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Evaluation of beeswax based petroleum bioremediation products

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Abstract

PRP® is physically modified beeswax that stimulates the natural microbial population to degrade oil. The focus of this study was to demonstrate the use of PRP in the form of a small boom that can retain and enhance the degradation of the retained oil. Diesel fuel was used as the model oil. The experimentation was performed using a custom designed model bilge reactor in a microcosm study. We demonstrate that the observed accelerated degradation was due to the PRP stimulating the natural microbial population capable of degrading hydrocarbon.

We describe a series of experiments designed to determine the efficacy and mechanism of the commercial product PRP/BioBoom®. Experiments were carried out in specially constructed tanks, which exposed the oil spill response product to near-environmental conditions (mesocosm). We found that: PRP enhanced biodegradation of the model oil compared to the non-stimulated natural population; 97 and 76 percent reduction in measured aliphatic and aromatics, respectively, compared to the non-stimulated natural population which degraded the aliphatic and aromatics 48 and 5 percent, respectively. PRP used as a boom (BioBoom) was observed to absorb free oil and enhance its biodegradation within the boom. Eighty-three percent of the aliphatics and 51 percent of the aromatics were degraded within the boom.

A theory for the efficacy of the material and its potential benefits in marine management and pollution control is proposed.

1 Introduction

The discharge of oily wastes into waterways is a common and global concern. Estimates place United States petroleum consumption at over 290 billion gallons each year. Due to the challenge of transport, refining, distribution, and

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petroleum product use an average of 13,000 spills are reported annually. Major spills generate public awareness, but the United States Environmental Protection Agency (EPA) has realized that non-point source discharge of oil results in a considerable amount of pollution. The EPA estimates that 150 – 450 million gallons of oil/fuel are discharged into US waterways annually. To put this figure into perspective, the Exxon Valdez accident released approximately 10.1 million gallons, only $1/15^{th}$ of what the US releases on an annual basis.

Petrol Rem, Inc. is commercializing a series of oil spill response bioremediation products under the name of PRP. PRP has been listed on the U.S. Environmental Protection Agency (EPA) National Contingency Plan's Product Schedule since November 1990 as a biological additive. Melted beeswax is converted into hollow microscopic spheres. The spheres are left loose or encased in oblong fabric bags of various diameters and lengths depending on the intended application. Due to the oleophilic properties of the beeswax, and its overall bulk density, the products float on the surface of the water allowing oil and other petroleum products to bind on contact.

Petrol Rem's product line includes various forms of PRP including their containment booms and bilge socks. The purpose of the products is to contain, absorb and facilitate the biodegradation of the spilled oil. Because both the beeswax and petroleum biodegrade, disposal is limited to the fabric bag.

2 Literature Review

2.1 Bacteria

The details of physiology and ecology of microbiological hydrocarbon metabolism, has been documented [1,2]. Knowledge of how bacteria can use oil as a carbon source, and the metabolic pathways associated with its use has led to the possibility of exploiting these processes to aid in oil degradation. This can be accomplished by stimulating the indigenous microbial population (biostimulation) or by adding specific microorganisms (bioaugmentation), Atlas, Leahy and Colwell, Dietz, and Swannell [1,2,3,4], have all reviewed the practice and techniques of these two strategies.

Most environments contain hydrocarbon-degrading microbes. The most common metabolic pathway for microbial consumption of petroleum is terminal oxidation, and subsequent β-oxidation [6] to permit the microorganism to get the carbon into its intermediary metabolism. Adding petroleum causes the bacterial population to shift to a higher percentage of hydrocarbon-degrading organisms [3,5,7].

Microorganisms are known to exist in both free floating (planktonic) and "bound" states (sessile). According to Kirchman and Mitchell [8] bacteria bound to particles or surfaces provide more than 40% of the heterotrophic activity while only comprising 10% of the population. This is particularly important in the case of PRP, an oleophilic particle. When in contact with oil the wax and petroleum create a semi-solid matrix of oil and wax. The material creates a surface area, allowing bacteria to bind stimulating the bacterial activity [9].

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2.2 Metabolism

Metabolic activity is dependent on the concentrations of specific nutrients, and may be sub optimal if one or more are deficient [9]. Bacteria and other microorganisms require phosphorus and nitrogen to perform normal metabolic activities [10].

Other compounds not expressly required for metabolic functions, have been shown to have a stimulatory effect on bacterial metabolism. Research suggests that the presence of fatty acids can actually help to initiate and increase the amount of hydrocarbon degradation [12], which partly explains the positive benefits of beeswax's presence on biodegradation rates.

2.3 Beeswax

Beeswax composition is complex and varies geographically, seasonally and with indigenous vegetation [13,14]. It is composed of primarily a mixture of fatty acid esters averaging carbon chains of approximately 40 carbon atoms [13]. Tulloch [15] further explained composition in 1980 when he determined that the esters were present in both monoester and more complex forms, such as diesters, triesters, and hydroxyesters. In total, there are more than 300 different compounds in natural beeswax, only four are present in an amount more than 5% of the total: C_{40} (6%), C_{46} (8%), and C_{48} (6%) as monoesters, and C_{24} acid (6%). Beeswax is biodegradable [16,17].

3 Objectives

Evaluations by the US EPA demonstrated that PRP enhanced biodegradation of oil but only slightly, and this research sought to confirm product performance in a "real world environment." A previous *in-situ* test verified the ability of PRP to hold and not release oil into the environment, but a true measure of the biodegradative capabilities wasn't determined [18]. A flowing mesocosm test, mimicking a Tier III evaluation as described by NETAC [19], designed to simulate a fresh water stream was used to. The full report covers a review of the product testing methods, a description of both the field and laboratory-generated data, and an analysis (with statistics) of the experimental results.

Following are the objectives the study: evaluate the biodegradation of petroleum hydrocarbons by products manufactured with and without added microorganisms, determine if the product is beneficial regarding biodegradation when compared to a synthetic absorbent boom, and investigate the effectiveness of the loose beeswax product alone.

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4 Methods and Materials

4.1 Biodegradation analysis using the Bilge Model Reactor

4.1.1 Bilge model reactor set-up

The bilge model reactor (BMR) as used in the experimentation is shown in Figure 1. The beaker size was 400 mL to accommodate $^1/_4$ inch glass tubing for the inlet and outlet. To prevent the contents from draining completely when flow was started required the addition of a "siphon break" on the outlet.

Water was sampled from Point State Park located at the confluence of the Allegheny, Monongahela and Ohio rivers in Pittsburgh, PA on the day of the test. The water was immediately transferred to the laboratory and dispensed into the BMR's. Two hundred milligrams of fresh diesel fuel was added to the reactors. BMR's requiring Beeswax products were given 1 gram of material to each flask. [See results for experimental design.] At this point, time 0 samples were sacrificed for analysis. At time 2 weeks and 4 weeks BMR's were sacrificed for analysis. The BMR's were gently shaken for the appropriate time on a rotary shaker – the rate was below the calibration of the shake table but gently moved the liquid on the glass but did not cause any losses through the opening in the BMR's. All BMR had covers that prevented contamination of the experiment but air could readily enter the reactor.

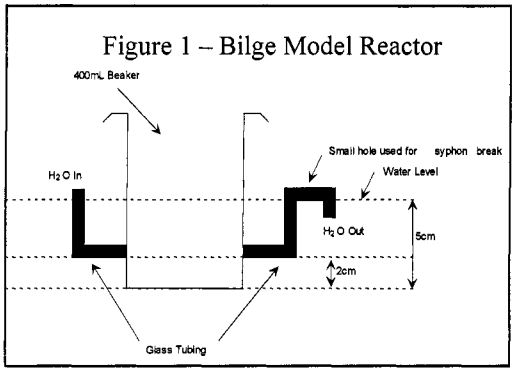

Figure 1 – Bilge Model Reactor

4.1.2 Extraction methods

The extraction method followed EPA SW 846 Method 3510 – Separatory Funnel Method modified to accommodate our sampling strategy. The liquid from the Bilge Model Reactors (BMR's) was transferred to a 500 mL separatory funnel making sure that samples containing the materials were transferred to the funnel. A 50 mL aliquot of methylene chloride (CH_2Cl_2) was added. The contents were mixed. After extraction, the funnel was permitted to stand and the 2-phases to separate. The organic phase was collected for immediate GC analysis.

The extraction efficiency for the diesel fuel was compared versus a standard curve at various concentration of diesel, which would represent a 90% decrease during the course of the experimentation. The idea was determine if similar extraction efficiencies would occur in the presence or absence of the Beeswax. The efficiencies ranged from for 90 to 103% based on comparison to a 5-point standard curve (25, 75, 100, 150, 200 mg diesel/BMR). Based on the calibration curves and the known concentrations of diesel present in each

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standard, it was possible to ascertain the new concentration of diesel fuel based on the GC's measurement of the diesel range organic (DRO) compounds.

The extraction process recovered the diesel linearly. However, the presence of the beeswax presented a number of unique problems. A significant amount of time was spent solving the impact of the Beeswax on all of the analytical methodology, particularly recoveries. The impact of the wax on the instrument is reflected in modification of the instrument conditions as described in the methods. Extraction routinely recovered more diesel range organics in the presence of beeswax than without. This is counterintuitive since one would expect due to the hydrophobic nature of the beeswax it would hold on to the hydrocarbon preventing good extraction. Separate standard curves were developed extracting known quantities of diesel fuel with and without Beeswax. In this way extraction efficiency and impact of the beeswax were accounted for during the experiment.

4.1.3 Chemical analysis

The analytical work was done on a Hewlett-Packard 5890 Gas Chromatograph (GC) equipped with a Hewlett Packard 7673 Autosampler, a 30 m, 0.25 μm ID RTX-5 column (Restek Corporation) and a hydrogen flame ionization detector. The salient parameters were as follows: For the Gas Chromatograph: Injector Temperature: 300° C, Detector Temperature: 325° C, Initial Oven Temperature: 50° C, Final Oven Temperature: 320° C, Oven Rate: 5.0° C/min, Helium Carrier Flow Rate: 4 mL/min, Purge Flow Rate: 7 mL/min, and Split Vent Flow Rate: 2.5 mL/min. For the Autosampler: Sample Washes: 2, Sample Pumps: 5, Viscosity: 7 seconds, and Volume: 1 μL.

The oven temperature was maintained at 250° C when not in use. Periodically the oven was maintained at 320° C to remove all the heavy compounds preventing the buildup of PRP components on the column that would degrade the instrument's sensitivity. The auto-injector syringe also had to be removed every 20 samples and cleaned mechanically with cleaning wires and an ultrasonic cleaner. The septum and injector lining were also to ensure that there was no carryover from the previous samples. Two-way analysis of variance (ANOVA) was used to verify results.

4.1.4 Standard preparation

For data interpretation it was necessary to use a calibration curve. There were several different curves that were constructed to demonstrate the relationship between mass and the area value read by the gas chromatograph.

4.1.5 Most probable number calculation

Samples from each experimental vessel were used to calculate the bacterial populations.

Total Hydrocarbon-degrading Organisms: A 6-tube MPN will be performed by dispensing 0.1 mL aliquots of each dilution into 2 mL of Bushnell-Haas broth dispensed into 6 wells/dilution of a 6 row by 4 well micro-titer plate. Twenty μL of filter sterilized diesel fuel will be added to each well and the MPN plates

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covered and incubated at room temperature. After a 14 day incubation period, 100 µL of a 5mg/mL solution of *p*–iodonitrotetrazolium violet will be added to each well to determine growth. Development of a pink or purple color upon standing for 45 minutes is considered a positive response. The MPN Calculator program will be used to provide the number of microorganisms per mL of the sample.

Total Heterotrophic Organisms: Microbial analysis was performed using MPN determination. A "6-tube MPN" was set up by dispensing 0.1 mL aliquots from each dilution into 1 mL of Trypticase Soy Broth (per Standard Methods, 18th Edition, 1992, p.9-33) that was previously dispensed into 6 wells/dilution of a 4 row by 6 well micro-titer plate. The MPN plates will be incubated at room temperature. After a 48 hour incubation period, 100 µl of a 5 mg/mL solution of *p*-iodonitrotetrazolium violet is added to each well to determine growth. Development of a pink or purple color upon standing for 20 minutes was considered a positive response. The number of positive wells and the related dilutions will be entered in a computerized enumeration method, "MPN Calculator" program (version 2.3). The software was developed by Albert J. Klee, (U.S. EPA, Office of Research and Development, Risk Reduction Engineering Laboratory, Cincinnati, OH) and provides the number of microorganisms per mL of the sample.

4.2 Mesocosm study and biodegradation analysis via GC/MS

4.2.1 Mesocosm Description

The mesocosm consisted of tank spilt into three relatively equal sections where water from a natural source could be constantly pumped through to simulate a "typical" fresh water stream. The tanks were constructed of plywood and supporting lumber lined with a polyvinyl swimming pool liner. The base of the tanks were structurally supported by a poured concrete base and covered by a PVC tarpaulin on 2 m supports to protect against rain and casual debris from entering the system. The three tanks were used for the experimental treatment and the controls. Each tank had the same general dimensions (i.e., 3 m x 1 m x 1.3 m), and was filled to a depth of about three feet. The tanks were separated one from another by a fiberglass wall sealed by silicone caulking. The design permitted sampling of the slick, water column at a depth of approximately 0.3 m below the slick, and incoming and outgoing water from each tank.

4.2.2 Water Source

The source of water for the system was provided on a continual basis by one of two near-by streams. Discharge from the system was sent to a large (95,000 L) holding tank to assure that no oil would be inadvertently discharged to the receiving stream. Water from the holding tank was intermittently discharged to a stream via an overland discharge of about 90 m subsequent to physical observation that no oil sheen was present in the holding tank's water.

Water was pumped from the stream at a rate of approximately 49,000 L per day into a an intermediate reservoir. Three identical submersible pumps into the

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influent section of each test tank pumped the water from the reservoir at the rate of about 11 L per minute per tank. Total flow in the system was about 2,000 L per hour. Turnover in each tank occurred approximately once every 3.2 hours.

To reduce the likelihood of short-circuiting in the tanks, a weir was used to direct water to flow downward beneath the slick, through the tank and then upward past a second weir prior to discharge to the large holding tank. Water was pumped for several days prior to the initiation of the test to allow for stabilization and acclimatization of the system.

4.2.3 Test Conditions

As previously described, the test was conducted in tanks using natural stream water. Weather and water conditions were recorded on a daily basis. These data were collected to provide a daily record for the physical conditions experienced during the experiment. Each of the three test tanks was fitted with a containment boom in a circular fashion to restrict the movement of the oil about the tank and to focus on the effects of the treatment. Each tank was dosed with 3.8 L of fresh (non-weathered) diesel fuel, from a local distributor.

Tank #1 contained the absorbent control, consisting of two one-pound sleeves of polypropylene absorbent within its boom. Tank #2 contained the "no-action" control where the oil was allowed to sit within the boom with no treatment. Tank #3 used the BioBoom for the oil absorption/containment as well as providing the area where the PRP could be applied as the primary means of treatment. In addition to the use of the B system, the oil in Tank #3 was directly treated with 90 g of PRP about 10 minutes after the introduction of the oil.

4.2.4 Chemical analysis

One mL of the *n*-hexane diluted sample or extract was placed into a 1.5 mL vial for use on the autosampler of the GC/MS instrument (HP 5890 with MSD). To this solution, 20 mL of a 500 ng/mL solution of the internal standards was added. The final concentrations of the internal standards in each sample were 10 ng/mL. This solution contained four deuterated compounds: d_8-naphthalene, d_{10}-anthracene, d_{12}-chrysene, and d_{12}-perylene, in methylene chloride.

4.2.5 Instrument configuration and calibration

A 1 μL aliquot of the hexane extract prepared by the above procedure was injected into a Hewlett-Packard 5890/5971 GC/MS instrument. This instrument was equipped with a DB-5 capillary column (30 m, 0.25 mm I.D., and 0.25 mm film thickness) and a split/splitless injection port operating in the splitless mode. Prior to the sample analysis, a five-point calibration was conducted on a standard mix of compounds to determine relative response factors (RRF) for the analytes. Data from the GC/MS runs are presented as relative amount versus the amount of hopane in the sample: the following relation can estimate the percent depletion of the selected analytes in the oil:

$$\text{Percent Analyte Depletion} = [1-(C_1/C_0) \text{ X } (H_0/H_1)] \text{ X } 100$$

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Where C_1 is the analyte amount in the degraded oil, C_0 is the analyte amount in the time-zero oil, H_1 is the hopane amount in the degraded oil and H_0 is the hopane concentration in the time-zero oil.

Individual peaks were normalized to hopane because it is a non-biodegradable compound over the course of the test. The total alkanes (aliphatics) and the total aromatics were summed and an analysis of variance (ANOVA) was conducted. Quality assurance and quality control techniques used were based on the methods described by the National Environmental Technology Applications Corporation [20].

5 Results

5.1 Comparison of Beeswax with and without added bacteria

A series of tests were run to evaluate the performance the natural population (Natural BioD), the Beeswax formulation with added microorganisms (Beeswax w/ microbes) and Beeswax with out added microorganisms (Beeswax w/o microbes).

Table 1 shows the results of the chemical analysis for diesel range organics (DRO) for the BMR studies. Samples were taken over a 4 week period and DRO results are presented as μg diesel/BMR. Each treatment was performed in triplicate at each time point. As can be seen all treatments resulted in the DRO reduction. The experimental average reduction for all treatments grouped together was 77%. The Beeswax w/o microbes treatment performed slightly better having a reduction of 84% compared to 71% and 77% for the Natural BioD and Beeswax w/ microbes, respectively. Statistical analysis using a 2 way analysis of variance (ANOVA) indicated that the decreases in DRO over time was significant for $P = 0.1$.

Table 1. DRO results for product formulation evaluation

Time 0	1	2	3	Avg
Natural BioD	142	151	145	146
Beeswax w/o microbes	145	127	166	146
Beeswax w/ microbes	113	112	106	110
Time 2 weeks	1	2	3	Avg
Natural BioD	76	84	67	76
Beeswax w/o microbes	64	76	115	85
Beeswax w/ microbes	60	50	74	61
Time 4 week	1	2	3	Avg
Natural BioD	51	40	35	42
Beeswax w/o microbes	29	15	28	24
Beeswax w/ microbes	31	25	21	26

The differences between treatments were not shown to be significant. However, the 2–way ANOVA groups all data sets to evaluate overall variability. To more accurately evaluate difference between individual treatments a student's T-test was run to compare the Natural BioD vs. Beeswax w/o microbes, Natural BioD vs. Beeswax w/ microbes and Beeswax w/ microbes vs. Beeswax w/o microbes. The analysis indicates the amount of reduction in the Beeswax w/ microbes and Beeswax w/o microbes were significantly different form the

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Natural BioD at the 90% confidence interval. There were no significant differences between the Beeswax w/ microbes and Beeswax w/o microbes.

Table 2. Marker Analysis for BMR studies.

Time 0	**1**	**2**	**3**	**Avg**
Beeswax w/ Microbes				
C_{17}/Pristane	1.27	1.29	1.32	1.29
C_{18}/Phytane	2.34	2.34	2.26	2.31
Natural BioD				
C_{17}/Pristane	1.24	1.29	1.27	1.27
C_{18}/Phytane	2.25	2.31	2.28	2.28
Beeswax w/o Microbes				
C_{17}/Pristane	1.21	1.30	1.34	1.28
C_{18}/Phytane	2.40	2.38	2.48	2.42
Time 2	**1**	**2**	**3**	**Avg**
Beeswax w/ Microbes				
C_{17}/Pristane	1.24	1.16	1.25	1.22
C_{18}/Phytane	2.33	1.94	2.14	2.14
Natural BioD				
C_{17}/Pristane	1.09	1.04	1.69	1.27
C_{18}/Phytane	1.77	2.00	1.98	1.92
Beeswax w/o Microbes				
C_{17}/Pristane	1.23	1.16	1.33	1.24
C_{18}/Phytane	2.12	2.26	2.28	2.22
Time 3	1	2	3	Avg
Beeswax w/ Microbes				
C_{17}/Pristane	0.83	0.76	0.54	0.71
C_{18}/Phytane	1.65	1.78	1.67	1.70
Natural BioD				
C_{17}/Pristane	0.63	0.44	0.10	0.39
C_{18}/Phytane	1.84	1.75	0.99	1.53
Beeswax w/o Bugs				
C_{17}/Pristane	0.66	0.08	1.06	0.60
C_{18}/Phytane	1.61	0.87	1.63	1.37

The overall decline in DRO could be the result of physical/chemical loss. The use of ratios of biodegradable to non-biodegradable makers is commonly used to demonstrate the loss is due to biodegradation. The most common method is the ratio of the alkane C_{17} to the isoprenoid pristane and C_{18} to phytane. These data are present Table 2. As can be seen in all cases the highly degradable alkanes decreased in relationship to the highly branched and recalcitrant isoprenoids. These data are indicative of biodegradation where more readily degradable components are metabolized in preference to less degradable materials. If the loss were physical no change in these ratios would be expected.

Bacterial numbers were followed during the course of the tests. The bacterial numbers (both heterotrophic and hydrocarbon degraders) increased over time. The high levels of microorganisms at time 0 indicated that there were a significant number of microorganisms present in the river water at the time of sampling. The level hydrocarbon degraders increased as a percentage of the population over the course of the experiments. At the end of the tests, however, treatments containing Beeswax showed higher numbers of organisms (both heterotrophic and hydrocarbon degraders). Considering that the DRO numbers suggest that the majority of the diesel has been degraded and the bacterial numbers in the Natural BioD had fallen off to level found in the original water, the presence of the Beeswax may be providing a nutrient source to sustain the population. This fact has a couple of positive implications: (1) the Beeswax is

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being degraded, an integral part of the Beeswax's application and (2) it is maintaining a very high hydrocarbon degrading population to respond to the next release of oil into the bilge.

The overall conclusion from these data is that Beeswax does enhance the natural degradation of the diesel in the BMR system. In addition, the added bacteria placed in the PRP appear to have no impact on the natural biodegradation rate. Finally, the Beeswax can maintain a high population of hydrocarbon degraders for a long period of time even after all oil has been degraded.

5.2 Mesocosm study and analysis via GC/MS

The following section describes the results of the experiment by segment and provides an analysis of the data as demonstrated by the accompanying data charts.

5.2.1 Control Slick

The control slick remained visually unchanged during the course of the experimentation. The hydrocarbon aroma, however, disappeared within the first week. The C_3–phenanthrene normalized GC/MS analysis indicated a change in the alkane envelope (on average a 47 percent decrease in total alkanes) during the three week period. The loss was due mainly to volatilization. This is indicated by the lack of change in the C_{17}/pristane ratio. Pristane is assumed to be non-biodegradable. Thus, a biodegradable analyte will decrease with time in relationship to the pristine. If loss is due to volatilization, the ratio will remain constant. The C_{17}/pristine ratio for the control slick was 1.1, 1.2, 1.2, and 0.99 for the four sampling events (over time). The data suggest volatilization is the main mechanism of loss during the first three weeks of the test. Biodegradation appears to have begun during the fourth week.

5.2.2 Absorbent Slick

The absorbent slick was affected immediately by the presence of the absorbent boom. The slick began to diminish and at 7 days virtually all the oil (visually estimated greater than 95 percent) had been lost from the system, presumably to the boom. Little change in the composition of the oil was detected during the first week and the overall loss was attributed to volatilization. Biodegradation was detected during the 14 and 21 day samplings via a change in the C_{17}/pristine phytane ratios (1.07, 1.03, 0.61, and 0.90 for Days 0, 7, 14 and 21, respectively.) There was an 80 percent decrease in the total aromatics. The "enhanced" biodegradation, compared to the control, detected in the absorbent slick can be attributed to less total oil being present in the tank. When following biodegradation using an analysis based on relative change, the sensitivity of detected changes is enhanced with smaller amounts of oil. As an example, if bacteria degrade 10 mg of oil a day and the initial oil was 1000 mg, 0.1 percent of the oil was degraded over the one day period. However, if only 100 mg of oil is used in 10 percent change will be seen even though the rate of biodegradation

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remains constant. Overall, the amount of biodegradation occurring in the absorbent slick for both the alkanes and the aromatics was greater than the control but less than the beeswax product slick.

5.2.3 Beeswax Product Slick

The GC/MS data for the product slick showed extensive loss of both the alkanes and aromatics, 96 and 76 percent, respectively. The majority of the loss occurred within the firs week (Figure 4). The loss was due to biodegradation (C_{17}/pristine ratio of 1.16, 0.97, 0.96, 1.23 for time 0 and 1 through 3 weeks, respectively). The decrease and then increase of the ratio's is typical of an oil that is extensively degraded resulting in a reduction in pristine to very low levels. The higher alkanes ($C_{24} - C_{25}$) appear to be slightly more resistant to biodegradation but it is anticipated that these would be removed with time. The high amount of alkanes as compared to the control (Figure 2) and absorbent slick (Figure 3) is possibly the result of a loss of aromatics to the PRP. Because the data is normalized to C_3-phenanthrene, the loss of the PAH fraction would mathematically increase the relative amount of all other constituents. An alternative explanation is that the results are an analytical anomaly, however, the actual relative abundances for n-alkanes are similar for other samples. This strengthens the explanation of selective absorption of aromatics by the encapsulating material. The encapsulating material having a slightly polar nature would have a tendency to interact with the more polar PAH's rather than the n-alkanes. At this point, more work would be needed to provide a definitive answer. Overall, the amount of biodegradation occurring in the product slick for both the alkanes and the aromatics was greater than the control and the absorbent slick.

5.2.4 Absorbent Boom

Little change in oil composition was detected in the absorbent boom during the course of the test. A 30 percent loss in alkanes and a 21 percent increase in aromatics were observed during the course of the test. The increase in aromatics is related to low aromatic values at the time zero. This could be attributed to an analytical anomaly or to selective absorption of alkanes by the polypropylene matrix. At this point, not enough data are available to distinguish between the two alternatives. No biodegradation was detected within the boom (C_{17}/pristine ratio of 1.02, 1.06, 1.06, 1.03 for time 0 and 1 through 3 weeks, respectively).

5.2.5 Beeswax Product Boom

The GC/MS data for the product boom showed loss of both the alkanes and aromatics, 83 and 51 percent, respectively. The majority of the loss occurred within the first week. The loss was due to biodegradation (C_{17} /pristine ratio of 0.92, 1.14, 0.82, 1.03 for time 0 and 1 through 3 weeks, respectively). The pristine ratio is unusual in the time zero data and may be affected by the absorption processes occurring in the slick exterior to the BioBoom. The higher alkanes ($C_{24} - C_{25}$) appear to be slightly more resistant to biodegradation but it is anticipated that these would be removed with time. The relative amount of

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alkanes decreased as compared to the product slick data and is lower than the time zero control slick. This again cold be the results of selective absorption of the PAH's which would be more likely retained in the sample and analyzed during laboratory procedures. Overall, the amount of biodegradation occurring in the product boom for both the alkanes and the aromatics was greater than the absorbent boom.

6 Conclusions

The overall conclusions of the study are:

1. Beeswax (the active ingredient in Petrol Rem Inc.'s products), in the form of PRP, selectively absorbs significantly enhances the natural degradation of the diesel fuel.
2. The degradation rate of diesel is significantly higher when using a BioBoom compared to the use of an absorbent boom in mesocosm studies.
3. The added bacteria placed in past Petrol Rem, Inc. products had no impact on the natural biodegradation rates and could be safely removed from the product without diminishing the products' effectiveness.
4. This beeswax based line of products could prove to be effective in handling isolated oil spills unavailable to conventional techniques or stationary treatment of small amount of petroleum pollution in marine and freshwater applications.

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