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Bench-Scale Treatability Study of the Slurry Phase-Bioremediation of Hamilton Harbour Sediments

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I. Introduction

Pollutants from a myriad of point and non-point sources, including industrial, agricultural, and municipal, emanating from water, land, and air, have accumulated in the water and sediments of the Great Lakes. The subsequent impairment within the Great Lakes Ecosystem has led to the designation of particular sites as Areas of Concern (AOCs). Of the 42 AOCs, 17 have been identified on the Canadian side of the Great Lakes. The Great Lakes Action Plan (GLAP), administered by Environment Canada, and supported by the Great Lakes Cleanup Fund through the Wastewater Technology Centre (WTC), has designated its resources to develop a rational approach to the assessment, removal, and remediation of the contaminated sediment.

This treatability study addresses the applicability of bioaugmentation technology in remediation of contaminated sediments. Specifically, this study focuses on the bioremediation of toxic organic contaminants (i.e. polynuclear aromatic hydrocarbons [PAHs]) in sediments dredged from the Hamilton Harbour.

Coal tar contamination in harbour sediments can exceed 1,000 parts per million (ppm). Coal tar is a heterogeneous mixture of compounds, including PAHs, formed as a biproduct of gasification. PAHs are of a significant health concern because many are suspected human and animal carcinogens. PAHs have been found to be acutely toxic *in vitro* to various prokaryotic and eukaryotic test species, including zooplankton, bacteria, rainbow trout, and mayfly nymphs. The toxicity observed in experimental bioassays has been positively correlated with PAH concentration. Further, concentrations in Hamilton Harbour sediments have been shown to exceed the LD_{50} 4- to 50-fold. Even at levels below those established for acute toxicity, the carcinogenic PAHs are implicated in wildlife tumorigenesis.

Toxicity and mutagenicity/carcinogenicity of contaminated sediments was presented by various researchers at: "The 34th Conference of The International Association for Great Lakes Research," June 2-6, 1991, State University of New York at Buffalo, Buffalo, NY. Amphipods and midges exposed to sediments from Indiana Harbor, Buffalo River, Saginaw River, and Waukegan Harbor had reduced survival after chronic but not acute exposure (Ingersoll, C.G., et al. The acute and chronic effects of contaminated Great Lakes sediment on the amphipod *Hyalella azteca* and the midges *Chironomus riparius* and *Chironomus tentans*).

Sediment extracts from the Saginaw River, Buffalo River, and Indiana Harbor were toxic to bacteria and reduced the sensitivity of the Ames/salmonella assay. Further, sediments were found to be mutagenic (Papoulias, D., et al. Mutagenic assessment of

contaminated Great Lakes sediments for the ARCS Program). Researchers have also established an association between PAHs, PCBs, and pesticides in sediments from the Buffalo River, and the carcinogenic and non-carcinogenic risk from the consumption of contaminated fish (Laniak, G.L., et al. Baseline human health risks in the Buffalo River, NY, Area of Concern).

In addition to PAHs, the Hamilton Harbour sediments have substantial concentrations of metals, including iron, zinc, lead, cadmium, copper, and mercury. The effect of these metals on sediment bioremediation cannot be firmly established without performing a feasibility study. Although the concentrations of most metals are below toxic thresholds, and none of the concentrations are known to completely inhibit bacterial activities, this study will, in part, determine their net affect on bacterial degradation of PAHs.

The object of this treatability study is to determine if the PAHs in the Hamilton Harbour sediments can be remediated in a slurry phase reactor. The success of this treatment is dependent on the ability of bacteria to degrade PAHs in an aqueous environment, and the effect of the myriad of metals on the bioremediation process. This information will aid in the development of a treatment train, addressing the relevance of this and other technologies in sediment remediation.

II. Materials and Methods

A. Experimental Overview

The proposed treatment methodology employs an augmented bioremediation approach, specifically developed to rapidly reduce polynuclear aromatic hydrocarbons (PAHs) in sediments. This proprietary process, developed by Waste Stream Technology Inc. (WST), will determine the rate and extent of contaminant reduction.

There are several factors responsible for the innovative nature and advantages of bioaugmentation versus biostimulation of indigenous organisms. The ecosystem associated with contaminated sediments results from an ability of organisms to either survive in their presence, or utilize the contaminant. Thus, there may not be an indigenous contaminant-degrading population to stimulate. If there is an indigenous contaminant degrader, it may be stimulated along with all other members of the ecosystem. Therefore, the niches which have already been established increase, resulting in the same proportions of all members, and not necessarily greatly increasing the rate of degradation (due to population dynamics which keep any individual population from outgrowing another via competitive inhibition). Biostimulation is more time demanding since time is required for natural selection of degraders, as well as for their activities to be identified. Stimulation of pathogenic organisms may also occur. The outcome of treatment is somewhat unpredictable, and the fate of the organisms and the contaminants are unknown.

Bioaugmentation has many advantages. Large-scale on-site production rapidly increases bacterial concentrations to levels associated with optimal biodegradation. The bacteria which are chosen for production are target-specific, non-pathogenic, and proven safe. They are applied when they are actively growing, and they are induced to rapidly utilize the contaminant(s). Continued applications lead to their predominance, virtually overgrowing indigenous species, including pathogens. If required, the indigenous bacteria can be reduced to non-competitive concentrations.

The activity of the amended bacteria is well understood, and their growth is completely controllable. They can be tracked throughout the process, and their activities can be correlated with contaminant degradation. The end products of their activities are known, leading to a predictable, safe resolution of the contamination. Finally, costs associated with this treatment are diminished by its rapidity, predictability, and technical merit.

B. Microcosm Design

Samples of contaminated sediment were analyzed upon receipt to establish a baseline physical, chemical, and biological profile (i.e. soil type, pH, microflora, nutrients, contaminants, etc.). Sediments were segregated into individual microcosms, consisting of 18.2 kG each. Three distinct treatment regimens were used to define each microcosm. Microcosms consisted of either (1) water alone, (2) WST Nutriblend solutions, or (3) WST Nutriblend and WST Bioblend solutions. Microcosm sizes were chosen based on sampling requirements for analyses, allowing proper biological and analytical sampling throughout the study period.

All of these studies were performed in a 60 liter slurry reactor (Eimco, Salt Lake City, Utah). Sediments were prescreened in a 30 mesh sieve, and added to dechlorinated tap water to a concentration of 30% (w/v). The slurry reactor was provided with a constant supply of air. Sediments were mixed continuously by an impeller located along the ventral surface of the reactor, and by a rotating air lift that moved sediments vertically through the apparatus.

Untreated Control. This microcosm served as a control to determine non-biological contaminant losses. These losses may occur via volatilization, adsorption to the test apparatus, and oxidation and photolysis.

Biostimulation Studies. In this study, we amended the microcosm with **WST Nutriblends.** This solution is specially designed to promote bacterial growth and replication, as well as buffer the sediment environment from pH fluctuations. It contains nitrogen, phosphorous, and trace minerals at the appropriate oxidative state to maximize degradation by indigenous microflora.

Bioaugmentation Studies. In the bioaugmentation microcosm, a specific contaminant-degrading bacterium, **WST Bioblend M-3MCA,** was added concomitant with WST Nutriblends. This organism was chosen based on historical data, as well as the pre-screen treatability performed previously. Bacteria for the bioaugmentation microcosm were grown in mineral media + carbon for 18 hours at 22°C to an OD_{600}=1.0 (late logarithmic growth phase). A 1% (v/v) inoculum was used for inoculation into the Eimco slurry reactor.

C. Biological and Chemical Analyses

Sediments were extracted according to EPA method 3550, and analysis for semi-volatiles were performed according to EPA method 8270. Briefly, 2 g of sediment was dried over sodium sulfate, then suspended in methylene chloride and sonicated. Samples were analyzed for PAH concentration by gas chromatography and mass spectroscopy. Internal standards, surrogates, method blanks, reference samples, and matrix spikes were all performed in accordance with this standardized procedure. Triplicate samples were taken on the sediment immediately after addition to the reactor, and at seven day intervals thereafter.

Nutrient profiles and pH analyses were performed on the initial sediment sample using the LaMotte water/soil test system (LaMotte Chemical Products, Chestertown, MD). The pH analyses was performed using EPA SW 846 method 150.1. Nutrients and pH were amended *ad libitum* appropriately to provide an environment that maximized microbial growth and replication. Specifically, the pH of the biostimulate and M-3MCA microcosms had to be monitored and adjusted throughout the study.

Microbial viability was monitored according to the procedures outlined in the *Standard Methods for the Examination of Water and WasteWater Method 907* and were expressed as colony forming units per gram of sediment (CFUs). Briefly, 1 gram of sediment was removed, suspended in 10 ml of sterile saline, and vortexed for 5 minutes at 22°C. The suspension was then centrifuged for 3 minutes at 1,000 x g. One ml of the supernatant fluid was removed and serially diluted 10-fold, and 100 μl was streaked onto nutrient agar plates. Plates were incubated for 48 hours at 22°C. Samples for analyses were taken on the sediment immediately after addition to the reactor, and at seven day intervals thereafter.

To determine the contaminant utilizing capability of the isolates, a contaminant-utilizing population (CUP) assay was performed periodically during the study by examination of bacterial growth on a target molecule (naphthalene) and one non-target molecule (fuel oil). Briefly, bacteria obtained from the CFU analysis plates were streaked onto minimal media and exposed to a saturated atmosphere containing either naphthalene or fuel oil. As a negative control, bacterial isolates were inoculated onto plates of MM alone. Utilization was defined as growth on test plates exceeding growth on negative control plates.

D. Statistical Analysis

The significance of the reduction in TPAH for each slurry was determined by the Analysis of Variance (ANOVA) method. The ANOVA test indicates the similarity of statistical means for differing populations. ANOVA was used to test the similarity of means for two populations: (1) Initial TPAH, (2) Final TPAH.

ANOVA was used to test the hypothesis that these normal populations all have the same mean.

The method of testing this null hypothesis is to compute two estimates for the (common) variance of the two populations in two different ways. The former involves the assumption that the means are the same. The latter does not. If the two estimates for the variance are too far apart, the assumption that the population means are the same must be wrong, so the null hypothesis is rejected. If the two estimates are not too far apart, we do not reject the null hypothesis. The F-statistic or F-ratio (derived from the F-distribution) is used to measure whether the estimates are *far apart* or *close together*.

The ANOVA table method was used to compute the F-statistic for each slurry since it permits ANOVA with unequal sample sizes.

The computed F-statistic was then compared to an F-test value which was determined from a table for 0.05 critical values of the F-distribution. An F-ratio less than the critical value indicates a non-significant statistical occurrence and the null hypothesis is not rejected, i.e. the means are similar (close together). This was interpreted as a non-significant reduction in TPAH. An F-ratio greater then the critical value indicates a significant statistical occurrence and the null hypothesis is rejected, i.e. the means are not similar (far apart). This was interpreted as a significant reduction in TPAH.

III. Results

A. Nutrient Profile

Nutrient analyses were performed on Harbour sediments using the LaMotte Chemical Test. Results showed the sediment to be silty clay, consisting of 10% sand, 46% silt, and 44% clay. The pH of the sediment was 7.7, within the physiological range of 6.0 and 8.0. Further, the sediment contained 20 ppm nitrate nitrogen, 5 ppm ammonia nitrogen, 62 ppm inorganic phosphorous, 205 ppm potassium, and 100 ppm sulfate. In addition, the sediment had high concentrations of ferric iron and aluminum, which concurs with previous analysis of the sediment.

Nitrate Nitrogen:	20 ppm	Ferric Iron:	50 ppm
Nitrite Nitrogen:	<1 ppm	Potassium:	205 ppm
Ammonia Nitrogen:	5 ppm	Sulfate:	100 ppm
Phosphorous:	62 ppm	Aluminum:	125 ppm

B. Untreated Control Microcosm

1. Analytical Data. Semi-volatile analysis of the sediment from this microcosm showed it to contain 1,039 ppm total PAH (TPAH) concentration, including 830 ppm low MW compounds, and 209 ppm high MW compounds (Figure 1A, Table 1). Low MW PAHs decreased consistently during the study, to 329 ppm at day 28. High MW PAHs fluctuated during the same period, to a final concentration of 386 ppm. Overall, there was a 31% decrease in TPAH by day 28; since there was an apparent increase in the High MW PAHs, this decrease is attributable to the loss of the volatile low MW molecules. The standard deviation was quite large for many of the samples (Table 1A). This is typically observed in highly contaminated samples, and is the result of sampling variability.

2. Biological Data. Microbial analysis showed the initial concentration of indigenous bacteria to be approximately 10^6 CFU per gram of sediment (Figure 1B, Table 2). This concentration increased an order of magnitude by day 7, then did not change appreciably through the remainder of the study. In addition, none of the organisms isolated from this sediment were able to utilize either naphthalene of fuel oil, as indicated by *in vitro* CUP analyses (Tables 3-5). This data, together with the observed disappearance of two and three ringed compounds, suggests that the loss of low MW molecules observed in this study was attributed to non-biological, rather than biological, means.

Table 1.
Hamilton Harbour Treatability
Slurry Reactor, Distilled Water Microcosm

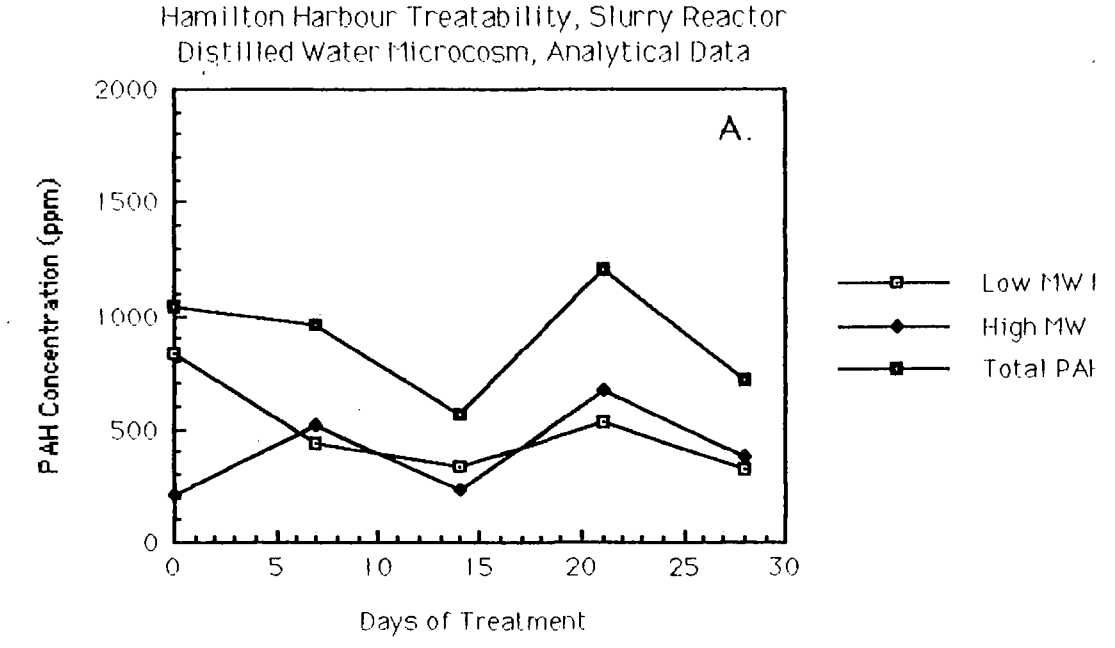
Compound	Initial	Day 7	Day 14	Day 21	Day 28
Naphthalene	551,449,616	313,145,122	233,152,245	195,174,283	89,128,267
2-Methylnaphthalene	30,27,31	15,0,0	0,0,0	8,8,15	4,5,3
Acenaphthylene	7,7,8	23,10,7	10,7,12	13,13,33	6,7,23
Acenaphthene	18,15,17	14,4,3	0,3,6	5,5,14	3,3,8
Dibenzofuran	14,12,14	11,4,3	4,3,5	5,5,12	3,3,8
Fluorene	20,17,20	20,5,3	0,0,0	7,7,23	4,4,12
Phenanthrene	85,76,86	135,31,21	25,35,47	42,46,144	26,25,76
Anthracene	29,27,30	76,15,10	11,17,21	21,27,81	13,13,38
Carbazole	0,0,0	7,2,2	0,0,0	3,3,8	1,1,4
Fluoranthene	79,72,83	222,51,32	34,55,72	72,88,219	47,44,122
Subtotal	**833,752,905**	**836,267,203**	**317,272,408**	**371,376,832**	**183,233,571**
Pyrene	64,58,67	226,46,29	27,43,35	64,79,224	40,37,115
Benzo(a)anthracene	30,28,31	137,33,21	21,30,44	47,57,149	29,28,82
Chrysene	26,26,28	140,38,24	24,33,47	54,64,162	32,33,95
Benzo(b)fluoranthene	22,24,27	128,37,27	0,13,48	48,58,149	29,30,86
Benzo(k)fluoranthene	21,21,21	133,44,34	0,31,40	53,58,154	26,28,63
Benzo(a)pyrene	26,26,27	159,50,35	40,13,59	63,72,177	36,38,107
Dibenzo(a,h)anthracene	0,0,0	20,0,0	0,0,0	11,11,25	8,9,22
Indeno(1,2,3)pyrene	10,10,10	68,23,16	24,21,17	29,31,67	22,23,57
Benzo(g,h,i)perylene	9,6,0	66,21,0	25,22,33	28,28,61	23,24,35
Subtotal	**218,199,211**	**1077,292,186**	**161,206,323**	**397,458,1168**	**245,250,662**
Total PAH	**1051,951,1116**	**1913,559,389**	**478,478,731**	**768,834,2000**	**428,483,1233**

1039

715

Table 1A.
Hamilton Harbour Treatability
Slurry Reactor, Distilled Water Microcosm
Standard Deviations of Sample Points

Compound	Initial	Day 7	Day 14	Day 21	Day 28
Low MW PAHs	833,752,905 SD±77	836,267,203 SD±349	317,272,408 SD±69	371,376,832 SD±265	183,233,571 SD±211
High MW PAHs	218,199,211 SD±10	1077,292,186 SD±487	161,206,323 SD±84	397,458,1168 SD±429	245,250,662 SD±239
Total PAHs	1051,951,1116 SD±83	1913,559,389 SD±835	478,478,731 SD±146	768,834,2000 SD±693	428,483,1233 SD±449


Hamilton Harbour Treatability, Slurry Reactor
Distilled Water Microcosm, Analytical Data
A.
PAH Concentration (ppm)
2000
1500
1000
500
0
0
5
10
15
20
25
30
Days of Treatment
Low MW I
High MW
Total PAI

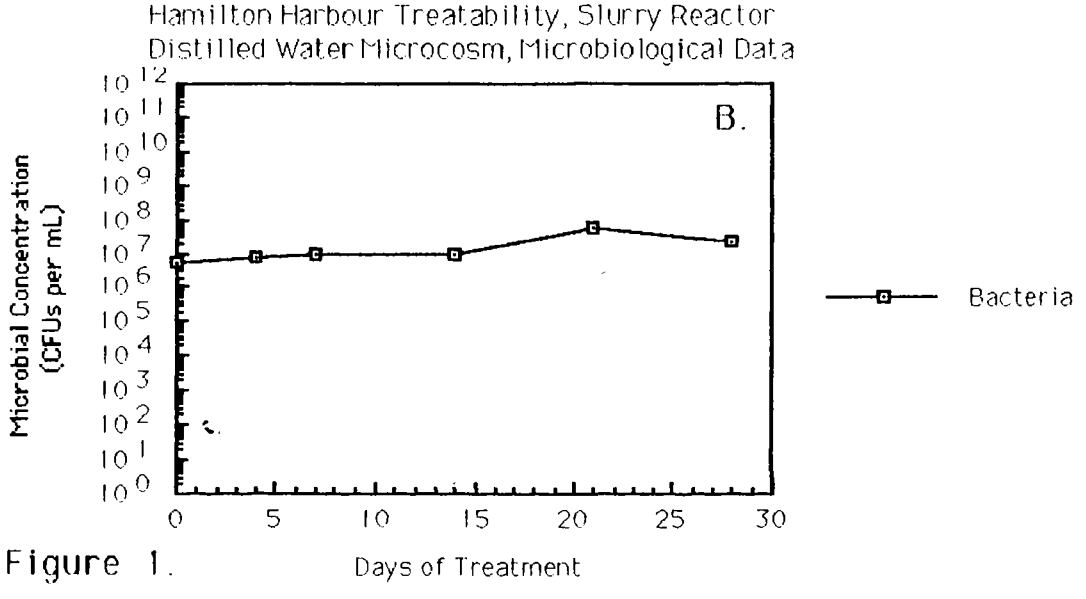

Hamilton Harbour Treatability, Slurry Reactor
Distilled Water Microcosm, Microbiological Data
B.
Microbial Concentration
(CFUs per mL)
0
5
10
15
20
25
30
Days of Treatment
Bacteria

Figure 1.

Table 2.
Hamilton Harbour Treatability
Slurry Reactor, Distilled Water Microcosm
CFU Analysis

Initial	Day 4	Day 7	Day 14	Day 21	Day 28
5.0×10^6	7.5×10^6	1.0×10^7	1.0×10^7	5.1×10^7	2.4×10^7

Table 3.
Hamilton Harbour Treatability
Slurry Reactor, Distilled Water Microcosm
CUP Analysis

Initial Sample

Colony Form	% Overall Population
1. Beige, medium, wrinkled, circular	15%
2. Medium, flat, orange, semi-transparent	2%
3. Medium, beige, transparent	38%
4. Medium, shiny, tan, irregular shape	26%
5. Large, yellow, shiny, opaque	6%
6. Medium, star shaped	13%

Each isolate was tested for naphthalene utilization on MM/naphthalene and MM/fuel oil plates. None of the isolates grew after 7 days incubation.

Table 4.
Hamilton Harbour Treatability
Slurry Reactor, Distilled Water Microcosm
CUP Analysis

Day 14 Sample

Colony Form	% Overall Population
1. Large, pink, shiny, pink	17%
2. Medium, creamy, opaque, shiny	11%
3. Small, yellow, opaque, shiny	8%
4. Medium, yellow. opaque,	3%
5. Medium-large, white, shiny	55%

Each isolate was tested for naphthalene utilization on MM/naphthalene and MM/fuel oil plates. None of the isolates grew after 7 days incubation.

Table 5.
Hamilton Harbour Treatability
Slurry Reactor, Distilled Water Microcosm
CUP Analysis

Day 28 Sample

Colony Form	% Overall Population
1. Small, creamy, shiny, translucent	72%
2. Medium-sized, pink, shiny, convex	11%
3. Medium, yellow, shiny, opaque	13%
4. Medium, orange, shiny	4%

Each isolate was tested for naphthalene utilization on MM/naphthalene and MM/fuel oil plates. None of the isolates grew after 7 days incubation.

C. Biostimulation Microcosm

1. Analytical Data. Semi-volatile analysis of the sediment from this microcosm showed it to contain 522 ppm TPAH, including 323 ppm low MW compounds, and 199 ppm high MW compounds (Figure 2A, Table 6). This total PAH concentration is much less than in either the distilled water or M-3MCA microcosm, and is most likely due to sampling error from the pooled site sediments. By day 28, the low MW PAHs were at 191 ppm, representing a 41% reduction over the initial sample. As in the distilled water microcosm, there was an apparent increase in the concentration of high MW PAHs, to 315 ppm. There was a 3% decrease in TPAH by day 28, and this decline may again be attributed to loss of volatile low MW molecules. The standard deviation was calculated for each data point, and was less than that determined for the distilled water microcosm (Table 6A), and is consistent with standard deviations observed in data analyzed from lesser contaminated samples.

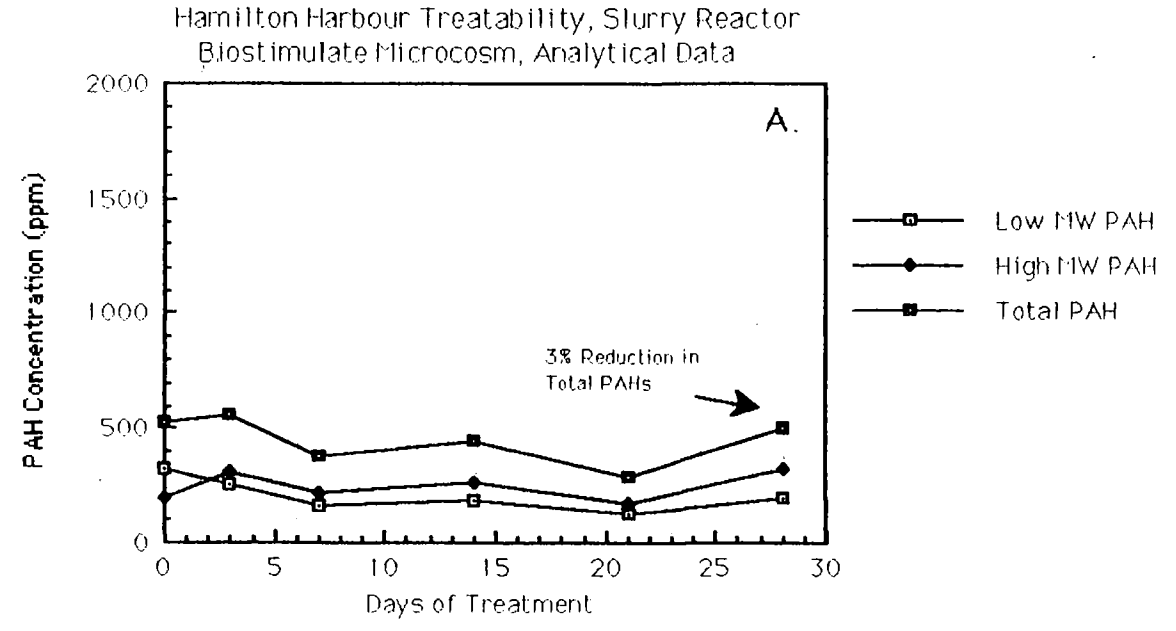
2. Biological Data. Microbial analysis showed the initial concentration of indigenous bacteria to be approximately 10^7 CFU per gram of sediment (Figure 2B, Table 7). This concentration increased 4 orders of magnitude by day 3, then fluctuated between 10^{11} and 10^7 through the remainder of the study. Despite the extraordinarily high concentration of bacteria none of the isolates were able to utilize the contaminant, as indicated by *in vitro* CUP analyses (Tables 8-9). Again, this suggests that the loss of low MW molecules observed in this study was attributed to non-biological, rather than biological, means.

Table 6.
Hamilton Harbour Treatability
Slurry Reactor, Biostimulate Microcosm

Compound	Initial	Day 3	Day 7	Day 14	Day 21	Day 28
Naphthalene	91	65,50	40,44,42	56, 39, 43	37,36,43	41,48,36
2-Methylnaphthalene	10					
Acenaphthylene	6	6,8	3,4,4	8, 5, 8	4,4,5	7,9,5
Acenaphthene	15	10,7	4,3,4	5, 3, 3	3,2,3	3,6,2
Dibenzofuran	12	6,5	3,3,3	5, 3, 4	2,2,3	4,4,3
Fluorene	18	9,7		7, 4, 7	4,3,4	6,7,13
Phenanthrene	74	53,38	28,27,28	47, 25, 48	24,21,23	44,54,27
Anthracene	21	23,17	10,13,12	7, 9, 17	9,8,8	17,23,10
Carbazole				2, 1, 3	1,1,1	2,2,1
Fluoranthene	76	101,86	61,73,70	87, 42, 76	40,35,37	69,92,47
Subtotal	**323**	**273,218**	**149,167,163**	**224,131,209**	**124,112,127**	**193,245,134**
Pyrene	56	89,65	50,61,61	72, 41, 66	41,39,42	65,80,47
Benzo(a)anthracene	25	42,37	24,30,28	40, 21, 35	21,20,22	31,50,27
Chrysene	33	37,38	25,30,28	42, 23, 36	21,21,22	37,53,29
Benzo(b)fluoranthene	21	39,33	24,25,24	33, 19, 33	20,19,18	34,47,25
Benzo(k)fluoranthene	20	43,33	21,30,27	35, 19, 29	19,19,22	34,49,28
Benzo(a)pyrene	24	48,42	27,32,29	43, 23, 38	22,21,23	40,57,31
Dibenzo(a,h)anthracene	3			2, 4, 7	4,2,2	9,12,7
Indeno(1,2,3)pyrene	9	20,21	13,14,12	24, 15, 23	12,11,12	24,31,19
Benzo(g,h,i)perylene	8	19,20	12,14,6	25, 16, 25	12,11,11	25,31,18
Subtotal	**199**	**337,289**	**196,236,215**	**316,181,292**	**172,163,164**	**304,410,231**
Total PAH	**522**	**610,507**	**345,403,378**	**540,312,501**	**296,275,291**	**497,655,365**

Table 6A.
Hamilton Harbour Treatability
Slurry Reactor, Biostimulate Microcosm
Standard Deviations of Sample Points

Compound	Initial	Day 3	Day 7	Day 14	Day 21	Day 28
Low MW PAHs	323	273,218 SD±39	149,167,163 SD±9	224,131,209 SD±50	124,112,127 SD±8	193,245,134 SD±56
High MW PAHs	199	337,289 SD±34	196,236,215 SD±20	316,181,292 SD±72	172,163,164 SD±5	304,410,231 SD±90
Total PAHs	522	610,507 SD±73	345,403,378 SD±29	540,312,501 SD±122	296,275,291 SD±11	497,655,365 SD±145


Hamilton Harbour Treatability, Slurry Reactor
Biostimulate Microcosm, Analytical Data
A.
PAH Concentration (ppm)
2000
1500
1000
500
0
3% Reduction in
Total PAHs
0
5
10
15
20
25
30
Days of Treatment
Low MW PAH
High MW PAH
Total PAH

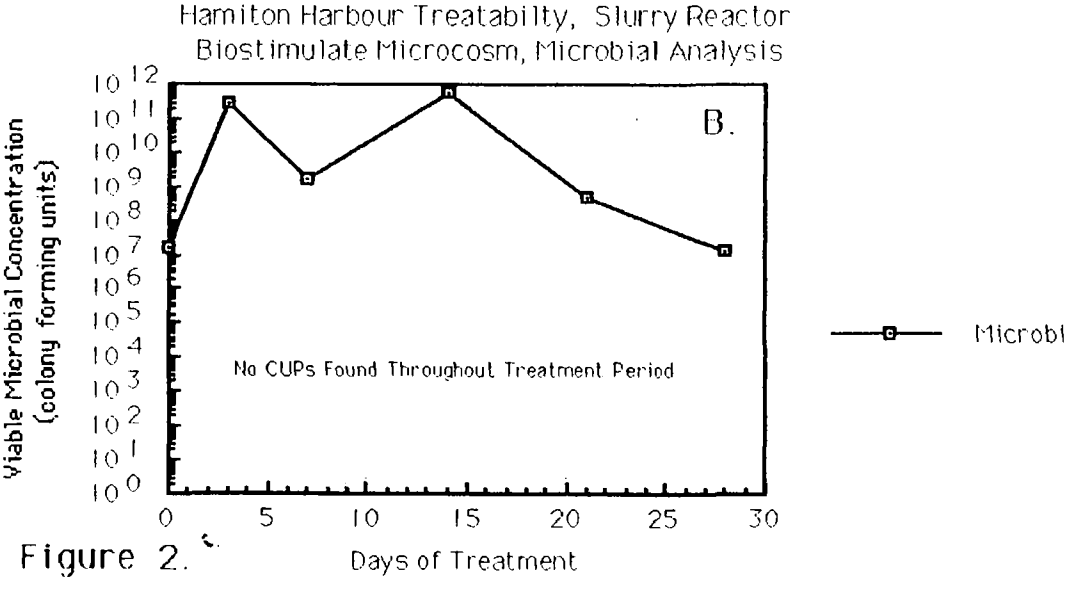

Hamiton Harbour Treatabilty, Slurry Reactor
Biostimulate Microcosm, Microbial Analysis
B.
Viable Microbial Concentration
(colony forming units)
No CUPs Found Throughout Treatment Period
0
5
10
15
20
25
30
Days of Treatment
Microbl

Figure 2.

Table 7.
Hamilton Harbour Treatability
Slurry Reactor, Biostimulate Microcosm
CFU Analysis

Initial	Day 3	Day 7	Day 14	Day 21	Day 28
1.5×10^7	2.9×10^{11}	1.7×10^9	5.4×10^{11}	4.8×10^8	1.4×10^7

Table 8.
Hamilton Harbour Treatability
Slurry Reactor, Biostimulate Microcosm
CUP Analysis

Day 7 Analysis:

Colony Form	% Overall Population
1. Large, irregular, flat, creamy	3%
2. Medium, orange, shiny, translucent	4%
3. Medium, opaque, yellow	10%
4. Medium, creamy, shiny, convex	53%
5. Medium, creamy, convex, opaque, shiny	14%
6. Small, white, convex	16%

Each isolate was tested for naphthalene utilization on MM/naphthalene and MM/fuel oil plates. None of the isolates grew after 7 days incubation.

Table 9.
Hamilton Harbour Treatability
Slurry Reactor, Biostimulate Microcosm
CUP Analysis

Day 14 Analysis:

Colony Form	% Overall Population
1. Large, flat, tan semi-transparent	1%
2. Medium, orange, shiny	2%
3. Medium, irregular, wrinkled	2%
4. Small, creamy, pink	20%
5. Small, white, opaque, shiny	69%
6. Medium, creamy, opaque, shiny	6%

Each isolate was tested for naphthalene utilization on MM/naphthalene and MM/fuel oil plates. None of the isolates grew after 7 days incubation.

D. Bioaugmentation **(M-3MCA)** Microcosm

1. Analytical Data. Semi-volatile analysis of the sediment from this microcosm showed it to contain 1,801 ppm TPAH, including 760 ppm low MW compounds, and 1,041 ppm high MW compounds (Figure 3A, Table 10). Low MW PAHs decreased to 97 ppm by day 28, after which they fluctuated slightly until day 42. Overall, there was an approximately 87% reduction in these 2 and 3 ring compounds. In addition, the concentration of heavier, less volatile 4 and 5 ring compounds decreased consistently to 107 ppm by day 28. By day 42, the concentration was 159 ppm, representing an 85% reduction from the initial sample. The standard deviation was calculated for various sample points, and in general was large during the early phase of the study, and was smaller and showed less variability as the soil was remediated.

2. Biological Data. Microbial analysis showed the initial concentration of indigenous bacteria to be approximately 10^5 CFU per gram of sediment (Figure 3B, Table 11). This concentration to 10^8 by day 3, then fluctuated between 10^{10} and 10^7 through the remainder of the study. Unlike the two control microcosms, a naphthalene utilizer was isolated from the augmented microcosm, as determined by *in vitro* CUP analyses (Tables 12-15). Further, the growth characteristics and colony morphology of this organism resembled that of WST Bioblend M-3MCA, suggesting this organism was responsible for the PAH degradation observed in this microcosm.

Table 10.
Hamilton Harbour Treatability
Slurry Reactor, M-3MCA Microcosm

Compound	Initial	Day 3	Day 7	Day 14	Day 21
Naphthalene	122,126,141	47,45,106	55,68,76	53,41,64	29,33,26
2-Methylnaphthalene	13,15,17	0,4,10	4,6,8	0,0,0	0,0,0
Acenaphthylene	20,30,31	8,16,24	9,11,25	7,5,10	3,3,0
Acenaphthene	22,28,33	5,9,18	8,9,17	5,3,6	2,2,1
Dibenzofuran	18,22,24	4,8,15	6,7,22	4,3,5	2,2,2
Fluorene	31,41,43	7,16,28	12,13,43	6,4,9	0,0,0
Phenanthrene	150,183,194	40,90,147	70,80,233	42,28,62	15,19,12
Anthracene	78,97,102	21,38,78	26,37,76	16,10,26	6,7,4
Carbazole	12,15,16	4,10,10	5,5,20	2,0,3	0,0,0
Fluoranthene	192,226,238	97,113,199	93,119,244	8,5,14	2,3,16
Subtotal	**658,783,839**	**233,349,635**	**288,355,794**	**143,99,199**	**59,69,61**
Pyrene	171,221,236	65,84,205	96,120,224	77,59,99	31,40,27
Benzo(a)anthracene	106,138,144	32,48,122	49,61,102	39,26,51	14,19,13
Chrysene	110,146,151	32,51,127	51,61,104	39,26,54	14,18,13
Benzo(b)fluoranthene	99,148,144	29,42,124	44,55,97	35,24,48	14,17,12
Benzo(k)fluoranthene	114,149,184	27,47,130	52,60,103	36,22,44	13,15,12
Benzo(a)pyrene	122,166,173	33,54,145	57,68,115	44,27,60	16,20,14
Dibenzo(a,h)anthracene	0,26,6	1,9,10	10,4,18	0,0,0	0,0,0
Indeno(1,2,3)pyrene	57,71,63	16,26,60	26,27,46	0,0,42	0,0,0
Benzo(g,h,i)perylene	55,67,56	15,27,19	25,25,43	0,0,44	0,0,0
Subtotal	**834,1132,1157**	**250,391,942**	**410,481,852**	**270,184,490**	**102,129,91**
Total PAH	**1492,1915,1996**	**483,740,1577**	**698,836,1646**	**413,283,699**	**161,198,152**

1801

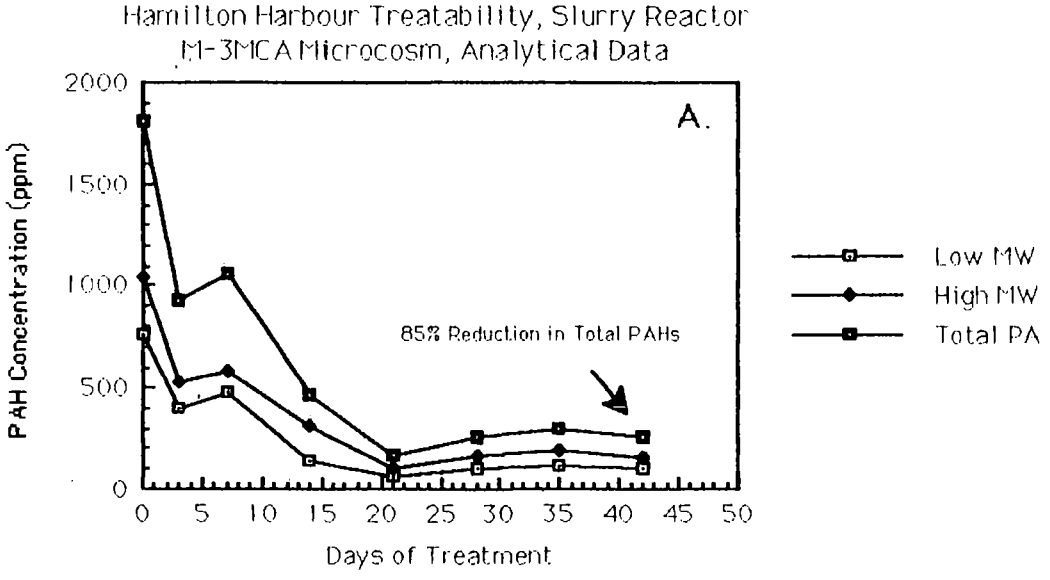
Table 10 (cont'd).
Hamilton Harbour Treatability
Slurry Reactor, M-3MCA Microcosm

Compound	**Day 28**	**Day 35**	**Day 42**
Naphthalene	39,38,29	30,34,33	34,34,30
2-Methylnaphthalene	0,0,0	0,0,0	0,0,0
Acenaphthylene	4,5,4	0,0,0	4,4,4
Acenaphthene	2,2,2	2,2,4	2,3,2
Dibenzofuran	3,3,2	2,2,3	2,2,2
Fluorene	3,3,2	0,0,0	0,0,0
Phenanthrene	17,21,15	19,22,34	20,23,23
Anthracene	6,8,6	7,8,15	7,9,9
Carbazole	1,1,1	0,0,0	0,0,0
Fluoranthene	27,33,24	28,33,52	29,34,37
Subtotal	**102,114,75**	**88,101,141**	**98,109,107**
Pyrene	46,45,39	29,33,47	26,30,30
Benzo(a)anthracene	23,24,19	21,22,32	20,22,22
Chrysene	22,24,19	21,22,33	21,23,22
Benzo(b)fluoranthene	22,25,19	20,22,29	17,21,15
Benzo(k)fluoranthene	17,18,15	17,15,28	18,18,19
Benzo(a)pyrene	22,25,19	22,23,34	22,24,23
Dibenzo(a,h)anthracene	0,0,0	0,0,0	0,0,0
Indeno(1,2,3)pyrene	11,13,9	12,11,16	13,14,13
Benzo(g,h,i)perylene	6,12,4	22,11,30	14,15,14
Subtotal	**169,186,143**	**164,159,249**	**151,167,158**
Total PAH	**271,300,228**	**252,260,390**	**249,276,265**

Table 10A.
Hamilton Harbour Treatability
Slurry Reactor, M-3MCA Microcosm
Standard Deviations of Sample Points

Compound	Initial	Day 3	Day 7	Day 14	Day 21
Low MW PAHs	658,783,839 SD±93	233,349,635 SD±207	288,355,794 SD±275	143,99,199 SD±50	59,69,61 SD±5.3
High MW PAHs	834,1132,1157 SD±180	250,391,942 SD±366	410,481,852 SD±237	270,184,490 SD±158	102,129,91 SD±20
Total PAHs	1492,1915,1996 SD±271	483,740,1577 SD±572	698,836,1646 SD±512	413,283,699 SD±213	161,198,152 SD±24

Compound	Day 28	Day 35	Day 42
Low MW PAHs	102,114,75 SD±20	88,101,141 SD±28	98,109,107 SD±5.9
High MW PAHs	169,186,143 SD±22	164,159,249 SD±51	151,167,158 SD±8.0
Total PAHs	271,300,228 SD±36	252,260,390 SD±78	249,276,265 SD±14


Hamilton Harbour Treatability, Slurry Reactor
M-3MCA Microcosm, Analytical Data
A.
PAH Concentration (ppm)
2000
1500
1000
500
0
85% Reduction in Total PAHs
0 5 10 15 20 25 30 35 40 45 50
Days of Treatment
Low MW
High MW
Total PA

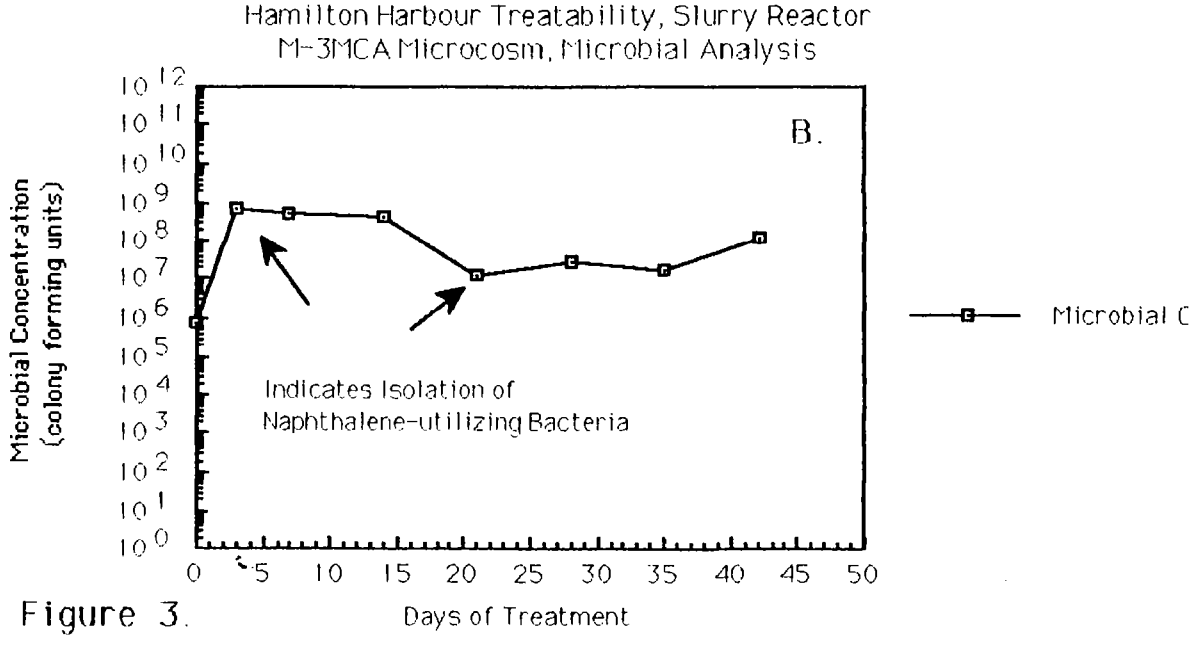

Hamilton Harbour Treatability, Slurry Reactor
M-3MCA Microcosm, Microbial Analysis
B.
Microbial Concentration
(colony forming units)
Indicates Isolation of
Naphthalene-utilizing Bacteria
0 5 10 15 20 25 30 35 40 45 50
Days of Treatment
Microbial C

Figure 3.

Table 11.
Hamilton Harbour Treatability
Slurry Reactor, M-3MCA Microcosm
CFU Analysis

Initial	Day 3	Day 6	Day 7	Day 14	Day 21
7.8×10^5	6.3×10^8	5.5×10^{10}	4.6×10^8	4.4×10^8	1.3×10^7

Day 24	Day 27	Day 35	Day 41
5.4×10^8	2.8×10^7	1.8×10^7	1.2×10^8

The pH was adjusted on days 14, 21, and 23 with additions of dibasic potassium phosphate to neutrality.

Table 12.
Hamilton Harbour Treatability
Slurry Reactor, M-3MCA Microcosm
CUP Analysis

Day 3 Analysis:

Colony Form	% Overall Population
1. Small, white, opaque, convex	29%
2. Medium, creamy-pink, irregular, opaque	19%
3. Medium, beige, flat, rough, opaque	38%
4. Large, flat, transparent, pink-orange, flat	4%
5. Small, yellow, opaque, convex	6%
6. Small, orange, flat, opaque	6%

Each isolate was tested for naphthalene utilization on MM/naphthalene and MM/fuel oil plates. Isolates 3 grew after 2 days incubation, and growth was comparable to the *in vitro* M-3MCA isolates.

Table 13.
Hamilton Harbour Treatability
Slurry Reactor, M-3MCA Microcosm
CUP Analysis

Day 7 Analysis:

Colony Form	% Overall Population
1. Small, yellow, translucent, convex	10%
2. Medium, orange, translucent, convex	3%
3. Medium, creamy, opaque, convex	4%
4. Small, beige, round, convex, shiny	23%
5. Medium, flat, white, rough	16%
6. Medium, creamy-yellow, translucent	19%
7. Medium, pink, opaque, irregular	25%

Each isolate was tested for naphthalene utilization on MM/naphthalene and MM/fuel oil plates. None of the isolates grew after 7 days incubation.

Table 14.
Hamilton Harbour Treatability
Slurry Reactor, M-3MCA Microcosm
CUP Analysis

Day 24 Analysis:

Colony Form	% Overall Population
1. Large, irregular, lobate, creamy-pink	9%
2. Medium, flat edge, beige	18%
3. Small, translucent, tan, shiny	32%
4. Small, translucent, white, shiny	21%
5. Medium, flat, irregular, rough, white	14%
6. Medium, shiny, tan, convex, opaque	6%

Each isolate was tested for naphthalene utilization on MM/naphthalene and MM/fuel oil plates. Isolates 2 grew after 2 days incubation, and growth was comparable to the *in vitro* M-3MCA isolates.

Table 15.
Hamilton Harbour Treatability
Slurry Reactor, M-3MCA Microcosm
CUP Analysis

Day 35 Analysis:

Colony Form	% Overall Population
1. Medium, white, opaque, convex, shiny	13%
2. Small, white, shiny, semi-translucent	72%
3. Large, creamy, dull, rough	½%
4. Medium, flat, star-shaped, creamy-pink	12%
5. Large, flat, wrinkled, creamy-yellow	5%
6. Medium, convex, shiny, creamy-yellow	1½%

Each isolate was tested for naphthalene utilization on MM/naphthalene and MM/fuel oil plates. None of the isolates grew after 7 days incubation.

E. Statistical Analysis

1. Distilled Water Microcosm. The computed F-ratio, 1.5, is less than both the F-test values at the $0.05_{(1,4)}$ level of significance, 7.71, and $0.01_{(1,4)}$ level of significance, 21.2 (Figure 4). Since the F-ratio value falls within the confidence region of the F-distribution, the null hypothesis is not rejected. Therefore the means are not significantly different (they are close together) and there was no significant reduction in TPAH.

2. Biostimulate Microcosm. The computed F-ratio, 0.0764, is less than both the F-test values at the $0.05_{(1,2)}$ level of significance, 18.5, and the $0.01_{(1,2)}$ level of significance. Since the F-ratio value falls within the confidence region the null hypothesis is not rejected. Therefore the means are not significantly different (they are close together) and there was no significant reduction in TPAH.

3. M-3MCA Microcosm. The computed F-ratio, 95.6, is greater than both the F-test values at the $0.05_{(1,4)}$ level of significance, 7.71, and the $0.01_{(1,4)}$ level of significance, 21.2. Since the F-ratio value falls in the critical region the null hypothesis is rejected. Therefore the means are significantly different (they are far apart) and there was a significant reduction in TPAH.

Distilled Water Microcosm

Source of Variance	d.f.	Sum of Squares	Mean Square	F-ratio
Treatment	1	158,112.6	158,112.6	1.5
Error	4	418,333.4	104,583.4	
Total	5	576,446.0		

$F\ test_{.05(1,4)} = 7.71$

$F\ test_{.01(1,4)} = 21.2$

Biostimulate Microcosm

Source of Variance	d.f.	Sum of Squares	Mean Square	F-ratio
Treatment	1	1,610.05	1,610.05	0.0764
Error	2	42,162.7	21,081.35	
Total	3	43,772.75		

$F\ test_{.05(1,2)} = 18.5$

$F\ test_{.01(1,2)} = 98.5$

M-3MCA Microcosm

Source of Variance	d.f.	Sum of Squares	Mean Square	F-ratio
Treatment	1	3,546,628.1	3,546,628.1	95.6
Error	4	146,870.7	36,717.7	
Total	5	3,693,498.8		

$F\ test_{.05(1,4)} = 7.71$

$F\ test_{.01(1,4)} = 21.2$

Figure 4. Statistical Analysis of Treatment, by Microcosm.

IV. Discussion

The goal of this treatability study was to determine the feasibility of bioremediation technology in the remediation of PAHs in material dredged from the Hamilton Harbour. A slurry phase microcosm design was chosen in order to replicate field conditions. Dredged sediments would have a high water content, and dewatering or drying may be logistically difficult and expensive when comparing alternative treatment technologies.

The results of this study indicate that slurry-phase treatment of dredged Hamilton Harbour sediments with WST Bioblend M-3MCA resulted in a significant degradation of PAHs as measured by ANOVA. In addition, the soil metals did not appear to interfere substantially with the remediation process. However, this can only be firmly established by removing the sediment metals and comparing the degradation rates to native sediments.

Bioremediation of PAH-contaminated sediment was dependent on bioaugmentation with Waste Stream Bioblends, as there was no significant degradation in either the distilled water microcosm, or the biostimulate microcosm. The rate of degradation was maximum during the first 14 days of treatment, and became asymptotic thereafter. Consequently, slurry phase bioremediation may be expected to clean the sediment in a timely manner. Although slurry reactors allow less treatment of material when compared with ex situ solid phase, the loss in material handling is made up in the quick treatment turnaround. The Eimco reactor enables a relatively high capacity slurry treatment, with an allowable solids concentration of 40%.

Further degradation, beyond the concentrations observed at the termination of the microcosm, may be expected with additional nutrient supplements, prolonged incubation times, and additional inoculations with WST Bioblends. The additional inoculations would be expected to re-establish a predominant population of contaminant-utilizing microorganisms. After several months, there is a natural attrition of organisms due to the toxic nature of some contaminants, and idiosyncratic population dynamics. Without additional inoculations, the indigenous population would ultimately overtake the bioaugmented bacteria.

The preliminary bench-scale treatability study was performed in order to measure the applicability of bioremediation technology. In addition, prescreening several WST Bioblends simultaneously predicted the most effective WST Bioblend in slurry-phase remediation of Hamilton Harbour sediments. WST Bioblend M-3MCA showed usefulness in the preliminary screening, as well previous slurry-phase treatability studies performed in this laboratory.

There was a significant decrease (up to 60%) in the

concentration of the volatile 2 and 3 ring PAH compounds in both the distilled water and biostimulate control microcosms. This loss, together with the lack of identifiable CUPs in these microcosms, suggests that these changes are attributed to volatilization. The constant mixing and aeration of the sediments in the slurry reactor provided an ideal atmosphere to induce volatilization. In order to determine precise volatile losses, mass balance experiments should be performed while capturing volatile air emissions with a carbon trap.

A consistent feature of the analytical data from the distilled water and biostimulate microcosms is the apparent paradox comparing the change in concentrations of the low and high molecular weight PAHs over time. Specifically, as the low weight PAHs decrease in concentration, the heavier compounds appear to increase. Since PAHs were not routinely added to the sediment, this observation must be artifactual, and related to the concentration of PAHs and non-target compounds in the sediment. For example, there may be a difference in the extraction efficiency of these heavier compounds as other molecules volatilize or are degraded. Specifically, the initial dredged soil is contaminated with a plethora of petroleum compounds, including PAHs. As these and other compounds are lost, either through biotic or abiotic means, the remaining PAHs are more effectively solubilized by the extraction solvent. This observation is consistent in the pre-screening experiment and the Eimco slurry phase treatability, and is supported by examination of the chromatograms.

Chromatograms from the pre-treated sediments show a high baseline of unidentified material. Previous research has shown that similar profiles represent petroleum compounds, including straight and branched chain hydrocarbons. The baseline is greatly reduced after several weeks of treatment. The net result is to skew the concentrations of the residual molecules. This effect was not observed in the M-3MCA microcosm because the bacteria specifically target the contaminants, with little effect on the ancillary compounds.

Carbon, in sediment PAHs, served as an energy source and building block for macromolecular synthesis, and, in various oxidative states, is used by living organisms in macromolecular syntheses and as an energy source. During macromolecular syntheses, carbon is used during growth and replication in nucleic acid, carbohydrate, protein, and lipid synthesis. As an energy source, reduced carbon is biochemically oxidized during a complex series of reactions, and the energy released during each reaction is captured on the molecular level by $NADH_2$, $FADH_2$, or ATP. Therefore, bioremediation of sediment PAHs offers a permanent solution to for these pollutants. Contaminants are recycled into the ecosystem as CO_2, water, and biomass.

Biological analysis indicated modest concentrations of *in vitro* contaminant-utilizing microorganisms in the M-3MCA microcosm. CFU and CUP analyses are performed because these tests provide a good indicator as to the ongoing biological activity in the soil. The goal of bioaugmentation is to elevate the concentration of soil microorganisms above baseline levels, and maintain their growth and replication during the treatment regiment. This results in high concentrations of a contaminant utilizing population, which is further substantiated by *in vitro* CUP tests.

The results of this treatability address the utility of biostimulation bioremediation technology. Specifically, the stimulation of indigenous bacteria by WST Nutriblends resulted in a very high concentration of viable bacteria, as indicated by CFU analysis. However, analytical data showed no change in the concentration of total PAHs. Therefore, although there were significant numbers of bacteria, none of the strains effectively degraded the sediment PAHs. This hypothesis is supported by the lack of a CUP isolated during any of the sampling points of this microcosm.

In summary, based on data obtained in this treatability study, it may be concluded that slurry phase bioremediation of Hamilton Harbour sediments using WST Bioblend M-3MCA may be expected to result a timely and effective reduction in the concentration of target PAHs.