ARTICLE

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

Pedro A. Isaza, Andrew J. Daugulis

Department of Chemical Engineering, Queen's University, Kingston, Ontario, Canada K7L 3N6; telephone: 613-533-2784; fax: 613-533-6637; e-mail: andrew.daugulis@chee.queensu.ca

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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 fivefold, when delivered from Desmopan 9370 A (polyurethane). Similar effects were observed in Hytrel and Kraton® D4150 K 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.

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KEYWORDS: ultrasound; mass transfer; solid–liquid partitioning systems; PAHs; consortium; biodegradation

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, 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 bio-treatment 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; Daugulis and McCracken, 2003; Janikowski et al., 2002; Muñoz et al., 2003; Vandermeer and Daugulis, 2007; 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

Correspondence to: A.J. Daugulis

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 lipophillic and hydrophilic properties (Kost et al., 1988; Levy et al., 1989; Miyazaki et al., 1985, 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 (Wang et al., 1996; Wood et al., 1997).

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.

Materials and Methods

Chemicals and Polymers

All salts and spectrophotometric grade methanol (95+%) were 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). 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 supplied by DuPont (Kingston, 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 I.

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 (0–0.1 mg/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 0.1 g) in 5 mL 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.

Partitioning Coefficients

Partitioning coefficient procedures were adapted from those described by Prpich et al. (2006), as well as Rehmann et al. (2008). Methanol stock solutions containing all four PAHs at 500, 250, 125, and 50 mg/L concentrations were generated and distributed in 20 mL scintillation vials. Varying masses

Table I. Basic polymer properties.

	Polymer				
Commercial or common name	Chemical name	Source	Glass transition temperature (T_g, °C)	Density (g/mL)	Particle size distribution (shape)
KRATON® D4150K	Styrene/butadiene linear triblock copolymer	Kraton	Styrene: 90; butadiene: −90	0.920	4.6 ± 0.6 mm by 3.8 ± 0.85 mm (rice)
Hytrel® 8206	Polyether-ester	Dupont	−59	1.170	3.3 ± 0.17 mm by 3.8 ± 0.1 mm (rice)
Desmopan 9370A	Polyurethane elastomer	Bayer material science	−70	1.060	2.8 ± 0.15 mm by 2.4 ± 0.15 mm (rice)
Bridgestone tires	Polybutadiene rubber or styrene-butadiene rubber	Bridgestone	N/A (proprietary and product dependant)	0.44 (typical recycled tires)	<4.76 mm by 4.76 mm (square)

of Desmopan beads (1–3 g) were then introduced and vessels were sealed and agitated for 24 h at 180 rpm and 20°C. Equilibrium concentrations of PAHs in methanol were determined via spectrophotometry and polymer uptakes were calculated via mass balance.

PAH Release Tests

Polymer pellets (6 g equivalent to about 280 polymer beads), or used tires, were loaded by equilibrating a stock methanol solution containing all PAHs at a concentration of 500 mg/L for 24 h at 180 rpm and 20°C. Uptake of PAHs was then determined via mass balance by analyzing methanol, analogous to the partitioning coefficient procedure. After uptake, methanol was decanted, and polymer beads were washed with water for 3 min and allowed to air dry for 24 h 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°C (Fig. 1).

PAH Equilibrium Tests

Three grams of Desmopan were loaded to 4.0 and 5.5 mg/g of phenanthrene and pyrene respectively and allowed to equilibrate with 15 mL of methanol for 24 h at 180 rpm and 20°C. Concentrations were monitored for 30 min to establish equilibrium positions without sonication. After such a time, the vial was sonicated for 30 min, and PAH concentrations were measured. At 60 min, sonication was deactivated and measurements were obtained for an additional 30 min. At 90 min, sonication was re-activated and readings were obtained until time 120 min.

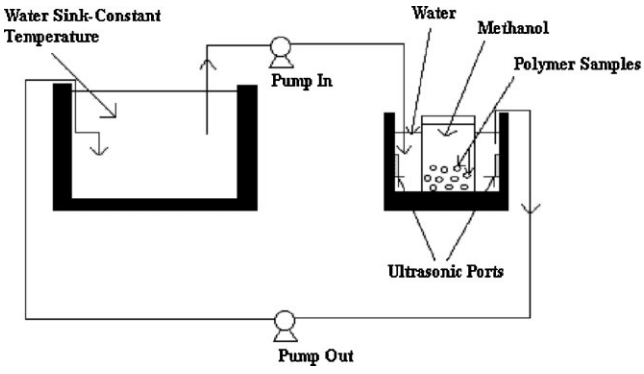

Figure 1. Schematic diagram of experimental setup used to determine the effect of ultrasound on release and degradation rates of PAHs. Note that methanol was replaced with medium during degradation experiments.

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). 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 0.25 g/L, were added as crystals and PAHs were added on a weekly basis to ensure selection pressure. After 2 weeks, 10 mL of liquid culture were filtered through glass wool, to remove PAH crystals, and added to a 250 mL flask containing 50 mL of medium and 10 mL of silicone oil with phenanthrene and fluoranthene dissolved at 500 mg/L, while pyrene and BaP were at 250 and 100 mg/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 24 h at 180 rpm and 20°C, and samples were removed daily and centrifuged at 3,500 rpm for 15 min. Separated silicone oil layer samples were then assayed via spectrophotometry for PAH concentrations and returned to maintain approximately constant aqueous and organic phase volumes.

Consortium Growth on Glucose in the Presence and Absence of Sonication

A stock sample of the enriched consortium was grown in 40 mL of medium with the inclusion of glucose (5 g/L) for 48 h. The cell culture was centrifuged, rinsed twice and re-suspended in fresh growth medium with glucose (1 g/L) to yield an optical density of $OD_{600} = 0.6$. The culture suspension was then divided into two 100 mL samples. The first solution was maintained as a control (no sonication) and OD was obtained for 300 min of growth at 20°C. In parallel, the second culture was exposed to continuous sonication at 20°C for 280 min while obtaining OD readings. After 280 min, sonication was switched off and cell regrowth was monitored for an additional 30 min, and after 24 h.

Degradation of Phenanthrene Delivered From Silicone Oil in the Absence of Sonication

Fifty milliliters of medium and 10 mL of silicone oil, loaded with PAHs at standard concentrations, along with a frozen sample of stock culture, preserved in DMSO (10 v/v%) at −80°C, were added to a 250 mL flask. The vessel was agitated

for 96 h at 180 rpm and 20°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 250 mL flask. In parallel, phenanthrene was loaded into silicone oil to a concentration of 500 mg/L and 10 mL of the resulting solution was introduced in the flask containing the re-suspended culture. Mixing at 600 rpm was applied at all times. Five-milliliters 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.

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 h growth period and introduction of phenanthrene loaded silicone oil. The cycle consisted of 25 min on and 3 h off, with the first cycle beginning at 5.5 h. 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 600 rpm except if/when being ultrasonically irradiated.

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, 20 g of Desmopan were loaded with phenanthrene (1 mg/g). Five grams of the loaded polymers were then added to the flask and degradation was allowed to proceed for 144 h at 600 rpm and 20°C. Small quantities of polymers (~0.1 g) 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.

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 600 rpm), commencing after the addition of Desmopan polymers, which consisted of 25 min on and 3 h 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.

Ultrasonic Treatment and Stirring

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

Results and Discussion

Enriched Consortium

Figure 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 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).

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 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), and Miyazaki et al. (1985, 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

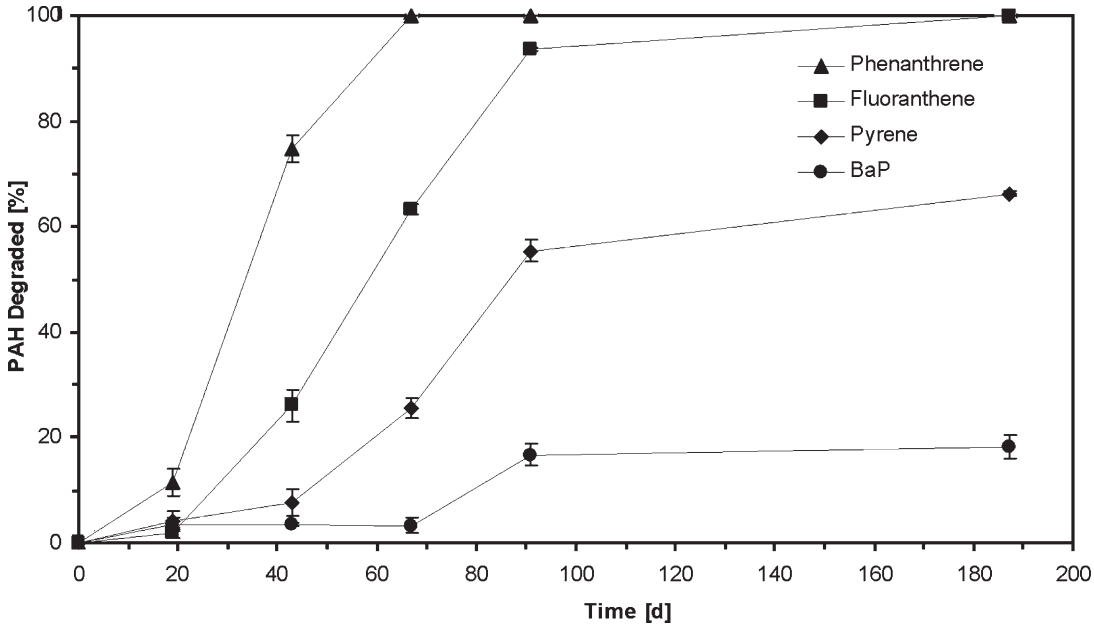

Figure 2. Degradation of PAHs delivered from silicone oil at 600 rpm by enriched microbial consortium. Triplicate measurements taken at each time point were used as a standard deviation.

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 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 min were required for BaP to achieve its equilibrium concentration or final percent release

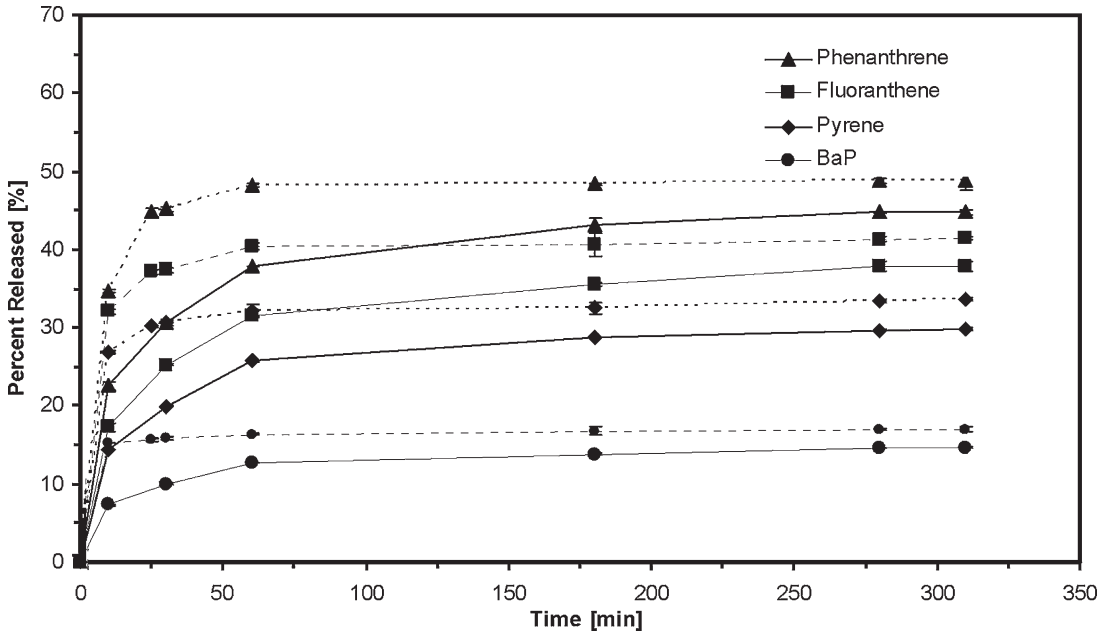

Figure 3. Release of PAHs from Desmopan polymer into methanol in the presence and absence of continuous sonication. Solid lines represent control/non-sonicated data while dashed lines represent sonicated results. Triplicate measurements taken at each time point were used as a standard deviation.

position, compared to 60 min 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 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 min were required to reach the ensuing equilibria compared to 300 min 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 500 m/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.

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. 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 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 (data not shown). 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.

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 min) are summarized in Table II. 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 increased from 4.16 to 4.6 mg/L min in the presence of sonication, representing a 10% increase in rates. Similarly, a 19% improvement for phenanthrene was observed in Kraton. Pyrene on the other hand, demonstrated a 13% increase in Hytrel and 28% in Kraton. Similar assessments could be made for the remaining data presented in Table II. It is difficult to quantify effects as a function of

Table II. Initial rates of release for three additional polymers examined, under the presence (labeled ultra) and absence of sonication (labeled control) [(mg/L min)].

	Hytrel polymer		Kraton polymer		Tires	
PAHs	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 ± 0.002	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

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.

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 h and the final equilibrium position was monitored for 30 min (corresponding to the initial 30 min in Fig. 4), followed by the initiation of sonication. From Figure 4 at least a 5 min 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 min. 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 min of exposure the ultrasound was switched off and the equilibrium position returned to original levels. A second irradiation cycle was then commenced at 90 min, 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.

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 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 4. Equivalent experiments were also conducted using Kraton, Hytrel, and recycled tires and shifts were observed in all systems (data not shown).

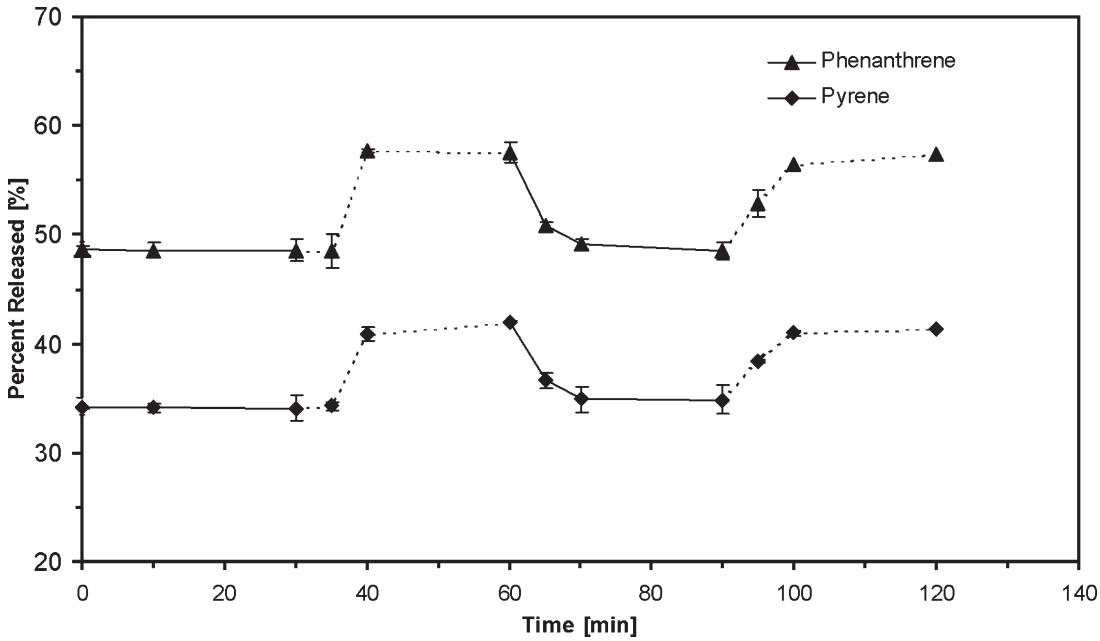

Figure 4. Equilibrium concentration of phenanthrene and pyrene, between Desmopan polymer pellets and methanol, in the presence and absence of sonication. Solid and dashed lines represent data obtained for non-sonicated (control) and sonicated periods respectively. Triplicate measurements taken at each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

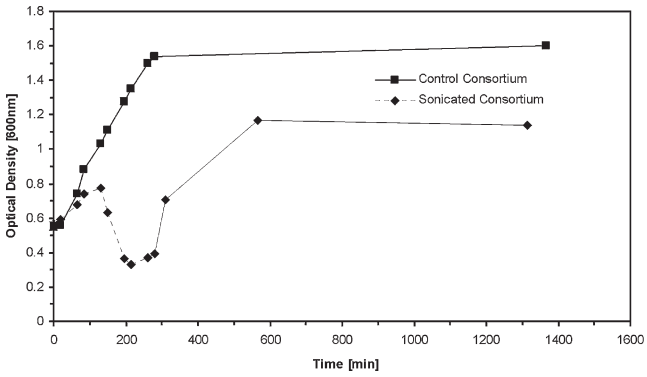

Figure 5. Optical Density values for growth on glucose under presence and absence of continuous sonication. Solid and dashed lines represent non-sonicated and sonicated periods respectively.

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 5, rates of OD increased in both the control and continuously sonicated consortia and were identical for the initial 90 min, suggesting that induced microbial inactivation was not pronounced 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, for 90 min of continuous exposure, effects on cell viability were indiscernible. However, further irradiation began to decrease OD readings until a plateau was reached and sustained up to 280 min 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.

A secondary aspect investigated was the extent of the cell damage and consortium ability for re-growth following irradiation. After 280 min, sonication was terminated and optical density changes were measured for an additional 1,200 min. From Figure 5, it is apparent that after 280 min, 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 5, demonstrated that continuous sonication effects for up to 100 min were non-lethal and induced only temporary cell damage. It therefore appears feasible to balance sonically enhanced mass transport and inactivation effects.

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 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 (600 rpm) 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 6. In contrast to the glucose experiments, exposures were cyclic and were not initiated for the sonic consortium until after 5 h 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 h 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 h, lasted 25 min and was followed by 3 h of non-sonicated mixing, a cycle adapted from Wood et al. (1997). From Figure 6, it is inconclusive whether sonication enhanced rates of phenanthrene degradation after the first cycle (finishing at 9 h). The rates of PAH disappearance for the sonicated culture, between times 0 and 5 h, were within error of being equivalent to

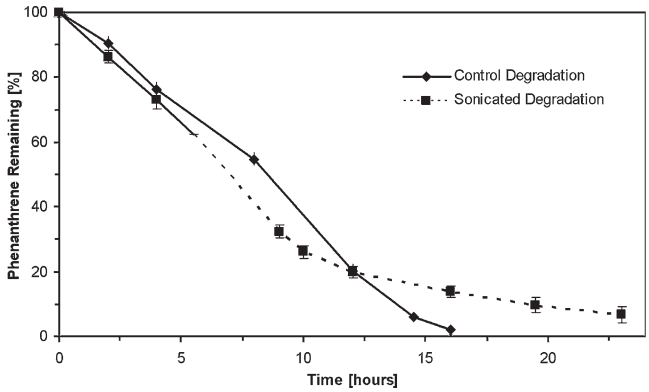

Figure 6. Phenanthrene degradation delivered from silicone oil in the presence and absence of sonication. Solid and dashed lines represent non-sonicated and sonicated periods respectively. Note that sonication was applied cyclically (25 min on followed by 3 h off) and began after 5.5 h as adapted from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

those between times 5.5 and 9 h (post first cycle). The second sonication cycle started at 9 h, and from Figure 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 h. Unlike growth on glucose, it appears as though the period of re-growth provided (3 h), 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 h 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.

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 min on and 3 h off were applied. From Figure 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 fourfold, since 144 h were required to reach the 30% remaining mark in the control degradation, compared to approximately 36 h 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

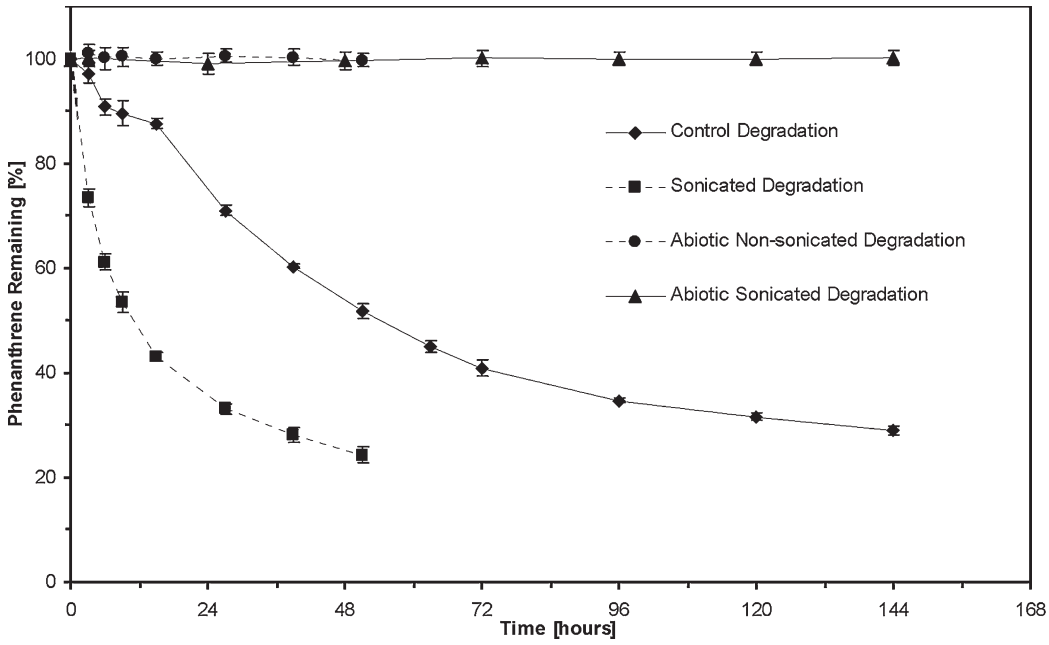

Figure 7. Biotic and abiotic phenanthrene degradation delivered from Desmopan in the presence and absence of sonication. Solid and dashed lines represent non-sonicated and sonicated periods respectively. Sonication was applied cyclically (25 min on followed by 3 h off) as adapted from Wood et al. (1997).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

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).

It is also important to note that inactivation effects arising from sonication in the liquid–liquid system (using identical sonication cycles), discussed for Figure 6, were not apparent in the solid–liquid set-up. Degradation profiles for both sonicated and control cases, seen in Figure 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.

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 fivefold relative to unmixed control cases when sonication was applied. This effect was also investigated and observed in Kraton and Hytrel 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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