**IN-SITU BIORESTORATION OF SEDIMENTS CONTAMINATED WITH POLYCYCLIC AROMATIC HYDROCARBONS (PAHS)**

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**ABSTRACT**

Restoration of contaminated sediments involves either mechanical removal of the sediment followed by treatment or *in-situ* treatment of the contaminants. Even after mechanical removal, considerable residues of the polluted material usually remain which require some form of *in-situ* treatment for eventual restoration. For *in-situ* treatment, conditions that appear to be rate determining are: (1) availability of oxygen; (2) availability of nutrients; and (3) nature of contaminants and sediment properties. There are significant variations in the redox potential of sediments as a function of depth. Aerobic bacterial biodegradation of PAHs is widely known and has been well studied. PAHs exhibit high octanol/water partition coefficient ($K_{OW}$), which results in the accumulation of these compounds in fatty tissues with subsequent biomagnification in the food chain.

In this paper, a novel method of controlling the redox potential in contaminated sediments using semi-permeable synthetic membranes will be presented. The method allows *in-situ* biodegradation of PAHs in contaminated sediments while preventing the membranes from fouling. Experimental data will be presented on biodegradation rates of 18 PAHs ( 2 ring to 6 ring compounds) as a function of redox potential. The rate of biodegradation decreased with increasing ring size and decreasing oxidation potential. Experimental testing of the synthetic membranes showed that *in-situ* PAH degradation could be increased several fold by utilizing the membranes to increase the sediment oxidation potential. The membrane technology is widely applicable for *in-situ* biotreatment of contaminated freshwater and marine sediments. A detailed simulation model to estimate the required membrane area and treatment time will also be presented.

**INTRODUCTION**

Previous attempts to treat contaminated sediments have mainly involved dredging followed by long-term storage (capping) or *ex-situ* treatment or *in-situ* treatment, using injection of nutrients, adapted microorganisms, chemicals, etc. While *ex-situ* treatment are very expensive, most of

the *in-situ* methods suffer from losses of the additives to the overlying water column, competition from indigenous microorganisms and difficulty in controlling environmental conditions, such as pH, temperature, alkalinity, etc. Luthy et al suggested through their findings that bioremediation could be the technically and economically feasible process for a realistic solution to the needs of contaminant removal both in *in-situ* and *ex-situ* operations. (Talley, Luthy and Ghosh et al 2001) The work being presented with the membrane promises a process, which can be used to enhance the rate of bioremediation, and control environmental conditions. Membranes being the part of biological systems' separations mechanisms provide a promise of long sought after feasible solution to the environmental problems. The studies done previously gave positive results for complete mineralization. Various studies have identified specific organisms capable of degrading PAH compounds (Heitkamp, M.A. et al 1989; Cerniglia, C.E. 1984). Information on aerobic pathways is generally limited to two-and three-ring PAH compounds including naphthalene (Fredrickson, J.K et al 1991), acenaphthene, and phenanthrene (Fredrickson, J.K et al 1991), (Brodkorb, T.S. et al 1992). Biodegradation of PAHs under anaerobic and sulfate reducing conditions have also been discussed (Young, L. 1999). The genotoxicity and mutagenecity of PAH and their dead-end metabolites in case of oxygen limiting condition has been a cause of concern. (Haeseler et al 2001).

**EXPERIMENT AND RESULTS:**

**Objectives:** The main objectives of this study were to demonstrate the feasibility of using micro porous membranes for manipulating the redox potential and deliver treatment agents into PAH contaminated sediments and aquifers, thereby enhancing the rate and extent of *in-situ* bioremediation. Table 1 summarizes the PAHs accumulated in the New York-New Jersey Harbor sediment, which was used in our experimental studies.

**TABLE 1. Selected polycyclic aromatic hydrocarbons (PAHs) in NY/NJ sediment .**

| Compound | Concentration (mg/Kg) (Percentage of Total PAHs) |
|---|---|
| Naphthalene | 3.8 (12.3) |
| 2-Methylnaphthalene | 2.6 (6.23) |
| Phenanthrene | 7.8 (20.3) |
| Acenaphthene | 2.8 (7.82) |
| Anthracene | 3.2 (8.23) |
| Fluorene | 1.8 (4.27) |
| Fluoranthene | 6.4 (14.5) |
| Pyrene | 3.9 (6.67) |
| Benzo [a] anthracene | 2.1 (3.46) |
| Chrysene | 2.7 (4.78) |
| Benzo[a]pyrene | 2.1 (3.19) |
| Benzo[k]fluoranthene | 1.7 (2.15) |
| Benzo[ghi]perylene | 0.8 (1.54) |
| TPAH | 46.8 (98.6) |

## MATERIALS AND METHODS

### Membrane Reactor Studies

Shaker vial extraction method (Huang et al. 1996) was used to analyze the soil concentration of each PAH. The extracts are analyzed using a Hewlett Packard Gas Chromatograph equipped with a flame ionization detector (GC-FID). A bench-scale micro-reactor system was developed to quantitate biodegradation rate of PAHs in sediment samples. A schematic of the micro-reactor system is shown in Figure 1. The length (L) was kept small (10 cm) to minimize axial profile along the length of the micro-reactor system. The diameter of the micro-reactor was 20 mm to minimize the effect of radial diffusion in the sediment. Preliminary experiments conducted with 10 cm diameter micro-reactors had shown that the PAH concentration profile exhibited less than 15% variation within a radial distance of 10 mm. Hence, the average PAH concentration was measured experimentally as a function of time, redox condition and addition of various biotreatment agents, such as air, argon, sulfate or nitrate containing water.

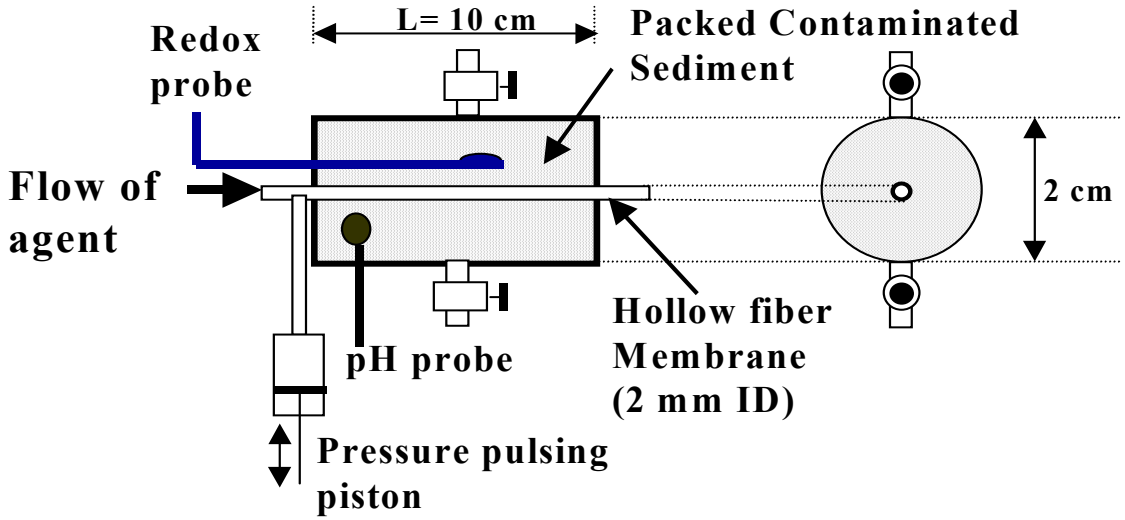


**FIGURE 1. Schematic of the membrane micro-reactor system.**

All micro-reactors were immersed in a constant temperature bath to maintain temperature during the experiment. 2 mm internal diameter polypropylene porous hollow fibers were used in the study. To minimize the impact of pore plugging due to microbial growth, two strategies were used: (1) the membrane hollow fibers were coated with a 1 to 3 micron thickness layer of palladium metal using electroless plating (Govind, et al., 1996), which prevented microbial attachment to the membrane surface; and (2) the pressure inside the hollow fiber was pulsed from 10 psia to 30 psia with a cycle time of 1 minute (membrane bubble pressure was 27 psia) to slough-off microbial growth from the pores and surface of the membrane hollow fibers. Application of these two strategies prevented membrane biofouling, a well-known problem with the application of membranes in biological environments.

The following biotreatment agents were studied: (1) air at 5 mL/minute flow rate to maintain aerobic conditions in the sediment; (2) argon gas at 5 mL/minute to maintain anaerobic conditions; (3) DI/DD water containing 15 mg/L sodium nitrate at a flow rate of 15 mL/minute; and (4) DI/DD water containing 12 mg/L of sodium sulfate at a flow rate of 15 mL/minute. The redox potential was measured by a platinum electrode calomel half-cell connected to a meter.

Chemical binding of PAHs to organic matter has been studied in the literature (Govind and Ramani, 2001). Almost all of the biodegradation studies in the literature

ignore the effect of chemical binding and measure disappearance of parent compound (not extractable) as indication of biodegradation. Mineralization studies, which involve the measurement of radiolabelled carbon dioxide from spiked radiolabelled PAHs, ignore the impact of aging, which results in chemical binding. Measurement of carbon dioxide evolution from aged contaminated sediments measures the combination of PAH mineralization and degradation of organic matter, and hence are not indicative of purely PAH mineralization. In our studies, chemical binding effects were measured by using sterilized sediments, by addition of sodium azide. Separate measurements of gas (carbon dioxide, methane, hydrogen sulfide, nitrogen) evolution (not presented here) demonstrated that addition of sodium azide did not result in any bioactivity for the experimental time periods used in our studies. Using sterilized sediments, non-extractability of PAHs was used as quantitation of chemical binding to sediment organic matter.

Percentage biotransformation in this paper is inclusive of chemical binding (also shown separately), since it is postulated that oxidative enzymes, produced by bacteria during the aging time period, rather than active bacteria are involved in initial hydrolysis of PAHs which results in chemical binding (Govind and Ramani, 2001).

## RESULTS AND DISCUSSION

### Experimental Results

#### *Membrane Reactor Studies*

Experimental studies conducted over a 90 days time period showed that all PAHs were biotransformed (only parent compounds were analyzed) under both low and high redox potentials, and the extent of biotransformation decreased under reducing conditions. The extent of biotransformation decreased with increasing ring size. Physical adsorption of PAH compounds on the surface of hollow fibers and glassware, measured by decrease in PAH concentration from aqueous solutions, was found to be negligible (less than 1%), compared to the extent of biotransformation.

Experiments with sterilized contaminated sediments revealed chemical binding to organic matter of sediments under only positive redox potential conditions (Govind and Ramani, 2001), as shown by hatched bars in Figure 2. The extent of chemical binding increased with increasing positive redox potential and with increasing ring size. Chemical binding was not observed under reducing

conditions, indicating that chemical binding of PAHs to organic matter is an oxidative process.
Relatively rapid biotransformation of PAHs was observed experimentally, (Figure 3) when air was passed through the membrane fiber. The oxidation-reduction potential exceeded +600. The extent of biotransformation decreased with increasing ring size. 2-ring PAHs were completely biotransformed in 60 days while about 57% of 6-ring PAHs were biotransformed.

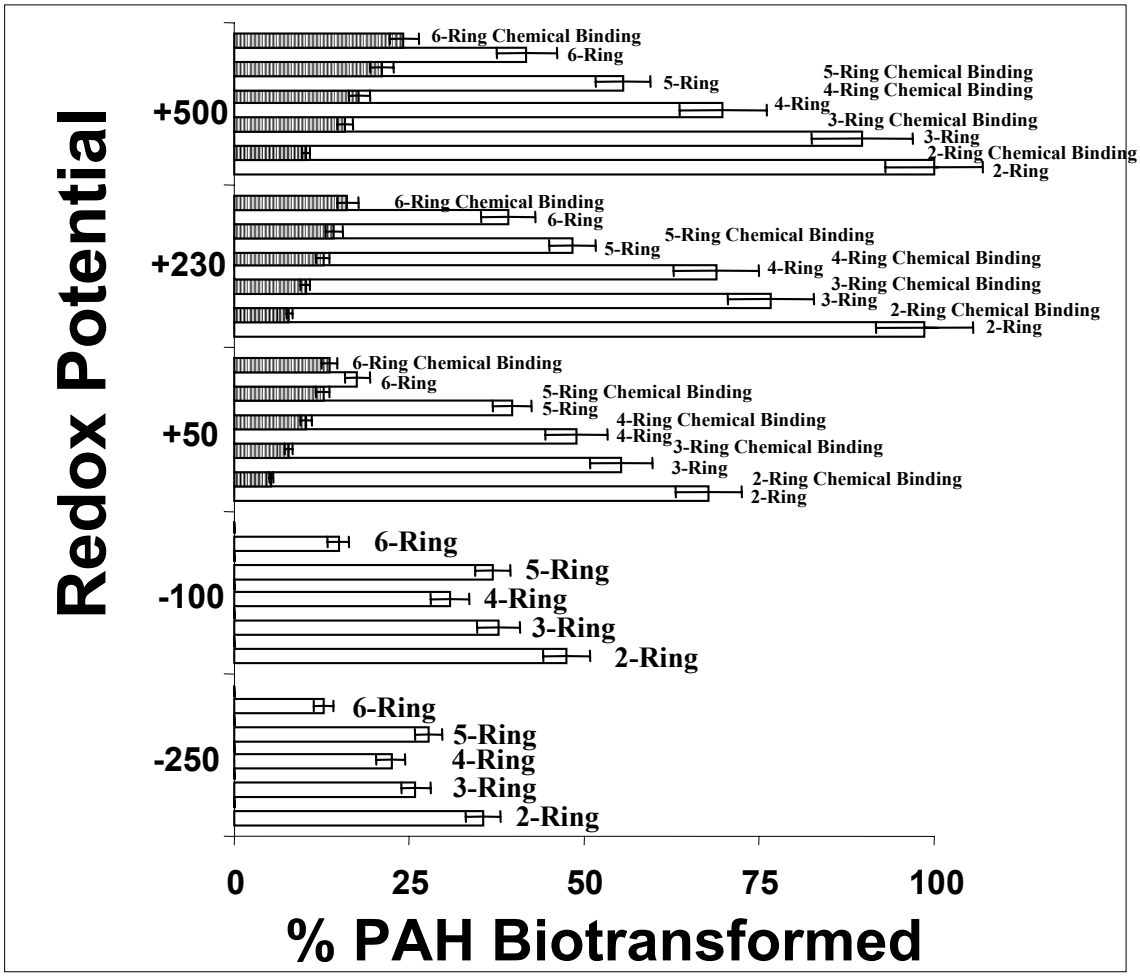


**FIGURE 2. Extent of PAH biotransformation under various redox potentials. Extent of chemical binding is shown by the hatched bars.**

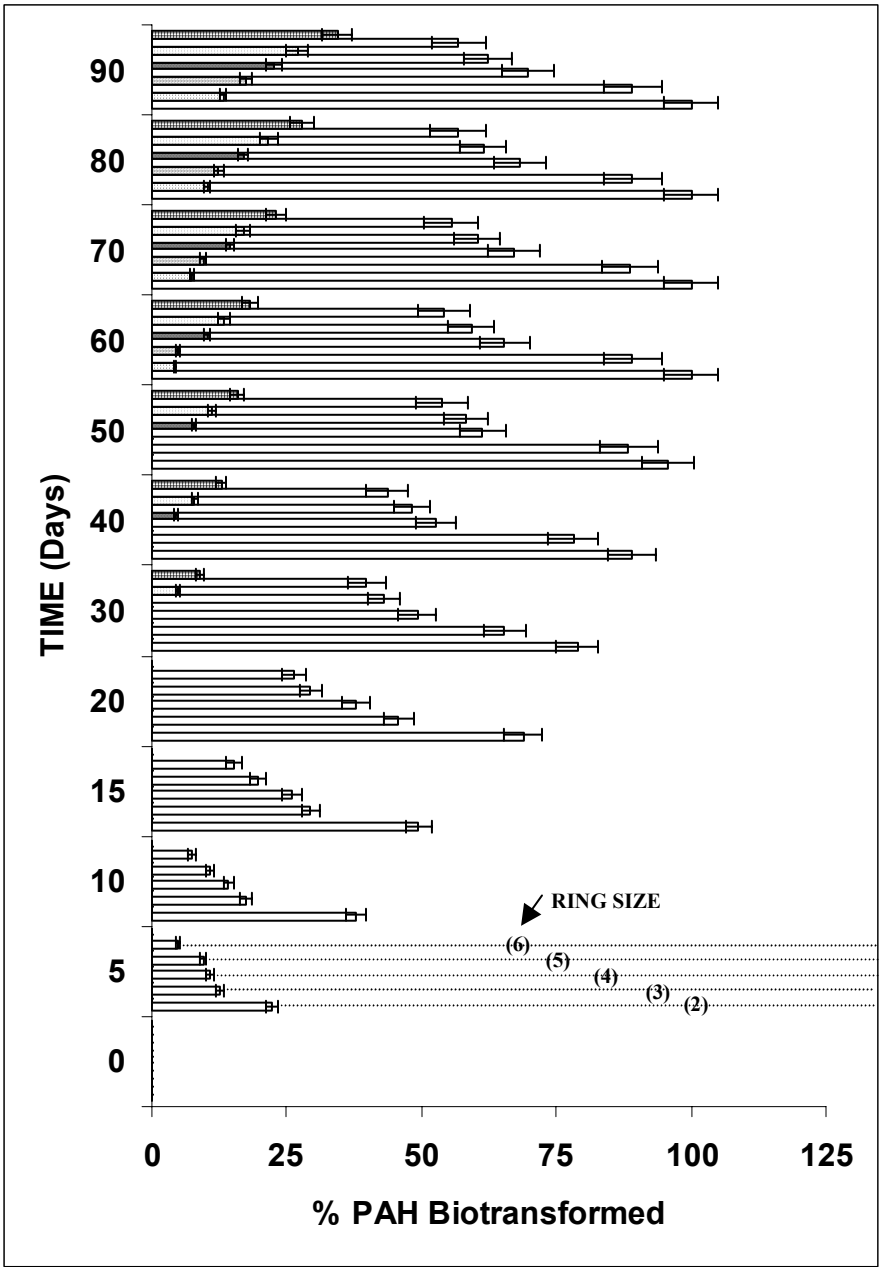


**FIGURE 3. Extent of PAH biotransformation under aerobic conditions. Chemical binding of PAHs to organic matter is shown by hatched bars.**

The extent of chemical binding under aerobic conditions increased with time, and with increasing ring size. The order of ring size, as shown for 5 days, is the same for all time periods. As mentioned earlier, % biotransformation is inclusive of chemical binding and in 90 days, almost half of the 6-ring PAHs are chemically bound to sediment organic matter.

When argon gas was passed through the hollow fiber membranes, anaerobic conditions prevailed in the sediment sample, with a redox potential below –240 mV. PAH biodegradation was observed, although the rate of biodegradation was considerably slower than the aerobic case. Figure 4 shows the extent of PAH biodegradation under anaerobic conditions. The acclimation time for the onset of biodegradation was about 35 days, and this time period is not shown in the graph. No chemical binding was observed under anaerobic conditions.

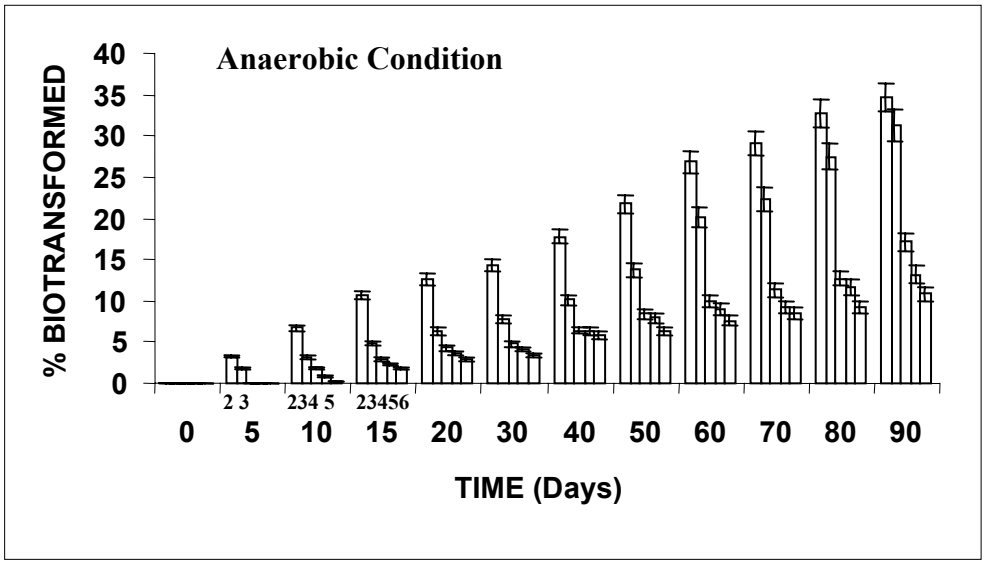


**FIGURE 4. Extent of PAH biodegradation under anaerobic conditions.**

**The order of ring size, as shown for 5, 10 and 15 days is the same for all remaining time periods.**

Under denitrifying conditions, with nitrate water as the biotreatment agent, the extent of biodegradation was high, although lower than in the case of aerobic conditions. The acclimation time period for the onset of biodegradation was 30 days (not shown in the graph). PAH biodegradation under denitrifying conditions has also been studied (Mihelcic and Luthy, 1988), and degradation of

acenaphthene and naphthalene occurred from an initial aqueous-phase concentration of 1 mg/L to nondetectable levels in less than 9 weeks. Figure 5 shows the extent of PAH biodegradation under denitrifying conditions. No chemical binding was observed under denitrifying conditions.

When sulfate water was used resulting in sulfate-reducing conditions in the micro-reactor, the extent of PAH biodegradation decreased considerably, and the acclimation time was 45 days (not shown in the graph). PAH degradation under sulfate reducing conditions has only been reported recently (Young, 1999). Figure 6 shows the extent of PAH biodegradation under sulfate-reducing conditions.

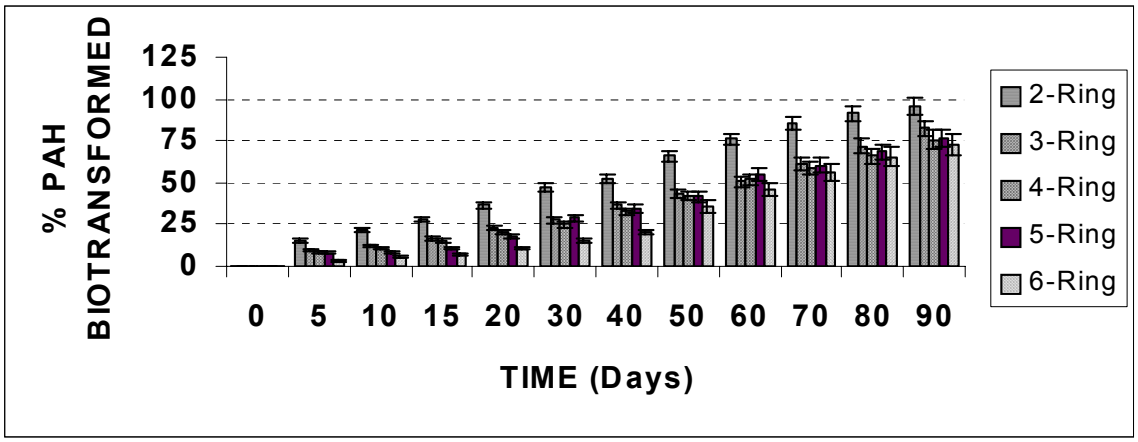


**FIGURE 5. Extent of PAH biodegradation under denitrifying conditions. For each time period, the PAH ring size is from 2 to 6 starting from the left.**

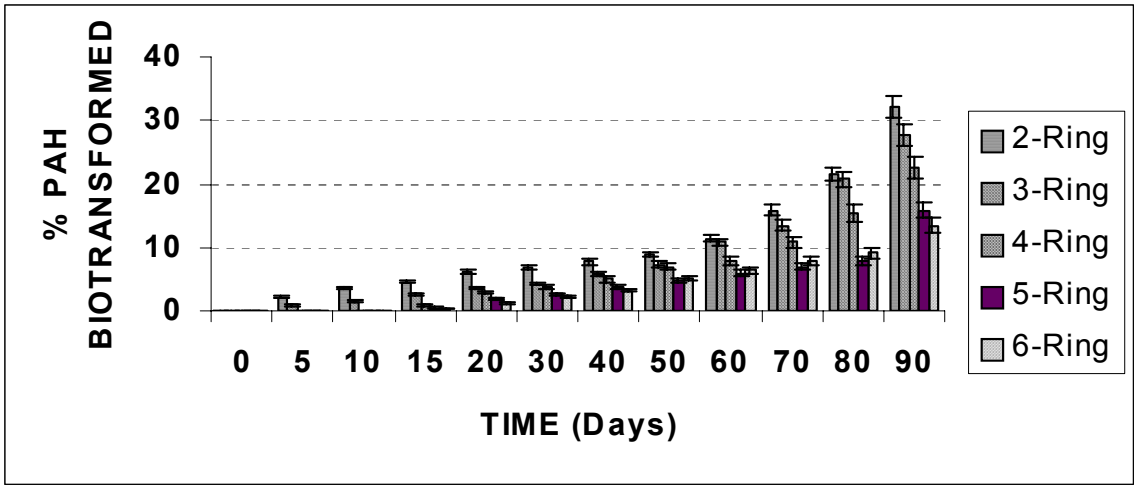


**FIGURE 6. Extent of PAH biodegradation under sulfate-reducing conditions. For each time period, the ring size is from 2 to 6, starting from the left.**

The above results showed that PAHs in contaminated sediments could be biodegraded successfully under various redox conditions using a membrane delivery system. Porous membranes can provide biotreatment agents to alter the intrinsic redox potentials in the contaminated zone, and the use of palladium coated hollow fibers with pressure back pulsing prevents biomass clogging effects usually observed with the use of membranes in environmental biosystems.

The effect of radial diffusion has shown that a single hollow fiber membrane can affect the intrinsic redox potential within a radial distance of about 10 times the fiber radius, and this is mainly due to the fact that the rate of biodegradation compared to aqueous diffusivity of the biotreatment agent is relatively slow. It is shown that chemical binding of PAHs to sediment organic matter is an important mechanism for PAHs, which previously has been largely ignored in biodegradation and bioavailability studies. Chemical binding of PAHs occurs only under positive redox conditions, increasing with increasing positive redox values and with increasing PAH ring size.

**Simulation Studies:**

Srivastava et al proposed a combined model, described in Table 2, of chemical binding and biodegradation for the membrane reactor used in the experiments above mentioned. (Srivastava et al 2004)

**Table 2: Mathematical Model for bioremediation using membrane**

| Eqn No. | Equation | Description |
|---|---|---|
| 1. | $\frac{\partial O(r,t)}{\partial t} = D_o \frac{1}{r}\frac{\partial}{\partial r} r \frac{\partial O(r,t)}{\partial r} - \frac{1}{Y_o}\frac{\mu_{max} x(r,t) C_H(r,t)}{K_c + C_H(r,t)}\frac{O(r,t)}{K_o + O(r,t)} - SOD$ | Oxygen Diffusion with Consumption |
| 2. | $-\frac{\partial C(r,t)}{\partial t} = K_1 C(r,t) E(r,t)$ | PAH Consumption |
| 3. | $\frac{\partial C_H(r,t)}{\partial t} = -\frac{1}{Y_H}\frac{\mu_{max} x(r,t) C_H(r,t)}{K_c + C_H(r,t)}\frac{O(r,t)}{K_o + O(r,t)} + K_1 C_H(r,t) E(r,t) - K_2 C_{org}^{\alpha} C_H$ | PAH-diol Consumption |
| 4. | $\frac{\partial x(r,t)}{\partial t} = \frac{\mu_{max} x(r,t) C_H(r,t)}{K_c + C_H(r,t)}\frac{O(r,t)}{K_o + O(r,t)}$ | Biomass Growth Rate |
| 5. | $\frac{\partial E(r,t)}{\partial t} = -\frac{1}{Y_E}\frac{\mu_{max} x(r,t) C_H(r,t)}{K_c + C_H(r,t)}\frac{O(r,t)}{K_o + O(r,t)} - K_1 C(r,t) E(r,t)$ | Enzyme Concentration |
| 6. | $\frac{\partial C_B(r,t)}{\partial t} = K_2 C_{org}^{\alpha} C_H(r,t)$ | Bound Contaminant |
| 7. | $\overline{O} = \frac{2\int_R^{R^*} O(r,t) r dr}{(R^{*2} - R^2)}$ | Oxygen Uptake |
| 8. | $\overline{C} = \frac{2\int_R^{R^*} C(r,t) r dr}{(R^{*2} - R^2)}$ | Average PAH in sediment |

The model was fitted to the experimental data. The model parameters not only fit the data well but also matched the reference literature biokinetics confirming the accuracy of the results obtained. The kinetics clearly is more favorable in case of two rings and relatively lesser as it progresses towards six rings. Moreover the effect of the redox potential affinity clearly seems to play a role in the biokinetics parameters.

Knightes et al (Knightes and Peters 1999, 2000) and Ghoshal et al (Ghoshal and Luthy 1997) tried to find the biodegradation kinetics of PAHs for aerobic biodegradation by fitting Monod model and by comparing with literature

values and concluded that the values of Ks, Y and $\mu_{max}$ could vary more than three orders of magnitude.

Table 3 gives the values of the representative simulation data. The data clearly shows the effectiveness of electron acceptor supply on the bioremediation. The variation in biodegradation time period with respect to the electron acceptor is also appreciable. In 60 days aerobic degradation causes PAH concentration to fall below detection level, though in denitrifying and sulfate reducing case the level is still of concern.

**Table 3: Representative Simulation data:**

| Time Elapsed (Days) | PAH Concentration remaining in the sediment (mg/Kg) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aerobic Condition | | | | | Denitrifying Condition | | | | | Sulfate Reducing Condition | | | | | Anaerobic Condition | | | | |
| | Ring Size | | | | | | | | | | | | | | | | | | | |
| | 2 | 3 | 4 | 5 | 6 | 2 | 3 | 4 | 5 | 6 | 2 | 3 | 4 | 5 | 6 | 2 | 3 | 4 | 5 | 6 |
| 0 | 6.4 | 15.6 | 15.6 | 3.8 | 0.80 | 6.4 | 15.6 | 15.6 | 3.8 | 0.80 | 6.4 | 15.6 | 15.6 | 3.80 | 0.80 | 6.4 | 15.6 | 15.6 | 3.80 | 0.80 |
| 10 | 3.8 | 11.0 | 13.5 | 3.5 | 0.75 | 5.8 | 14.2 | 15.0 | 3.5 | 0.79 | 6.2 | 15.5 | 15.5 | 3.75 | 0.80 | 6.3 | 15.4 | 15.5 | 3.75 | 0.80 |
| 20 | 2.5 | 7.5 | 11.0 | 3.0 | 0.65 | 4.5 | 12.4 | 13.5 | 3.2 | 0.78 | 6.1 | 15.4 | 15.4 | 3.70 | 0.79 | 6.0 | 14.8 | 15.2 | 3.70 | 0.80 |
| 30 | 1.5 | 6.0 | 9.5 | 2.5 | 0.55 | 3.5 | 11.5 | 12.0 | 2.8 | 0.72 | 5.9 | 15.0 | 15.0 | 3.65 | 0.78 | 5.5 | 14.2 | 15.0 | 3.65 | 0.79 |
| 40 | 0.8 | 3.5 | 7.5 | 2.0 | 0.45 | 2.8 | 9.8 | 10.5 | 2.4 | 0.62 | 5.7 | 14.5 | 14.6 | 3.60 | 0.78 | 5.2 | 13.8 | 14.4 | 3.60 | 0.78 |
| 50 | 0.2 | 2.5 | 6.0 | 1.6 | 0.35 | 2.0 | 8.0 | 9.0 | 2.2 | 0.44 | 5.5 | 14.0 | 14.4 | 3.55 | 0.77 | 5.0 | 13.0 | 14.2 | 3.50 | 0.76 |
| 60 | 0.0 | 1.5 | 4.2 | 1.2 | 0.30 | 1.5 | 6.5 | 7.8 | 1.8 | 0.40 | 5.3 | 13.5 | 14.0 | 3.50 | 0.76 | 4.8 | 12.2 | 14.0 | 3.45 | 0.74 |
| 70 | - | - | - | - | - | 1.0 | 5.5 | 6.2 | 1.5 | 0.38 | 5.1 | 13.0 | 13.8 | 3.45 | 0.75 | 4.6 | 12.0 | 13.8 | 3.40 | 0.72 |
| 80 | - | - | - | - | - | 0.6 | 4.5 | 5.5 | 1.2 | 0.30 | 4.9 | 12.2 | 13.2 | 3.40 | 0.74 | 4.4 | 11.5 | 13.5 | 3.35 | 0.71 |
| 90 | - | - | - | - | - | 0.3 | 3.5 | 4.2 | 1.0 | 0.25 | 4.7 | 11.8 | 12.8 | 3.30 | 0.72 | 4.2 | 11.0 | 13.0 | 3.30 | 0.70 |

Natural attenuation in shallow river simulation considering aerobic kinetics with saturated oxygen water interface providing the oxygen flux to the sediment gave representative data as described in Table 4.

**Table 4: Natural Attenuation in river sediment.**

| Time Elapsed (Days) | % PAH Concentration remaining in the sediment | | | | |
|---|---|---|---|---|---|
| | 2 Ring | 3 Ring | 4 Ring | 5 Ring | 6 Ring |
| 0 | 100 | 100 | 100 | 100 | 100 |
| 50 | 75 | 75 | 80 | 95 | 72 |
| 100 | 58 | 60 | 62 | 90 | 68 |
| 150 | 44 | 46 | 48 | 85 | 65 |
| 200 | 34 | 35 | 38 | 80 | 63 |
| 250 | 28 | 28 | 30 | 78 | 59 |
| 300 | 20 | 20 | 24 | 74 | 55 |
| 350 | 18 | 18 | 18 | 70 | 53 |

Natural attenuation with oxygen supply to air water interface is not going to give satisfactory sediment treatment even in years. PAHs will come to a plateau where they will be exist for a long time.

**CONCLUSION**

Using hollow fiber with pressure pulsing proved to be a successful treatment technology in the bench scale reactor. The aerobic degradation of PAH promises a treatment, which is much faster than other electron acceptor amendments. Kinetics data shows an enhanced affinity towards the oxygen as an electron acceptor. The affinity towards the substrate decreases as the ring size decreases and as we go towards no amendment (Anaerobic) from Aerobic, Denitrifying and Sulfate reducing conditions.

**NOMENCLATURE**

*Parameters:*

| | |
|---|---|
| $C^{\alpha}_{org}$: | Organic Matter in soil or sediment. $(mg/Kg)^{\alpha}$ |
| $D_0$: | Diffusivity of oxygen in sediment.$(cm^2/h)$ |
| K1: | PAH kinetics Parameter $(mg/Kg.day^{-1})$ |
| K2: | Binding Constant. $((mg/Kg)^{-\alpha}\ day^{-1})$ |
| K2': | $K2.C^{\alpha}_{org}$. $(day^{-1})$ |
| Kc: | Half Saturation Constant for PAH. (mg/Kg) |
| Ko: | Half Saturation Constant for electron acceptor. (mg/Kg) |
| SOD: | Sediment Oxygen Demand. $(gm/m^{-2}.day^{-1})$ |
| $\mu_{max}$: | Maximum Biomass Growth. $(day^{-1})$ |
| Ye: | Yield Coefficient for Enzyme. (mg/mg) |
| Yh: | Yield Coefficient for PAH. (mg/mg) |
| Yo: | Yield Coefficient for electron acceptor. (mg/mg) |

*Variables:*

| | |
|---|---|
| $\bar{A}$: | Average Quantity over the area. (Applied for O and PAHs). (mg/Kg) |
| C: | PAH Concentration. (mg/Kg) |
| $C_B$: | Bound PAH concentration. (mg/Kg) |
| $C_H$: | PAH-Diol Concentration. (mg/Kg) |
| E: | Enzyme Concentration. (mg/Kg) |
| O: | Electron Acceptor Concentration. (mg/Kg) |
| X: | Biomass concentration (mg/Kg) |

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