogenic polycyclic aromatic hydrocarbons (PAHs) in soil is an area of research that has been actively pursued over the last few decades. These organic moieties are comprised of two or more benzene rings fused in a linear, angular or cluster arrangement. They typically increase in genotoxic and carcinogenic character, as well as environmental persistence and resistance to biological degradation, as a function of increasing molecular weight (Cerniglia, 1992; Juhasz and Naidu, 2000). An efficient and feasible bioremediation process has been recently proposed and demonstrated by Rehmann et al. (2008) for PAH contaminated soils by means of solid-liquid two phase partitioning bioreactors (TPPBs).

TPPBs are comprised of an aqueous phase containing substrate degrading organisms, which coexists with an immiscible second phase acting as a reservoir for high concentrations of hydrophobic substrates, such as PAHs (Daugulis, 2001). The organic pollutants then partition into the aqueous phase as determined by mass transport and thermodynamic considerations, as well as metabolic demand. Recent advances have demonstrated that the sequestering phase is not restricted to organic solvents but can consist of solid polymeric materials (Amsden et al., 2003). This provided the critical feature which allowed Rehmann and colleagues (2008) to remove PAHs from soil by means of contacting it directly with polymers, followed by TPPB degradation. Although the overall remediation process was successful, degradations were subject to significant mass transport limitations (Rehmann et al., 2008). Improvements were proposed and demonstrated in Chapter 3, showing that a cyclic ultrasonic stimulus enhanced delivery of PAHs from polymers in partitioning systems without inducing noticeable biological inactivation effects or changes in the remediation scheme proposed by Rehmann and colleagues (2008). However, improvements were demonstrated at a flask level and the possibility of extrapolating these effects to bench scale bioreactors has yet to be demonstrated, along with a closer examination of the effects of intermittent sonication on microbial consortia.

The objectives of this study were: To evaluate the possibility of improving delivery and degradation of phenanthrene in polymer-liquid TPPBs by means of an ultrasonic probe immersed in a conventional bioreactor; and to identify and track consortium members through molecular techniques.

5.4 Materials and Methods

5.4.1 Chemicals, Polymers and Analytical Procedures

All salts, spectrophotometric grade methanol, PAHs and silicone oil were identical to those described in Chapter 3. Desmopan 9370A beads (polyurethane elastomer beads) were graciously donated by Bayer Material Science (Leverkusen, Germany). Analytical procedures for determining PAH concentrations in polymers and silicone oil were as described in Chapter 3.

5.4.2 Bioreactor Inoculum Growth Prior to Polymer Degradation Experiments

A 4L silicone oil (SO) stock solution was loaded with 4000, 2500, 100, and 50 mg/L of phenanthrene, fluoranthene, pyrene, and benzo[a]pyrene, respectively. 100 mL of medium was prepared and added to a flask along with 20 mL of stock SO, and two frozen samples of stock culture, enriched previously, were then introduced into the flask (as described in Chapter 3). The flask was agitated for 96h at 180rpm and 20^{o}C and after 4 days the entire solution was centrifuged (3500rpm, 15 min), the SO aspirated and cells were re-suspended in a new 1L flask. 500 mL of fresh media were then introduced along with 200mL of stock SO. The flask was then cultivated at the same conditions and after 72 hours the solution was again centrifuged, silicone oil aspirated and cells re-suspended. The cells were then introduced into the bioreactor for subsequent polymer-liquid degradation experiments, which in all cases followed an identical inoculum growth procedure to that described above.

5.4.3 Degradation of Phenanthrene in Polymer-Liquid TPPBs in the Presence and Absence of Ultrasonic Exposure

500g of Desmopan polymer beads were loaded, as described previously (see Chapter 3), with phenanthrene to a concentration of approximately 10mg/g. After loading, polymers were stored in the dark at 4^{o}C until needed. All solid-liquid degradations were conducted in a 5L Bioflo III reactor (New Brunswick Scientific, Edison, NJ, USA) containing three liters of medium, 50g of loaded polymers and seed inocula prepared as described above. The three degradation conditions were: A control case (no sonication), cycle sonication periods of 20min on followed by 4h off (20:4), and 20min on followed by 2h off (20:2). All other reactor conditions including mixing speed, aeration rate and temperature were maintained constant at 600rpm, 0.3L/min, and 30^{o}C respectively. Small quantities of polymers (approximately 0.1g) were periodically removed, in duplicate, and assayed for phenanthrene concentration.

5.4.4 Microorganisms and Molecular Analysis

Aqueous samples were removed from the bioreactor at the beginning and end of the control degradation, as well as at the end of the 20:4 degradation and frozen as previously described (see Chapter 3). The samples were then sent to Microbial Insights (Rockford, TN) for denaturing gradient gel electrophoresis (DGGE) analysis as well as bacterial identification.

5.4.5 Ultrasonic Equipment and Application

Sonication was applied in the bioreactor by means of a tuned 20 kHz Vibracell titanium extender designed and manufactured by Sonics and Materials (Newtown, CT). Its length and diameter were 38.1cm and 18mm respectively, and was introduced via the 19mm access port on the head plate of the vessel. A Sonics and Materials VC 750 processor (Newtown, CT) was used for ultrasound generation (maximum power output of 750W) and experiments were all conducted at a power output of 150W, as employed by Wood et al. (1997). Treatment times and exposures were controlled manually.

5.5 Results and Discussion

5.5.1 Degradation of Phenanthrene Delivered from Desmopan Under Various Sonication Cycles.

Figure 5-1 displays the degradation of phenanthrene delivered from Desmopan 9370A in the absence and presence of sonication applied using the on-off cycles described above (20:4 and 20:2). These conditions were selected due to their anticipated ability to maintain cellular activity as demonstrated by Wood et al. (1997), as well as in Chapter 3. The differences between sonicated and control degradations, displayed in Figure 5-1, indicate that the control condition (no sonication) required 16 hours to reach 50% substrate degradation, compared to approximately 8 and 6 hours in the 20:2 and 20:4 cases, respectively. These results demonstrate a 2 and 2.7 fold increase in degradation rates relative to the un-sonicated condition. This would suggest that an operating "window" for inducing improvements through variable sonication cycles is present and provides potential for optimization. The existence of a sonication cycle "window" that provides a positive effect arising from sonic energy input, while not unduly affecting microbial activity has been demonstrated by Wood and colleagues (1997) for ethanol producing bacteria. It can therefore be hypothesized that such enhancement cycles may not be affecting the cellular integrity of the consortium present here. Additionally, differences between the 2 and 2.7 fold improvements observed in the TPPB, relative to the 4 fold improvement seen at the flask scale (presented in Chapter 3) could be attributed to system configuration differences (ie. bath sonication-flask scale (see Chapter 3) vs. probe sonication-TPPB) as well as sonication intensity differences (70W-flask level (see Chapter 3) vs. 150W-TPPB).

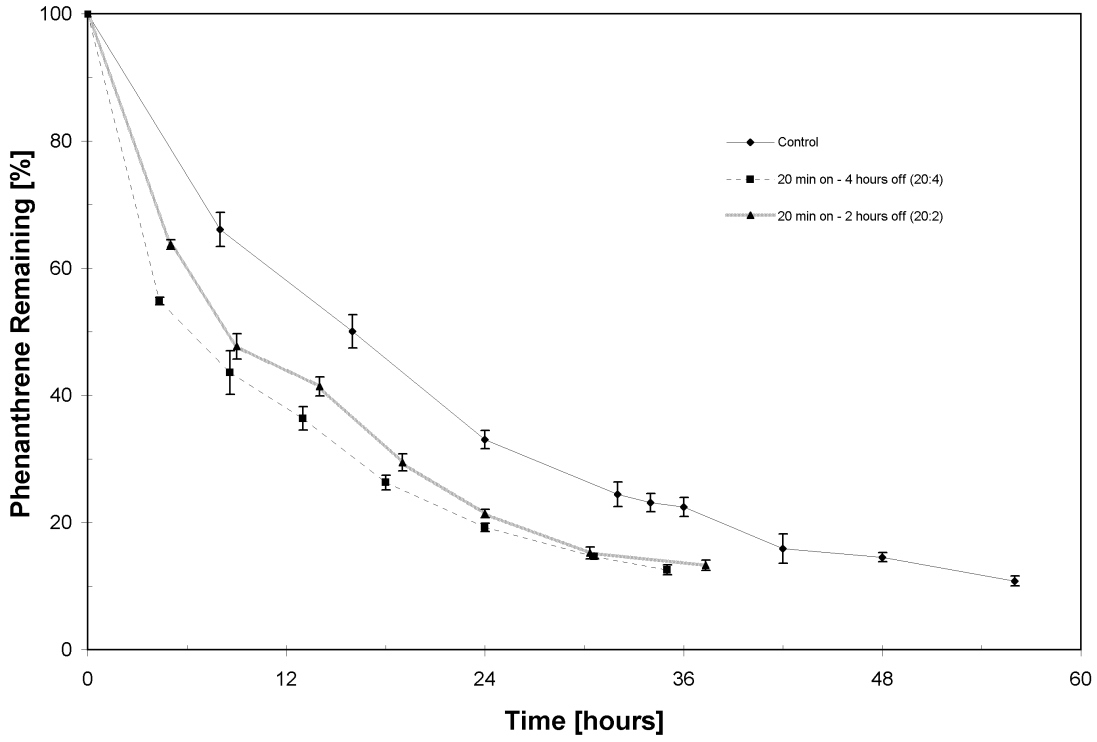

Figure 5-1: Phenanthrene remaining in Desmopan polymers (or reactor) as function of time under the different degradation conditions examined. Duplicate measurements taken at each time point were used as a standard deviation.

From Figure 5-1, it is also evident that the 20:4 case was capable of inducing significant delivery improvements even with longer deactivation periods. Additionally, it must be noted that the 20:2 case did not enhance degradation as effectively as the 20:4 case during the first 12 hours. Therefore, it can be surmised that increased sonic exposure was not beneficial at the initial stages of the degradation. Although more frequent cycles may theoretically improve delivery, the associated reductions in microbial viability are likely controlling overall rates of degradation at these initial stages when the cell population is at its lowest level. After 12 hours, rates of degradation in the 20:2 experiment increased relative to 20:4. It could, therefore, be hypothesized that mass transport limitations became more prominent at this stage due to decreased concentration gradients, and more frequent exposures improved substrate delivery. Also, bacterial

numbers at this latter period were likely higher, and effects on microbial viability were probably less pronounced.

It would be interesting to apply sonication in a more rational fashion to further optimize the process. For example, sonic exposure could be introduced once during the initial 12 hours, to maintain biological activity and "jump-start" delivery from the polymers, while in the final stages cycle frequency could be increased to improve mass transport limitations. Also, it is important to note that all equipment utilized such as the ultrasound generator, sonic probe, bioreactor and polymers are commercially available and can be used without retrofitting. Finally, since rate improvements were observed at small scale (as shown in Chapter 3) and bench scale here, applications at a larger scale seem feasible.

It is also interesting to note that degradation with or without any sonication plateaued at approximately 10% substrate remaining. The partitioning of phenanthrene into the aqueous phase is a thermodynamic phenomenon and a function of the concentration present in the polymer beads. Therefore, it could be hypothesized that at 10% residual substrate, the amount of phenanthrene equilibrated into the aqueous phase could not meet the metabolic demand in the reactor, and degradation subsided. Additionally, as demonstrated by Rehmann et al. (2008) partitioning of phenanthrene from Desmopan heavily favors the polymer phase. Therefore, at 10% remaining it is possible that the equilibrium aqueous concentration was below microbial uptake limits, resulting in essentially no more substrate degradation. Such effects were also observed and proposed in Chapter 3 at the flask level.

5.5.2 Molecular Analysis

The second section of this study utilized molecular techniques to examine effects of sonication on consortium composition, via DGGE, as it has been shown that various cellular properties make certain bacteria more resilient to sonic inactivation effects. Additionally consortium members were identified in order to correlate results with previous studies. Figure 5-2

shows the DGGE profiles obtained from Microbial Insights representing the consortium make-up at different stages of the degradation process. The gel on the left displays the consortium at time 0, the middle gel shows the community make-up at the end of the control degradation and the right gel displays the consortium at the end of the 20:4 sonic experiment. With the exception of one band, all three gels were similar. It can therefore be surmised that the consortium composition at the beginning and end of both sonicated and non-sonicated experiments were essentially equivalent. The exception noted is the appearance of a band in the middle and right gels, relative to the left gel, between bands 2.2 and 1.3. This suggests that a new microbial species flourished in the reactor and became prominent in the solid-liquid degradations with and without sonic exposure. However, an attempt to excise the band by Microbial Insights was unsuccessful and further characterization was not possible.

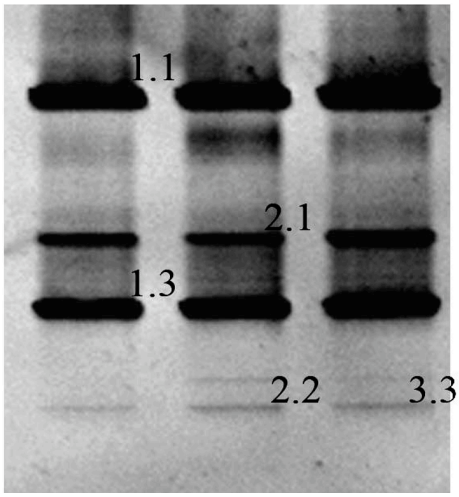

Figure 5-2: DGGE profile of amplified DNA from 16S rRNA gene portions. Bacterial bands constitute at least 1-2% of the total bacterial community. Labeled bands were excised and sequenced.

It must be also noted that since the middle and right gels displayed the same bands it can be concluded that sonication did not significantly alter the composition of the bacterial community. Such a possibility was hypothesized in Chapter 3 based on the fact that various microbial properties make certain cell lines more resilient to ultrasonic damage (Tiehm, 2001).

Therefore, the cyclic application of ultrasound utilized in this study ensured that the composition of the bacterial community was maintained. Such results are promising as they are in agreement with viability effects demonstrated by Wood et al (1997) and suggest that degradation capabilities will likely never be lost, as consortium compositions were maintained even after 36 hours of cyclic sonic exposure.

The numbered bands were excised, amplified a second time, sequenced and compared in the Ribosomal Database Project (RDP, 2009). Band 1.1 belonged to the genus *Pandoraea* and had a 98.5% similarity index with strain *Pandoraea* sp. R1717. Literature linking this particular strain to PAH degradations is not present to date (to our knowledge). However, other strains in the *Pandoraea* genus have been found to efficiently degrade various aromatics and PAHs (Ozaki et al., 2006; Viñas et al., 2005)

Bands labeled 1.3 and 2.1 were found to represent consortium members belonging to the *Sphingobium* genus. This was not surprising as this type of bacterium has been extensively isolated in PAH degradations (Cerniglia, 1992). Band 1.3 was found to have a 97% similarity index with 3 particular *Sphingobium* strains. *Pseudomonas paucimobilis* was the first match and such a strain has been reported to degrade phenanthrene (Mueller et al., 1997; Weissenfels et al., 1990) and fluoranthene (Mueller et al., 1997; Mueller et al., 1990). Additionally, its presence was not surprising since the consortium used here, developed in Chapter 3, introduced a *P. paucimobilis* strain, designated as EPA 505, which has been previously shown to efficiently degrade multiple PAHs (Vandermeer and Daugulis, 2007). The second match was with *Pseudomonas* sp. BRW2, which is closely related to strain EPA505, and has been shown to efficiently degrade pesticides (Leys et al., 2005). The final match was *Sphingobium herbicidovorans* strain FL, which to our knowledge has not been linked to PAH degradation. However, members of the same species have been found to degrade chlorinated phenoxyacids

(Stolz, 2009). Band 2.1 was found to have a 92.5% similarity index with *Sphingomonas paucimobilis* strain ZFJ-16, which has also not been reported in PAH degradations to date.

Band 2.2 was also sequenced and linked to 3 bacterial strains belonging to the *Pseudoxanthomonas* genus with a 97.7% similarity index. The first match was with *Stenotrophomonas* sp. MFC-C, a strain capable of co-metabolically degrading mefenacet, a popular aromatic herbicide (Harada et al., 2006). The second match was with *Pseudoxanthomonas mexicana*. This particular species has been demonstrated to degrade fluorene (Viñas et al., 2005) and since inoculum procedures exposed the consortia to various PAHs it is not surprising that such a strain was present. Additionally, this strain has been found to contribute to the degradation of 4-ringed PAHs (Lladó et al., 2009). The final match was *Pseudoxanthomonas* sp. PNK04, a biosurfactant producing bacterium (Anand et al., 2009), which suggests potential surfactant presence in the solid-liquid systems examined. However, additional studies are still required to address such a possibility.

Finally, band 3.3 was also found to belong to the *Pseudoxanthomonas* genus. This was to be expected as bands 2.2 and 3.3 were at equivalent heights on the gel and represent bacteria of similar genetic characteristics. A similarity index of 88.8% was found with *Pseudoxanthomonas mexicana*, which as previously mentioned is a strain common in PAH degradations.

5.6 Conclusion

The possibility of improving delivery and degradation of phenanthrene in a solid-liquid partitioning system at bench reactor scale, through the application of sonication, was examined. Rates of phenanthrene degradation, delivered from Desmopan, were improved by at least 2 fold in the presence of sonication cycles, and an operating window involving on/off sonication cycling capable of enhancing phenanthrene transport while minimizing microbial inactivation effects was established. Additionally, the success of the experimentation demonstrated that further rational

selection of ultrasonic cycling could lead to additional performance improvements. The experimental work was undertaken in a conventional bioreactor with commercial sonication equipment and readily available polymers, which suggests that extrapolation to larger scales is feasible. In addition, subsequent molecular analysis demonstrated that the ultrasonic selected cycles maintained consortium composition and it is therefore likely that degradative capabilities will not be reduced even after extended sonication periods. Genetic analysis also showed that consortium members belonged to the *Pandoraea*, *Sphingobium*, and *Pseudoxanthomonas* genera. Additional analysis revealed bacteria at the strain level, with some species being well known to be involved in PAH degradations, while others were not previously associated with such studies.

5.7 References

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Chapter 6
Conclusions and Recommendations for Future Work

6.1 Conclusion

The current work improved delivery (rate and extent) and degradation of PAHs in polymer-liquid partitioning systems at both flask and bench reactor levels. As a first contribution, a 5 fold improvement in delivery rates of PAHs from polymers via sonication (compared to non-sonicated cases) was successfully demonstrated at the flask level. Furthermore, the effect was displayed in a variety of commercial polymers, including recycled tires, opening the possibility for utilizing sonication to improve delivery of PAHs from polymers deemed to be economically feasible for environmental TPPB applications. This improvement by means of an external stimulus has never been shown for PAHs in solid-liquid systems and represents an innovative solution to the mass transport limitation hindering these novel systems and associated remediation schemes.

Additionally, sonication was found to not only improve delivery rates but also shift thermodynamic positions of solid-liquid systems. Enhanced extents of release were displayed by ultrasonic exposure, and results were in accordance with current sonochemistry knowledge. This particular phenomenon is of great importance as it demonstrates that sonication increased concentration gradients, driving mass transport via thermodynamics, and contributed to the faster delivery rates observed. Such a result has never been shown in two phase systems or examined in the field of biomedical engineering, and provides novel insight as to the manner in which sonication improves transport from polymers.

Although the above results are extremely relevant, the pivotal contribution of this work lies in the possibility of successfully incorporating the above physical improvements with biological systems capable of degrading these deleterious compounds. In itself, the consortium of

bacteria enriched in the present study was an important achievement, as PAHs with up to 5 benzene rings were readily degraded. Such an accomplishment has rarely been reported in literature. However, as mentioned above, at the flask level, the most important contribution of this work was the possibility of using sonication, applied in an intermittent fashion, to not only enhance transport of PAHs delivered from polymers, but also readily offset well known biological inactivation effects (associated with ultrasound) and result in degradation rates up to 4 times faster. This feat has double importance, as it demonstrates that sonic application not only improves delivery and masks cellular inactivation effects, but also provides significant potential for further optimizing ultrasonic cycling.

Additionally, molecular techniques (DGGE) were applied and provided further insight as to the effects of sonication at the microbial level. It was demonstrated that under the ultrasonic cycles selected, consortium compositions were maintained. In itself, this was a crucial contribution as it suggested that degradative capabilities would be unchanged, even after prolonged exposure periods, and provides motivation for further optimization research. Additionally, genetic analysis demonstrated that the consortium was comprised of bacteria belonging to the *Pandoraea*, *Sphingobium*, and *Pseudoxanthomonas* genera, which yielded an important point of comparison for other studies.

A mass transport analysis also helped to elucidate sonication effects on transport and thermodynamics. This was imperative to gain a better mechanistic understanding of effects on both external and internal resistances of PAH delivery as well as partitioning coefficients. Under standard delivery conditions (ie. no sonication), transport from polymers was found to be significantly restricted by the external resistance at the solid-liquid interface. As for sonication effects, rates of release under ultrasonic exposure were slightly faster than those obtained under internally controlled conditions, suggesting possible improvements in polymer transport properties. This revealed that sonication could address both external and internal transport

properties yielding delivery rates not possible through increased mixing. This was a break through contribution for two reasons. Firstly, it demonstrated that sonication removed the external resistance to transport, inherently controlling delivery of these aromatic compounds. Secondly, "improving" internal properties of polymers via an external stimulus is remarkable, and such a possibility was shown in the presence of sonication, which essentially induced the polymer to exhibit "different" properties. Such a feat cannot be achieved through mixing adjustment and is possibly the main attraction for continuing research in this area. The model also quantified diffusivities of PAHs in the polymer examined, and results and trends were well in agreement with previous literature reports. Thermodynamic effects were also examined, and partitioning coefficients estimated with and without sonication statistically differed from one another, while trends as a function of permeant properties were characterized. An additional contribution of this work was the expansion of predictive thermodynamic and transport knowledge in polymer-liquid systems. Previous work utilized octanol-water partitioning coefficients to estimate polymer-liquid interactions of PAHs. However, inconsistencies were sometimes observed. The present work revealed that polarizability was the most accurate descriptor for determining behavior of PAHs in solid-liquid systems. This yielded predictive knowledge critical for estimating uptake capabilities of polymers in "real" soil remediation scenarios where a large variety of PAHs are present.

Recall that the work presented here was motivated by the bottleneck in PAH delivery demonstrated by Rehmann et al. (2008) for solid-liquid bioreactor degradations. This limitation hampered the productivity of the proposed remediation strategy, and in order to address the applicability of the sonication solution, it was imperative to demonstrate equivalent effects at the bench bioreactor level. Results presented here confirmed such a possibility, as rates of phenanthrene degradation were improved by at least 2 fold with sonication (compared to non-sonicated systems), showing the potential for extrapolating results to an even larger scale. Although such improvements were not as significant as those obtained at the flask level, rational

selection of ultrasonic cycling may lead to additional performance enhancements, and further optimization research is therefore still necessary. The bench scale results are of great importance for various reasons. Firstly, they demonstrated that improvements could be achieved in a conventional bioreactor with commercial sonication equipment and readily available polymers, making the possibility for further scale up, and availability of equipment, a reality. Second, and most important, the use of sonication overcame the limitation of the novel remediation strategy proposed by Rehmann et al. (2008), bringing the technology one step closer to reaching commercial application.

6.2 Recommendations for Future Work

This work provides a basis for several future investigations. Although the current work has demonstrated the possibility of utilizing ultrasonic exposure to enhance delivery and degradation of PAHs in solid-liquid two phase partitioning systems, optimization studies of such schemes are still lacking. The possibility of optimizing ultrasonic cycling provides an independent variable potentially yielding more efficient deliveries with readily offset biological effects. As an example, decreasing the number of cycles at the initial stages of the degradations to "jump-start" mass transport, while maintaining microbial viability, and increasing the frequency at the latter stages, where delivery limitations are severely pronounced, could yield a more efficient process. Rational applications of such schemes have been described in more detail in Chapter 5. However, such investigations are still lacking to date.

Additionally, as described in Chapter 2, several ultrasonic wave properties affect the extent of delivery improvements. Such variables include frequency and intensity and have yet to be exploited in solid-liquid TPPB degradations. Importantly, sonic intensity is a property which can readily be controlled with the ultrasonic equipment detailed in Chapter 5, and provides a great opportunity for reducing the mass transport limitation present in these systems. On the other

hand, effects of increased intensity and frequency on the enriched bacteria have yet to be examined and could provide a better idea about the robustness of the consortium present.

Results presented in Chapter 5 suggested the possibility that consortium members in the current work could be producing biosurfactants. The possibility of such a situation has yet to be investigated, and effects on ultrasonically enhanced mass transport have also yet to be explored.

As an additional side note, effects of polymer porosity have not been examined in PAH deliveries and further research could be undertaken. Furthermore, the possibility of tailoring the porosity of polymers to improve delivery in two phase systems remains to be explored.

To determine the applicability of this novel work at a commercial level, it is important to examine its overall limitations. It is clear that microbial degradations are highly effective at degrading low and certain high molecular weight PAHs, and for the most part, it is safe to say that biodegradations are typically restricted by the poor aqueous solubility of these aromatics. However, the possibility that bacteria in soil have the entire enzymatic capacity required to address very large PAHs may be wishful thinking, which at one point may halt the applicability of these technologies industrially. In the case of TPPBs, such a devastating "deal breaker" could be readily circumvented. The possibility of combining physio-chemical and ultrasonically enhanced TPPBs opens the door to the complete degradation of PAHs. Photolysis is one of such attractive processes, which has already been combined with two-**liquid** phase TPPBs and improved degradation rates and extents of both high and low molecular weight PAHs have been reported (Guieysse and Viklund 2005). Additionally, results in these two-**liquid** phase systems showed the capability of photolysis to break down high molecular weight PAHs. Therefore, it is feasible to imagine that the introduction of such a technology in ultrasonically enhanced solid-liquid TPPB degradations could yield even more improved rates of degradation and the ability to degrade a wider range of PAHs, irrespective of whether the required enzymatic capacity exists or

not. Such work has yet to be undertaken and provides an interesting area of research which would take solid-liquid TPPBs one step closer to the industrial level.

6.3 References

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Appendix A

Equilibrium Shift Results for Additional Polymers Tested

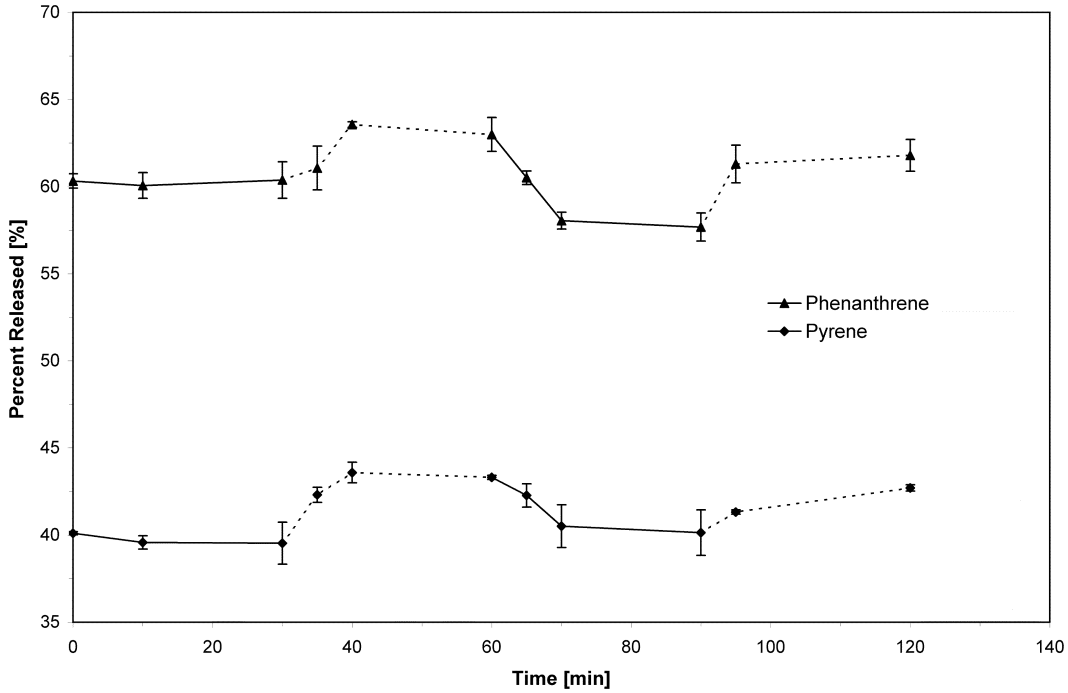

Figure A-1: Equilibrium concentration of phenanthrene and pyrene, between Hytrel® 8206 polymer pellets and methanol, in the presence and absence of sonication. Solid and dashed lines represent data obtained for non-sonicated (control) and sonicated periods respectively. Triplicate measurements taken at each time point were used as a standard deviation.

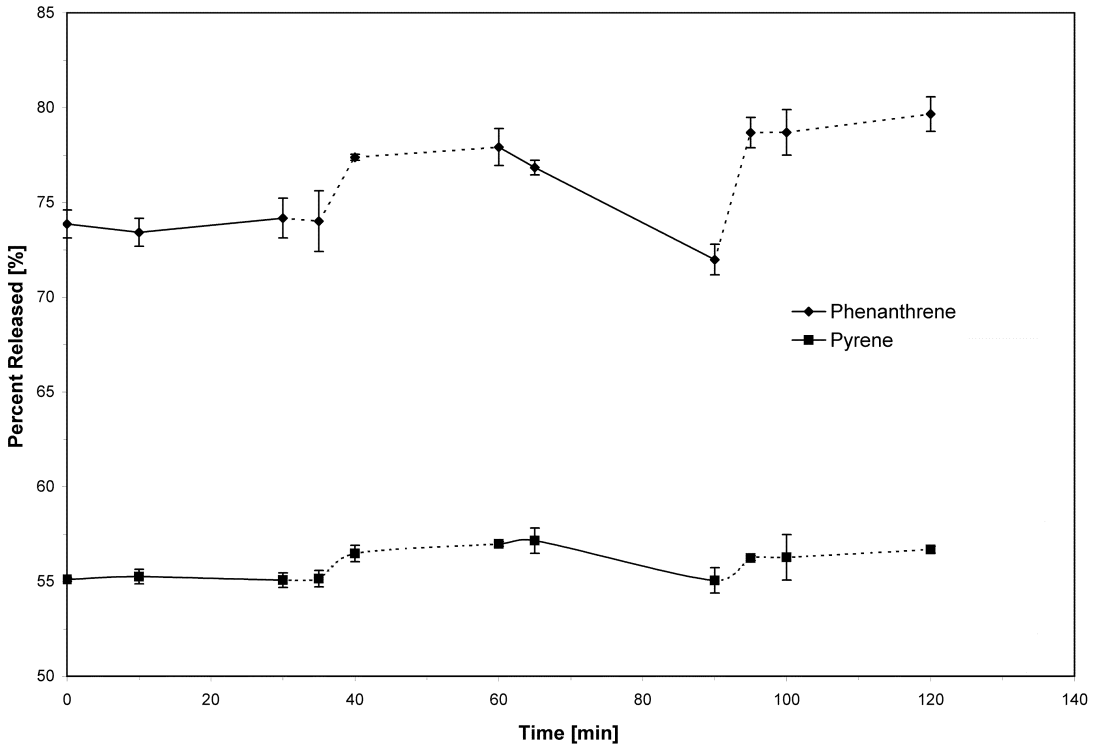

Figure A-2: Equilibrium concentration of phenanthrene and pyrene, between Kraton® D4150K polymer pellets and methanol, in the presence and absence of sonication. Solid and dashed lines represent data obtained for non-sonicated (control) and sonicated periods respectively. Triplicate measurements taken at each time point were used as a standard deviation.

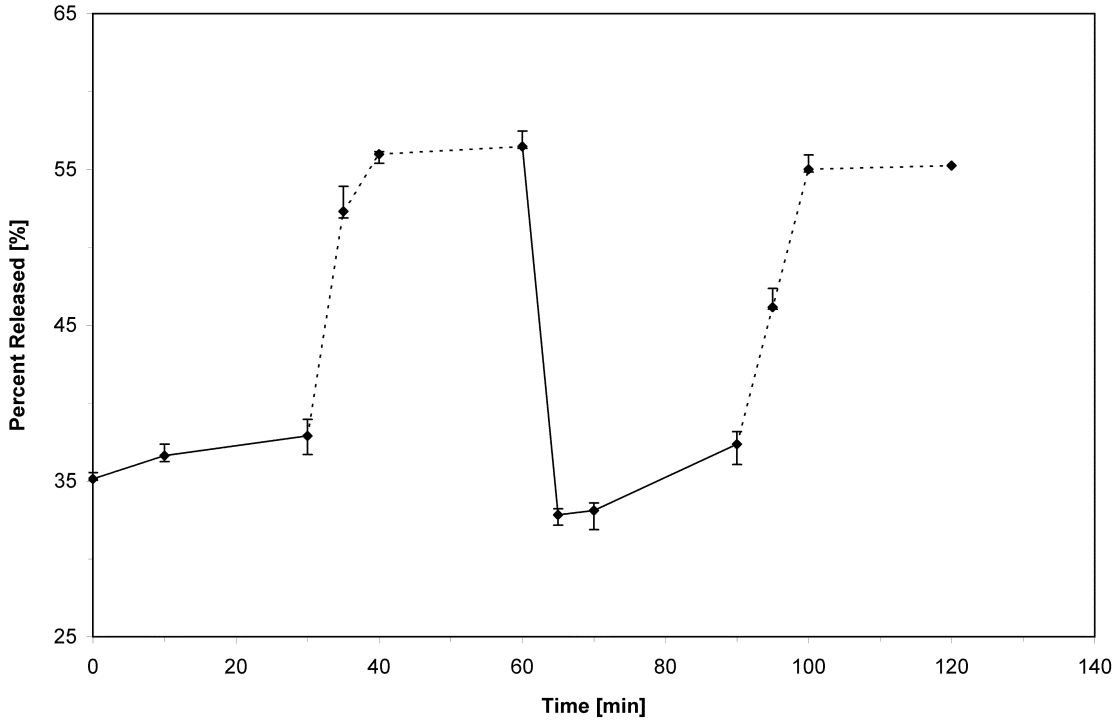

Figure A-3: Equilibrium concentration of phenanthrene, between recycled tires and methanol, in the presence and absence of sonication. Solid and dashed lines represent data obtained for non-sonicated (control) and sonicated periods respectively. Triplicate measurements taken at each time point were used as a standard deviation.

Appendix B

Calibration Curves of PAHs

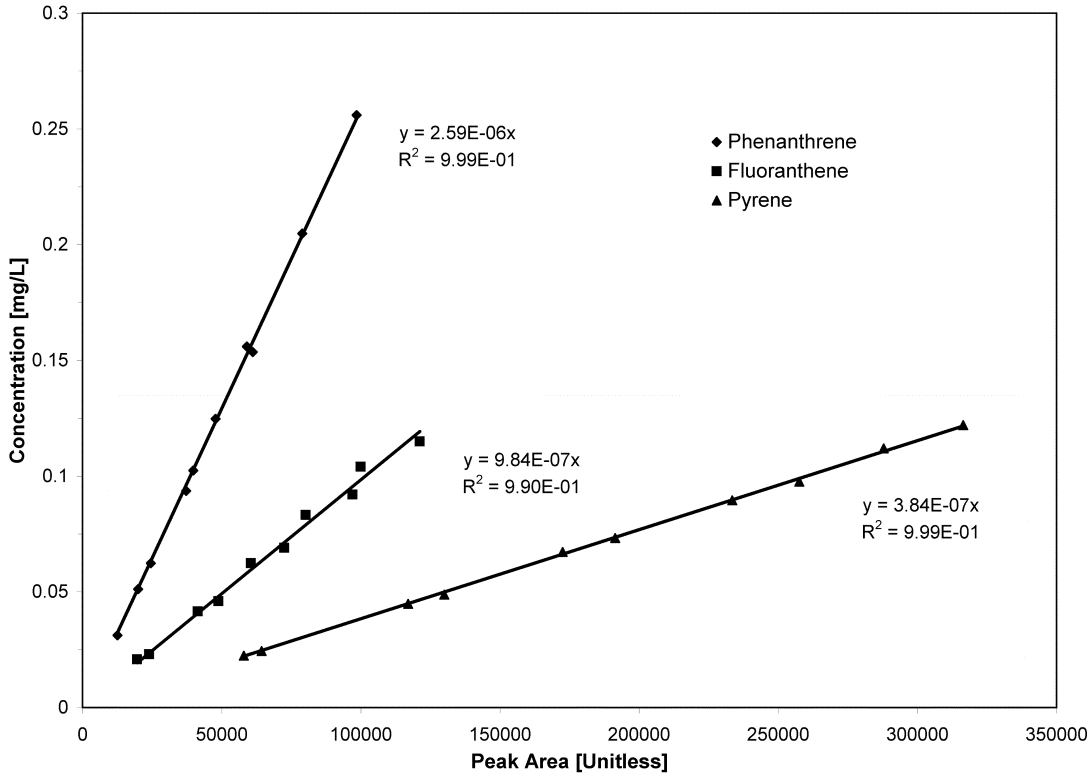

Figure B-1: Calibration curve generated for phenanthrene, fluoranthene and pyrene. The equation used and corresponding R^2 are shown on the graph.

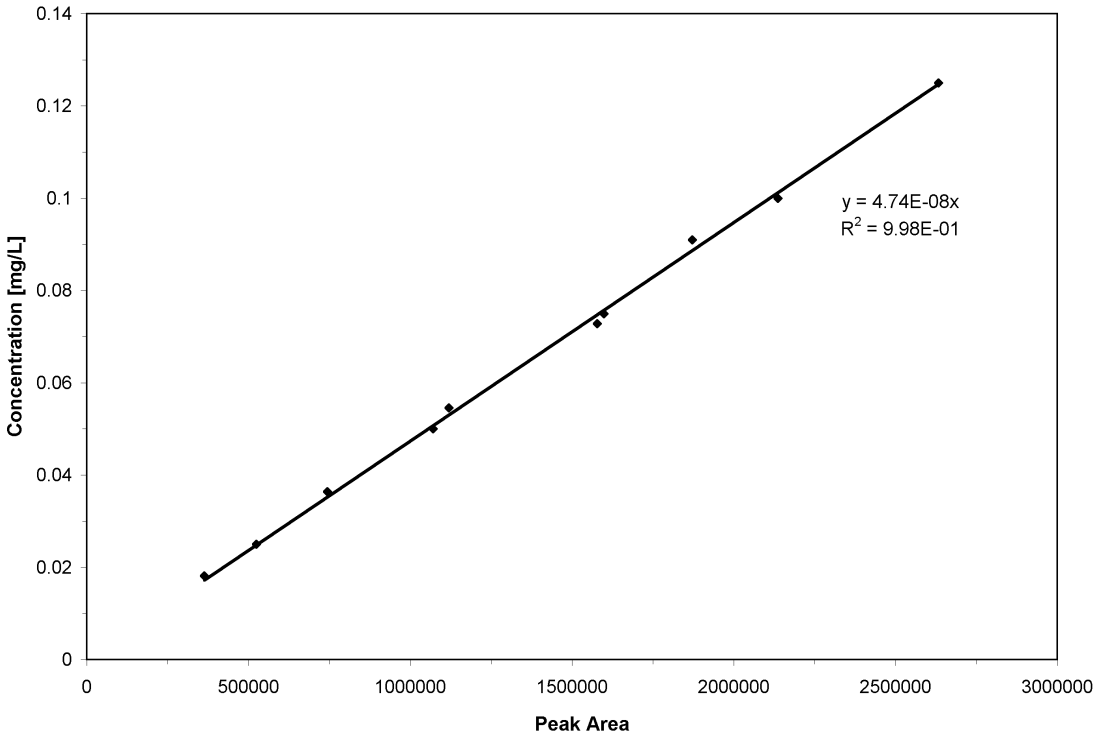

Figure B-2: Calibration curve generated for BaP. The equation used and corresponding R^2 are shown on the graph.