Laboratory Screening of Commercial Bioremediation Agents for the Deepwater Horizon Spill Response

Final Report

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3 Tier Executive Summary

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The following comprehensive report compares the top Bioremediation Agents available for National Contingency Plan (NCP), 40 CFR Part 300, and may be authorized for use by Federal On-Scene Coordinators in accordance with 40 CFR section 300.910. The research project was authorized by Biochem Strike Team, conducted by Louisiana State University (LSU) and funded British Petroleum (BP).

3 Tier has highlighted (In Yellow) the sections of the report that are specific to **Soil Rx.** The report also was reduced in size by eliminating the Chromatographs for all products except **Soil Rx** (All data is available upon request and these sections were eliminated to reduce the report from 104 pages to 39). All comparative data for all products remain for full review and disclosure.

The following are observations with respect to the three top performers of the study and a direct comparison of each. Top performing products were Product B, Product D, and Product J (**Soil RX**).

- Ease of use is a critical factor in properly choosing a product for field use. Out of the three products, Soil Rx offers the simplest use performa compared to Product D which requires a second product ("Nutrient Mix") to be mixed with the product and treatment rates of 2 pounds of product per 55 gallons of Gulf water (36,000 pounds per million gallons of water). Product B gets even more complicated with first having to mix the product in hot water and stir or mix for 20 minutes, then add to distilled water and add the "Optimizer" then apply. Not many response efforts will have hot water and/or distilled water available for mixing. With Soil Rx, simply dilute the concentrate 10 to 1 with available water (Can be diluted using ocean water) and apply evenly over the surface.
- On pages 6 & 7 of the report (underlined in green), the researcher clearly defines the significant differences the oil will go through as it weathers. The principal changes include reductions of potency and a reduction of most of the key contamination components (PAH). 3 Tier understands that it may be difficult to use the same material for all samples; it does note that the two other top performers used "Heavily Weathered Oil" while 3 Tier's samples were all "Lightly Weathered Oil". The performance of **Soil Rx** did not benefit from the natural reductions in the oil from weathering though our performance was virtually the same on the Alkanes but slightly less on the PAH due to no weathering.
- **Soil Rx** offers the latest advanced technology in the Bioremediation Agents category coupled with ease of use while being cost effective. 3 Tier products are designed to be extremely safe for both the applicator and the environment. This "Green Technology" is available today.

For additional information on the complete line of 3 Tier Environmental products, contact us directly at 877-226-7498, visit our website www.3tiertech.com, or email us at dburdette@3tiertech.com.

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1.0 Introduction and Summary of Methods

The current project focused on the testing of commercial bioremediation products with respect to their efficacy for degrading crude oil as compared to the process of natural attenuation in the Gulf of Mexico waters and coastline. Products were evaluated by a specialized team set up by the Alternative Response Technology (ART) program in response to the MC252 spill. The BioChem Strike Team (BCST) consisted of experts from USCG, BP, LSU, LDEQ, OSPR (California), NOAA, and highly experienced oil spill response consultants. The BCST determined that 10 products warranted further testing to determine their effectiveness in degrading oil under the specific environmental, climate and ecological conditions generated by the 2010 Gulf oil spill. The selected products were analyzed in a controlled flask study in the aquatic toxicology laboratory at LSU to determine their remediation potential on weathered crude oil recovered from south Louisiana marshes.

2.0 Materials and Methods

The experimental design protocol specified a 210 test flask study, incubated at room temperature on a consistently rotating, 200 rpm, orbital shaker. The samples were sacrificed over 5 separate sampling events including Time 0, 1, 2, 4 and 12. Each flask was analyzed for total nitrates (NO_3^-), total phosphates (PO_4^{3-}), total organic carbon (TOC), total alkanes, total polyaromatic hydrocarbons (PAHs), Diesel Range Organics (DRO), Oil Range Organics (ORO), Total Petroleum Hydrocarbons (TPH) and the physical parameters, pH, dissolved oxygen (DO) and temperature.

2.1 Chemical Analyses

2.1.1 *GC/MS Methods*

Extraction of PAHs and alkanes in water amended with oil follows methods outlined in EPA Method 8270 series. The entire 250 ml flask was sacrificed for oil extraction including approximately 80 ml of water and the all of the weathered oil remaining in the flask. The flasks were rinsed with dichloromethane (DCM) to ensure the complete solubilization of all oil into the final, extractable liquid fraction. Approximately 80 ml of water was poured into a 250-ml separatory funnel and adjusted to a pH of 7. A 30-ml aliquot of DCM was added to the separatory funnel and spiked with a known amount of standard surrogate. The funnel was capped and shaken for approximately 3 minutes, venting occasionally to remove solvent pressure. The solvent and water were allowed to separate and the solvent was drained through an anhydrous sodium sulfate funnel into a 250-ml flat-bottom flask. The solvent addition and draining step were repeated two more times. The sodium sulfate funnel was rinsed with DCM and allowed to drain completely. The flat-bottom flask was then placed on a rotary evaporation system and concentrated to a volume of 5-10 ml DCM and placed in a calibrated extraction thimble. If concentrating was necessary, the extract volume was placed under a nitrogen blow down concentrator and reduced to a volume of 1.0 ml. The DCM extract was exchanged to hexane using approximately 4-5 ml of hexane. A micro distillation column was added to the extraction thimble and placed in a hot water bath. The DCM was evaporated off and the remaining hexane extract was reduced to a volume of 1-2 ml. The hexane extract was placed beneath a nitrogen blow down device and reduced to a final volume of 1.0 ml hexane.

2.1.2 *GC/MS Instrumental analyses*

After addition of internal standards, samples were analyzed using an Agilent 7890A GC fitted with a 0.25 mm ID × 30 m HP-5MS column and an Agilent 7683B autosampler. The injector was set to 250°C and the detector to 280°C. Detection of analytes involves the utilization of a HP 5975C Inert XL Series Mass Selective Detector operating in the Selected Ion Monitoring mode. The column was held at 60°C for 1 min and then ramped at 25°C/min to 160°C followed by 3°C/min to 268°C and 12°C/min to 300°C, where it was held for 8 min. Concentrations of parent PAHs were calculated based on calibrations using a five-point curve which were checked for each batch of samples analyzed. Concentrations were reported on a dry weight basis. Approximate alkylated PAH concentrations were calculated assuming the same response factors for each parent and corresponding alkylated analogues. For alkylated phenanthrene/anthracenes, the results were reported as pairs to incorporate the uncertainty of the measurements and quantification based on the average response factor of the individual parent PAHs.

Table 1. 77 compounds quantified by GC/MS analysis in each of the 210 test flasks over the 5 designated time intervals.

Internal Standard	n-Alkanes	n-Alkanes	PAHs
Napthalene-d8	nC-10 Decane	nC-22 Docosane	Naphthalene
Acenaphthen-d10	nC-11 undecane	nC-23 Tricosane	Fluorene
Chrysene-d12	nC-12 Dodecane	nC-24 Tetracosane	Dibenzothiophene
Perylene-d12	nC-13 Tridecane	nC-25 Pentacosane	Phenanthrene
Surrogate Standard	nC-14 Tetradecane	nC-26 Hexacosane	Anthracene
Phenanthrene-d10	nC-15 Pentadecane	nC-27 Heptacosane	Fluoranthene
Androstane	nC-16 Hexadecane	nC-28 Octacosane	NBT
	nC-17 Heptadecane	nC-29 Nonacosane	Benzo (a) Antracene
	Pristane	nC-30 Triacontane	Chrysene
	nC-18 Octadecane	nC-31 Hentriacontane	Benzo (b) Fluoranthene
	Phytane	nC-32 Dotriacontane	Benzo (k) Fouoranthene
	nC-19 Nonadecane	nC-33 Tritriacontane	Benzo (e) Pyrene
	nC-20 Eicosane	nC-34 Tetratriacontance	Benzo (a) Pyrene
	nC-21 Heneicosane	nC-35 Pentatriacontane	Perylene
			Indeno (1,2,3-cd) Pyrene
			Dibeno (a,h) anthracene
			Benzo (g,h,i) perylene
			Pyrene

2.2 Other analytical approaches

A. Water quality analysis
   - DO, pH, temperature and initial salinity were measured using standard field equipment, (YSI 85-10 meter) appropriately calibrated.

B. Microbial analysis
   - Microbial activity was measured by epifluorescence direct cell count (EDCC) for Most Probable Number (MPN). Verifiable quantification was inconsistent between data sets due to clumping of weathered oil. Best estimates for all samples indicated acceptable biomass presence and density. All samples had MPN estimates in excess of 10^6 cells/ml.

C. Nutrients
   - Total inorganic phosphates (PO_4^{3-}) using EPA Method 365.4, total nitrates (NO_3^-) using EPA Method 4500-NO3 F modified and total organic (TOC) using EPA Method 9060. 20 ml of water were subsampled from the 100 ml test flasks in order to analyze each treatment for available nutrients. The remaining 80 ml and weathered oil from each flask was then extracted for hydrocarbon analysis.

D. Hydrocarbons
   - DRO includes C_{10} through C_{28} alkanes; ORO includes C_{20} through C_{35} alkanes as per modified EPA Method 8015. The integrated concentrations of each analyte over the delineated range were massed to obtain a representative concentration. TPH concentrations were calculated by massing the alkane and PAH concentrations for each triplicate treatment series. As per Haines e*t al.* 2003, a percent reduction was calculated using the following modified equation:

$$UCL_{90} = \left(\frac{t_{0.10,2Df} \times s}{\sqrt{n}} \right)$$

The UCL_{90} calculation changed the percent degradation rates less than 5% from those reductions calculated in Appendix A and reported in this report. This held true for all flasks, including those with the greatest standard deviations of the 14 treatment series. Therefore, the above equation was not used in the final percent reduction calculations.

3.0 Screening Protocol

3.1 Preparation of Oiled Flasks

The crude oil used in the study was recovered in Bay Jimmy (coordinates: 29°27’238” N, 89°53’510” W) on August 20, 2010. 0.5 g of weathered crude oil were weighed out and deposited into the bottom of a sterile 250 ml Erlenmeyer flask. Before the oil was added, each flask was rinsed with de-ionized water and autoclaved to ensure sterility. 10 ml of the solvent DCM was added and the flasks were placed on the shaker table for approximately 10 minutes until the oil had completely dissolved in the DCM. The flasks were then left uncovered under a ventilation hood to allow the DCM to flash off, leaving a ring of crude oil in the bottom of each 250 ml test flask.

Each of the 210 test flasks, including 60 control flasks and 150 product flasks, were prepared in this exact manner. After extraction, the triplicate samples were analyzed for % Relative Standard Deviation (% RSD). QA/QC measures stipulated that the % RSD among the triplicates for each test series must be less than 20% in order to fall within a statistically viable range. All values for % RSD at Time 0 for both the control and product flasks were well below the acceptable upper limit of 20% RSD.

3.2 Preparation of Controls

Four separate controls were prepared in triplicate for each of the five sampling events, resulting in 60 total control flasks.

- **Negative Control** treatments consisted of 100 ml of sterile Gulf water and 0.5 g of weathered crude oil per test flask. As in all other test flasks, 0.5 g of oil was dissolved in 10 ml of DCM, creating a coating of weathered oil in the bottom of each flask. 100 ml of autoclaved Gulf water was then added to each flask. No nutrients were added. The % RSD for TPH of the triplicate flasks at Time 0 fell within QA/QC limits at 1.67%.
- **Positive Control 1** treatments consisted of 100 ml of Gulf water and 0.5 g of weathered crude oil per test flask; no nutrients were added. As in all other test flasks, 0.5 g of oil were dissolved in 10 ml of DCM, creating a coating of weathered oil in the bottom of each flask. The % RSD for TPH of the triplicate flasks at Time 0 fell within QA/QC limits at 5.76%.
- **Positive Control 2** treatments consisted of 100 ml of Gulf water, 0.5 g of weathered crude oil and a nutrient blend per test flask. The nutrients consisted of 0.25 g KH_2PO_4 and 0.5 g NH_4NO_3 per flask. As in all other test flasks, 0.5 g of oil were dissolved in 10 ml of DCM, creating a coating of weathered oil in the bottom of each flask. The % RSD for TPH of the triplicate flasks at Time 0 fell within QA/QC limits at 8.87%.
- **Positive Control 3** treatments required a solution of 0.09 g of hexadecane and 0.01 g of chrysene per flask containing 100 ml of Gulf water. Based on the difficulty of accurately weighing 0.01 and 0.09 grams of each component, a stock solution of hexadecane and chrysene in DCM was prepared. The solution could then be accurately pipetted into each test flask. The calculations to produce 30 ml of solution are as follows:

3.3 Stock solutions

- 30 ml of DCM contained 0.3 g chrysene and 3.6 ml hexadecane
- 1 ml of DCM contained 0.01 g chrysene and 0.12 ml hexadecane

0.3 g of chrysene was first added to 30 ml of DCM and allowed to dissolve. Once dissolved, 3.6 ml of hexadecane was added to the chrysene-DCM solution. 1 ml of the composite solution was then added to each of the Positive Control 3 test flask, 15 flasks in total. Based on the passive volatilization of DCM as compared to hexadecane and chrysene, the DCM solution in the bottom

of each flask was flashed off under a vented hood. Only the desired amount of chrysene and hexadecane remained in the bottom of the test flask.

The final Positive Control 3 flasks consisted of 100 ml of Gulf water and 1 ml of the solution of nC-16 hexadecane and chrysene described above. % RSD for alkanes and PAHs of the triplicate flasks at Time 0 fell within QA/QC limits at 5.1% and 0.36% respectively.

3.4 Preparation of Products

The following products were added to triplicate flasks using formulations and approaches provided by product representatives to LSU. All products tested in the laboratory screening study are listed in the U.S. Environmental Protection Agency's (US EPA) Office of Emergency Management Regulatory and Policy Division's National Oil and Hazardous Substances Pollution Contingency Plan (NCP) Product Schedule.

Crude oils consist of hundreds to thousands of complex components. These hydrocarbon and non-hydrocarbon components can range from small, volatile compounds to large, semivolatile ones. Due to variations in geological formations, all crude oils and petroleum products, to some extent, have chemical compositions that differ from each other. This variability in chemical composition provides a unique chemical "fingerprint" for individual oils and provides a means for identifying the source oil following a spill. Monitoring the effects of oil weathering (e.g. photooxidation, dissolution, evaporation, and biodegradation) is achieved through comparison of the biodegradation indicators (such as *n*-C_{17}/pristine and *n*-C_{18}/phytane ratios) for the spilled oil and source oil over time. Weathering causes considerable changes in the chemical composition and physical state of spilled oil. The degree of weathering (lightly, moderately, and highly weathered) and rate of weathering is highly variable for each spill and set of conditions. As a result of weathering, the following chemical compositional changes typically occur during an oil spill:

- Significant losses occur in the low-molecular-weight *n*-alkanes (< *n*-C_{15}). The ratios of *n*-C_{17}/pristine and *n*-C_{18}/phytane are virtually unaltered in fresh to lightly weathered oils, but significant losses of alkanes and some isoprenoids do occur. Therefore, for fresh or lightly weathered oils, alkane and isoprenoid comparisons may be useful for determining source oil and rate of degradation.
- As much as 20-25% of aromatic, volatile organic compounds (VOC) are lost with 24-26 hours following a spill.
- Weathering produces a significant decrease in the naphthalene concentration relative to other alkylated PAH families.
- Development of a profile in each alkylated PAH family displaying the distribution of $C_0 < C_1 < C_2 < C_3$.

- Enrichment of the chrysene concentrations relative to other PAH series and significant decreases in the relative ratios of the sum of naphthalenes, phenanthrenes, dibenzothiophenes, and fluorenes to chrysene.

Type and identity of fresh to weathered oils and petroleum products can be readily revealed from GM/MS chromatograms especially where the spilled oil is heavy and background hydrocarbons are low in the impacted environment. Chromatograms provide a distribution pattern of petroleum hydrocarbons including individually resolved n-alkanes and major isoprenoids. Comparing biodegradation indicators such as *n*-C_{17}/pristane and *n*-C_{18}/phytane can be used to monitor the effect of both microbial degradation and physical weathering on the loss of hydrocarbons at the impacted site (Wang & Fingas 2003). In the current study, the degree of weathering for each test series was determined based on the chromatographic curve profiles of *n*-C_{17} and *n*-C_{18} hydrocarbons and their associated isoprenoids, pristane and phytane, at Time 0. The degree of weathering was assessed based on the elution height of *n*-$C_{17}$and *n*-C_{18} to their respective isoprenoids; if the first peak, the *n*-C_{17} and *n*-C_{18} hydrocarbon was the same height as the associated isoprenoid, the degree of weathering was classified as lightly or slightly weathered. If the first peaks were shorter than the second, the samples were considered heavily weathered. Reference: *ASTM Standards* D 573900 Standard Practice for Oil Spill Source Identification by Gas Chromatography and Positive Ion Electron Impact Low Resolution Mass Spectrometry

The Time 0 peak height ratio of the *n*-C_{17} and *n*-C_{18} hydrocarbons to their respective isoprenoids was analyzed for each test series and a degree of weathering was assigned. 9 of the flask series began the test period with a slightly weathered oil fraction, 1 consisted of a moderately weathered oil fraction and 3 demonstrated a heavily weathered oil profile (Table 2). As the degree of the initial weathering of oil impacts the biodegradability of the remaining oil including rates of microbial degradation, it is not appropriate to compare the treatment products analyzed in this test study to one another.

Product A

Nutrients Added: Yes
N: 0.5g/flask
P: 0.25g/flask

TPH % RSD at Time 0: *3.29*

The manufacturer's protocol suggested a 1:1 ratio of product to oil; 0.5 ml of the Product A product was added to each test flask.

Product B

Nutrients Added: No

TPH % RSD at Time 0: *16.84*

25 g of the provided microbe mixture were added to a 1000 ml graduated cylinder. 990 ml of de-ionized water was heated to 38°C and poured into the 1000 ml graduated cylinder. The mixture was stirred and allowed to settle for 20 minutes. 50 ml of the microbe and water mixture was added to 900 ml of room temperature distilled water. 50 ml of "Liquid Optimizer" was added to the solution for a final volume of 1 liter. 5 ml of the Product B solution was added to each test flask.

Product C

Nutrients Added: No

TPH % RSD at Time 0: *6.89*

0.15 grams of Product C for were added to each of the test flasks. The protocol called for concurrent inoculation of the flasks with pre-cultured indigenous oil degrading bacteria. However, no indigenous bacteria were cultured due to the pre-existing hydrocarbon degrading microbes present in the Gulf water collected for the study.

Product D

Nutrients Added: No

TPH % RSD at Time 0: *7.84*

To treat 55 gallons of Gulf water, the manufacturer suggested a ratio of 6 oz. of the provided nutrients mix with 2 pounds of the Product D. The ratio was reduced to 2.18 g of Product D and 0.75 g of nutrients in 500 ml of Gulf water. The two components were mixed thoroughly and 5 ml of resultant Product D solution were added to each flask.

Product E

Nutrients Added: Yes
N: 0.5g/flask
P: 0.25g/flask
Acetate: 0.1g/flask

TPH % RSD at Time 0: *9.04*

Pre-inoculated diatomaceous beads were added to a standard mineral salts broth and gulf water solution in a 500 ml bottle. The bottle was incubated for 3 days and 5 ml of the inoculated solution was added to each test flask.

Product F

Nutrients Added: No

TPH % RSD at Time 0: *8.17*

Based on the manufacturer's ratio describing the application of the product to dispersed oil, 0.5 ml of Product F was added to each test flask.

Product G

Nutrients added: No

TPH % RSD at Time 0: *5.12*

1.5 ml of Product G was added to each test flask based on the instructions that 0.3 ml of product be added to each 0.1 ml of oil.

Product H

Nutrients added: Yes
N: 0.5g/flask
P: 0.25g/flask

TPH % RSD at Time 0: *3.27*

50 grams of the Product H Hydrocarbon Digesting Microbe was stirred in 250 ml of de-ionized water for four hours. In the meantime, a 1% *[undisclosed product name]* solution (Surface Washing Agent listed on the NCP product schedule) was made by combining 1 ml of the *[undisclosed product name]* solution with 99 ml of de-ionized water. 1 ml of the Product H Hydrocarbon Digesting Microbe liquid fraction from the 250 ml flask and 0.05 ml of the *[undisclosed product name]* solution was added to each of the test flasks.

Product I

Nutrients added: Yes
N: 0.5g/flask
P: 0.25g/flask

TPH % RSD at Time 0: 2.46

1 ml of Product I was added to 99 ml of Gulf water in each test flask in order to obtain a 1% product solution.

Product J - 3 Tier Technologies Soil RX

Nutrients added: Yes
N: 0.5g/flask
P: 0.25g/flask

TPH % RSD at Time 0: *5.03*

Product J was mixed at a ratio of 1 part product to 10 parts water. 3 ml of the solution was then added to the test flasks.

Table 2. Preparation of test flasks included specific liquid and oiled fractions as well as nutrient amendments. As the source oil was not homogenized, test flasks demonstrated different degrees of weathering (slightly, moderately and heavily) as indicated in the above table.

Treatment	Liquid Fraction	Oiled Fraction	Nutrient Amendment
Negative Control	100 ml sterile Gulf water	0.5 g slightly weathered crude oil	None
Positive Control 1	100 ml non-sterile Gulf water	0.5 g slightly weathered crude oil	None
Positive Control 2	100 ml non-sterile Gulf water	0.5 g slightly weathered crude oil	0.25 g KH_2PO_4 0.5 g NH_4NO_3
Positive Control 3	100 ml non-sterile Gulf water	0.09 g hexadecane, 0.01 g chrysene spike	0.25 g KH_2PO_4 0.5 g NH_4NO_3
Product A	100 ml non-sterile Gulf water	0.5 g slightly weathered crude oil	0.25 g KH_2PO_4 0.5 g NH_4NO_3
Product B	100 ml non-sterile Gulf water	0.5 g heavily weathered crude oil	None
Product C	100 ml non-sterile Gulf water	0.5 g heavily weathered crude oil	None
Product D	100 ml non-sterile Gulf water	0.5 g heavily weathered crude oil	None
Product E	100 ml non-sterile Gulf water	0.5 g slightly weathered crude oil	0.25 g KH_2PO_4 0.5 g NH_4NO_3 0.1 g Sodium Acetate
Product F	100 ml non-sterile Gulf water	0.5 g moderately weathered crude oil	None
Product G	100 ml non-sterile Gulf water	0.5 g slightly weathered crude oil	None
Product H	100 ml non-sterile Gulf water	0.5 g slightly weathered crude oil	0.25 g KH_2PO_4 0.5 g NH_4NO_3
Product I	100 ml non-sterile Gulf water	0.5 g slightly weathered crude oil	0.25 g KH_2PO_4 0.5 g NH_4NO_3
Product J	100 ml non-sterile Gulf water	0.5 g slightly weathered crude oil	0.25 g KH_2PO_4 0.5 g NH_4NO_3

4.0 Findings

The ten commercial products tested demonstrated the ability to biodegrade and/or reduce total concentrations of Bay Jimmy weathered oil (including alkanes, PAHs). Additionally the flask study has verified that the remaining dispersed and weathered oil in coastal environments along the Louisiana and northern Gulf of Mexico will continue to biodegrade. This is not a new finding and has been the opinion of many scientists as a reasonable outcome for any oil spill affecting the coastlines of Gulf States. However, the study does demonstrate the capability to accelerate biodegradation strategies so as to minimize the toxicological legacy of the spill over time.

Data sets are included in *Appendix A* of the report. Representative chromatograms for the first four weeks of the study are in *Appendix B*.

Specific findings for control and commercial products are as follows:

Negative Control: The negative control flasks consisted of slightly weathered oil added to sterile Gulf water; neither ammonium nitrate nor potassium phosphate were added to the test series. The flasks indicated reductions in alkanes and PAHs over 28 days as 49.4% and 35.5% respectively. The TOC demonstrated a slight increase over the 12 week study; both nitrate and inorganic phosphate was limited over the duration of the 82 test days.

Positive Control 1: This series of control flasks consisted of weathered oil and non-sterile Bay Jimmy water with no additional nutrients. After 4 weeks, a 42.5% reduction in alkanes and an 81.7% reduction in PAHs were seen. Over the entire 12 week period, data sets demonstrated a 13.5% increase in total alkanes and a 28.7% reduction in PAHs. Based on the variability of 0.5 gram oil measurements within each flask, this slight increase is an acceptable result for the Positive Control 1 data series. Both nitrogen and phosphorous were limited and a slight increase in TOC from 7.05 mg/L at Time 0 to 17.19 mg/L was seen over the 12 week test period. Such data suggests microbial metabolism of the carbon source occurred to produce modest reductions in weathered oil. However the more complete degradation was inhibited by nutrient limitation; this is consistent with USEPA studies indicating the need for nutrient amendment so as to maintain steady biodegradation/mineralization.

Positive Control 2: The Positive Control 2 series of flasks consisted of site water from Bay Jimmy, weathered oil and nutrient additions of nitrogen and phosphorus. Indigenous aquatic microflora was the only active biological component in the flasks. After 28 days, a 96.3% reduction in alkanes and 74.3% reduction in PAHs was seen in the test series. After 82 days, 95.6% reduction in alkanes and 16.5% reduction in PAHs was demonstrated. Nutrients were not limited in the flasks and TOC increased from 7.85 mg/L at Time 0 to 98.68 mg/L at 12 weeks. The steady increase in TOC concentration suggests the conversion of carbon in the form of alkane chains and PAH structures to cellular carbon; microbial growth and an increase in cell density/biomass can be inferred from the data. Previous literature suggests that indigenous

hydrocarbon degrading microbial populations increase in response to an input of carbon and as in this case, weathered oil (Wilson *et al.* 1999*).* As demonstrated in the data set and suggested by the literature, adapted, acclimated and nutrient-amended microbial seed is able to produce significant reductions in both the alkane and PAH constituents of weathered oil (Boufadel *et al.* 1999).

Positive Control 3: Positive Control 3 consisted of site water with indigenous microflora and the chrysene/hexadecane additive as a primary carbon sources. No weathered oil or nutrients were added to the flask series. A 75.3% decrease in alkanes (nC-16 hexadecane) and a 69.5% decrease in PAHs (chrysene) were seen after 28 days; TOC did not increase over the 82 days of testing. A 78.1 % decrease in nC-16 hexadecane and a 74.9% decrease in chrysene was demonstrated over the 12 week test period.

Product A: Product A is identified as a bioremediation accelerator and as such, does not contain bacterial cultures; the product attempts to contribute to the establishment of a robust microbial population. As per manufacturer instructions, additional nutrients were added to the test flasks along with non-sterile site water and weathered oil; there was no nutrient limitation over the 82 test days. TOC concentrations were among the highest of the tested products and increased slightly over the 12 week period. After 4 weeks a 90.0% reduction in alkanes and an 82.4% reduction in PAHs was demonstrated. Considerable biodegradation of alkanes was seen over the course of treatment with 95.9% of these constituents reduced in 12 weeks. An 11.2% reduction in PAHs resulted from the Product A treatment over 12 weeks. Product A proved effective in degrading both the alkane and PAH components of weathered oil.

Product B: Product B is classified as a dual product**,** namely a bioremediation enhancer and an adapted microbe amendment with additional surfactant. The product was added to non-sterile site water and weathered oil. No additional nutrients were added, potentially limiting the biomass production. A 100% reduction of alkanes and an 85.1% reduction in PAHs were seen after 28 days; a 98.6% reduction in alkanes and a 38.9% reduction in PAHs were demonstrated over 12 weeks. TOC increased over the 82 day test period from 67.64 mg/L to 108.1 mg/L. Oil in the aqueous phase, as facilitated by the surfactant contained in the product, allowed for greater microbial colonization and therefore biodegradation/mineralization.

Product C: Product C is a nutrient amendment package optimized for indigenous petroleum hydrocarbons degraders in marine environments. The product was reconstituted in non-sterile site water and added to test flasks containing weathered oil. No nutrients were added to the test series and phosphorous and nitrogen was limited. After 28 days. 85.6% reduction in alkanes and a 48.9% reduction in PAHs were demonstrated. An 88.6% reduction in alkanes and a 78.6% reduction in PAHs were seen over 12 weeks. After 12 weeks, the TOC concentration doubled from 23.5 mg/L to 50.1 mg/L, an indication of the conversion of hydrocarbons to cellular carbon. Both the alkane and PAH constituents were substantially reduced and the product proved effective in degrading the components of weathered oil.

Product D: Product D is classified as viable, adapted petroleum hydrocarbon microbes and nutrient amendment. The product was provided as a dry powder of "biomass" and a proprietary nutrient blend. Both components were reconstituted in site water prior to addition to flasks containing site water and weathered oil. After 28 days, a 95.3% reduction in alkanes and a 68.9% reduction in PAHs were seen. The end of the test study saw nearly complete reduction of alkanes as 99.9% of the alkane constituents were degraded and 98.5% of the PAHs were degraded by the end of week 12. In total, approximately 99.8% of the weathered crude oil, both alkane and PAH constituents, were degraded by Product D by the end of 12 weeks. Analysis of nitrates and phosphates showed low concentrations throughout the duration of the 82 day test period. TOC concentration increased between Time 0 and Week 1, leveled out between Week 1 and Week 4, and doubled by Week 12. GC/MS analysis showed a substantial reduction in alkanes between Week 4 and Week 12 and an increase in biomass as seen in TOC concentrations corresponds to this hydrocarbon metabolism. Ultimately, the product was effective in reducing the total volume of heavily weathered oil over the test period.

Product E: Product E contains indigenous microflora isolated from Timbalier Bay, Louisiana. The product and nutrients were added to the site water and oiled test flasks. After 4 weeks, a 93.6% reduction in alkanes and a 61.0% reduction in PAHs were seen. A 98.5% reduction in total alkanes and 1.8% reduction in PAHs were seen at the end of 12 weeks, indicating a great degree of variability in the data set. The Product E test series was not nutrient limited with both nitrate and phosphate concentrations remaining high through the 82 test days. TOC concentration increased steadily over the test period, indicating constant mineralization of weathered oil to biomass as is expected with the addition of hydrocarbon degrading microbial strains. Overall, Product E demonstrates the ability to degrade both the alkane and PAH components of oil over 28 days.

Product F: Product F is a biological enzyme additive and surfactant and manufacturer direction called for the application of the product to dispersed oil rather than weathered oil. However the intention of the study was to determine the efficacy of a product in degrading weathered oil and therefore 0.5 ml of Product F was added to the oiled flasks. 78.8% of alkanes and 44.9% of PAHs were degraded by 28 days into the study. By the end of the 12 week test period, 80.1% of the alkane constituents and 79.3% of the PAH constituents were degraded. Product F demonstrated the ability to degrade both components of weathered crude oil equally well. Nutrients appeared to be limited with both nitrate and phosphate concentrations remaining low throughout the testing period. There was a slight increase in TOC from Time 0 to Week 1 with no increase in concentration seen in the following 11 weeks.

Product G: Product G is a biological additive, enzyme package and surfactant that was added per manufacturer's instruction to site water and oiled test flasks. After 28 days, a 62.4% reduction in alkanes and a 47.7% reduction in PAHs were demonstrated. An 81.2% reduction in alkanes and a 48.2% reduction in PAHs were seen by the end of the 12 week study. Nitrate concentrations were low through the duration of the study, approximately 1 mg/L, while

phosphate concentrations were slightly higher at about 30 mg/L. Like other products with a surfactant additive, no significant increase in TOC was seen in the Product G over the 82 test days. Even with no increase in TOC, an indicator of hydrocarbon conversion to biomass, further reductions in weathered oil constituents took place over 12 weeks.

Product H: Product H is listed as a proprietary microbial and enzymatic product. The product was added along with nutrients and the surface washing agent, *[undisclosed product name]*, to non-sterile site water and weathered oil. After 4 weeks, a 95.0% reduction in alkanes and a 36.8% reduction in PAHs were seen. A 95.2% reduction in alkane constituents and a 28.1% reduction in PAHs were seen over 12 weeks. Nutrient concentrations remained at non-limiting levels for the duration of the study with nitrate concentrations remaining slightly lower than phosphate. TOC increased at a steady rate over 82 days, and along with the reduction of hydrocarbons seen in the GC/MS analysis, a substantial conversion of crude oil to biomass can be inferred. The product demonstrated the ability to degrade both components of weathered crude oil without substantial inputs of amendment nutrients to the environment.

Product I: Product I is listed as a blend of proprietary microbes (*Bacillus*, *Pseudomonas*) and enzymes. The product was added to the oiled test flasks in order to achieve a 1% solution. After 4 weeks, a 95.2% reduction in alkenes and a 25.7% reduction in PAHs were seen. Alkane reductions reached 98.5% and PAHs were reduced 20.0% by week 12. There appeared to be no nutrient limitation as both nitrates and phosphates remained in high concentrations for the duration of the study. As expected for a product containing hydrocarbon degrading bacteria, a steady increase in TOC was demonstrated over the 82 day study. Product I exhibited the capacity to degrade both the alkane and PAH constituents of weathered oil to a great degree.

Product J (Soil RX): Product J is classified as a product containing humic acid, microbes, amino acids and a surfactant. As per manufacturer instructions, Product J was mixed in a 1:10 ratio with water; 3 ml of the solution was then added to each test flask. After 4 weeks of treatment with Product J, a 93.6% reduction in alkanes and a 31.1% reduction in PAHs were seen. A 98.8% reduction in alkanes and a 35.0% reduction in PAHs were demonstrated after 12 weeks. Nutrients were not limited as the concentrations of both nitrate and phosphate remained among the highest of all products tested over the 82 day study. As seen in the other products containing surfactants, the TOC did not increase over the test period, fluctuating between 1,600 mg/L and 2,500 mg/L over the 12 weeks. There is no increase in biomass corresponding to a reduction in weathered oil constituents.

5.0 General Discussion

Table 3. The 8 shaded boxes in the column labeled "4 weeks" indicate the products that attained both a >90% reduction in alkanes and a >20% reduction in PAHs by the end of 28 days of testing as recommended by Haines *et al.* 2003. The 5 shaded boxes in the column labeled "12 weeks" indicate the products that finished the 82 day test period with a >90% reduction in alkanes and a >20% reduction in PAHs. The green boxes indicate products without surfactants while the gray shaded boxes indicate products with a surfactant component. The yellow boxes indicate a supplemental surface washing agent was included.

Total Petroleum Hydrocarbon Percent Reduction									
		1 week		**2 weeks**		**4 weeks**		**12 weeks**	
Treatment	Initial TPH Concentration mg/kg	Alkane	PAH	Alkane	PAH	Alkane	PAH	Alkane	PAH
Negative Control	*28341*	*16.1*	***-10.7***	*64.2*	***-15.2***	*49.4*	*35.5*	*14.2*	*14.1*
Positive Control 1	*21637*	*10.7*	*20.4*	*60.5*	*2.9*	*42.5*	*81.7*	***-13.5***	*28.7*
Positive Control 2	*22312*	*74.2*	*16.7*	*94.7*	***-3.2***	*96.3*	*74.3*	*95.6*	*16.5*
Positive Control 3	*332667*	*78.8*	*70.9*	*75.4*	*74.1*	*75.3*	*69.5*	*78.1*	*74.9*
Product A	*24218*	*54.9*	***-10.4***	*86.9*	***-24.0***	*90.0*	*82.4*	*95.9*	*11.2*
Product B	*9178*	*79.0*	*14.4*	*97.6*	***-2.8***	*100.0*	*85.1*	*98.6*	*38.9*
Product C	*17828*	*51.2*	*20.7*	*84.6*	***-49.7***	*85.6*	*48.9*	*88.6*	*78.6*
Product D	*14253*	*82.8*	*34.6*	*94.1*	***-36.4***	*95.3*	*68.9*	*99.9*	*98.5*
Product E	*23688*	*65.8*	*7.0*	*93.0*	***-26.9***	*93.6*	*61.0*	*98.5*	*1.8*
Product F	*19073*	*52.8*	*32.8*	*79.3*	***-86.6***	*78.8*	*44.9*	*80.1*	*79.3*
Product G	*28555*	*27.3*	*41.8*	*64.0*	***-79.8***	*62.4*	*47.7*	*81.2*	*48.2*
Product H	*23283*	*67.7*	***-7.8***	*91.7*	***-40.1***	*95.0*	*36.8*	*95.2*	*28.1*
Product I	*28961*	*54.4*	***-4.8***	*82.6*	*17.7*	*95.2*	*25.7*	*98.5*	*20.0*
Product J	*28254*	*63.3*	*12.6*	*93.1*	*36.7*	*93.6*	*31.1*	*98.8*	*35.0*

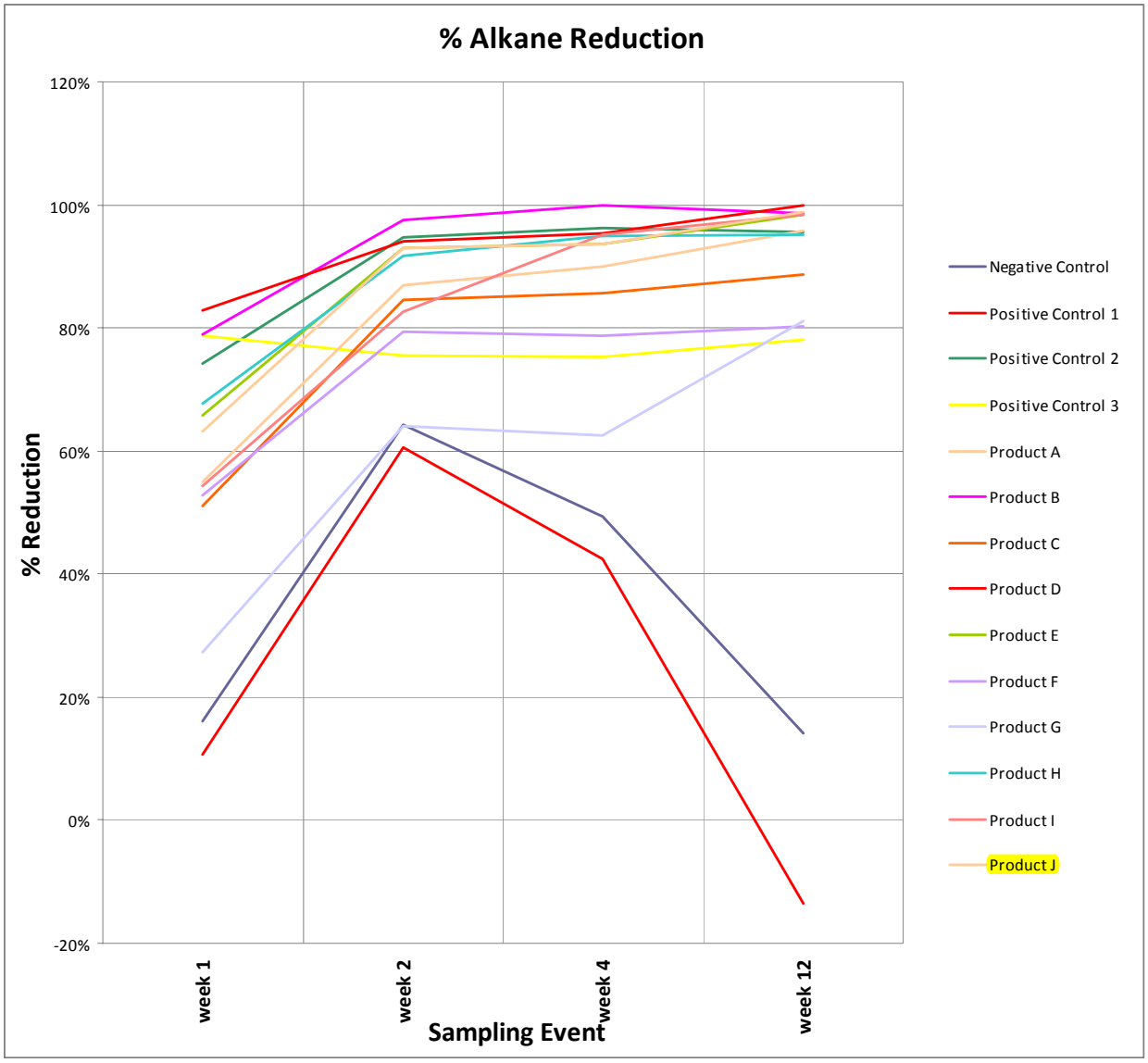


Figure 1. % Alkane reduction over time.

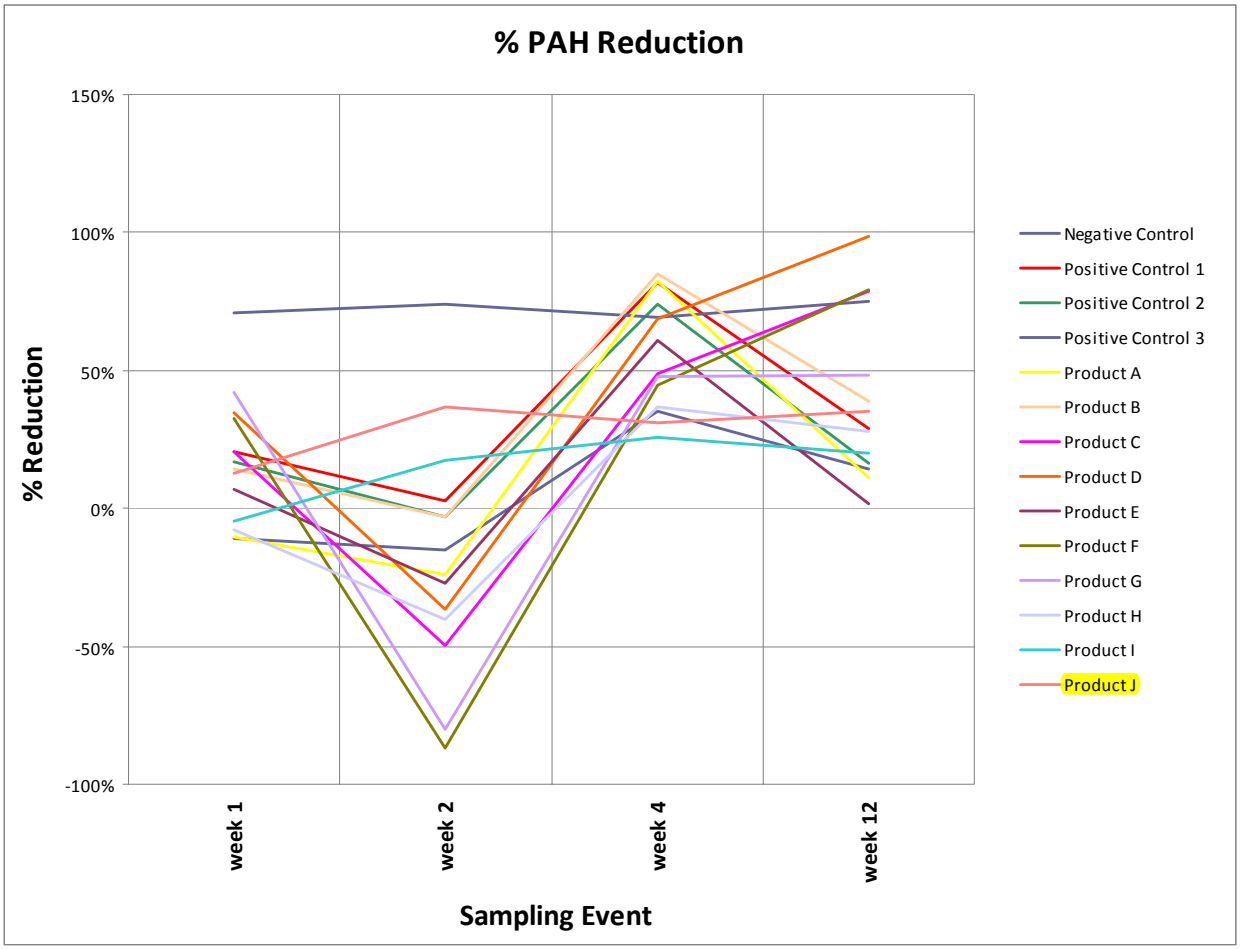


Figure 2. % PAH reduction over time.

Graphs are drawn based on the TOC concentrations of the 14 treatment flasks. Treatments ranged from approximately 6 mg/L to nearly 2,500 mg/L.

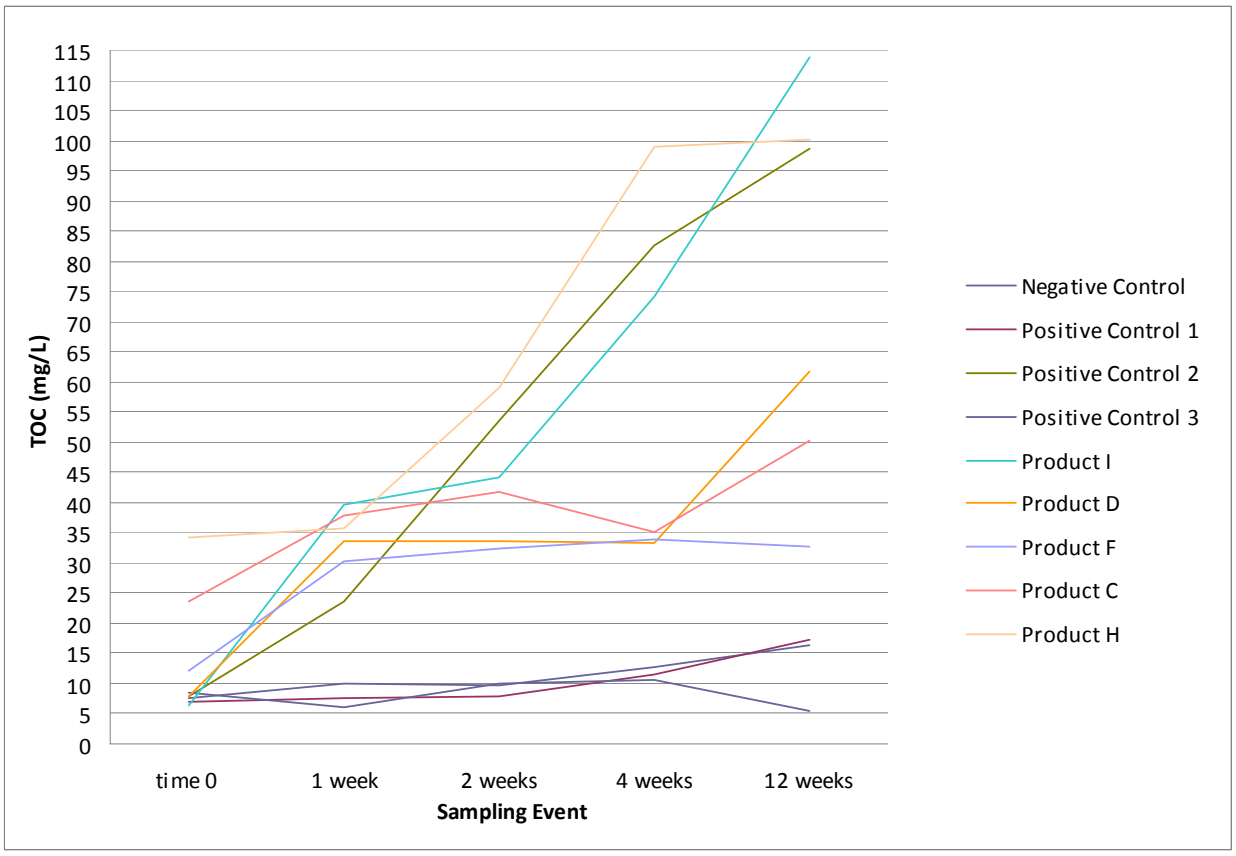
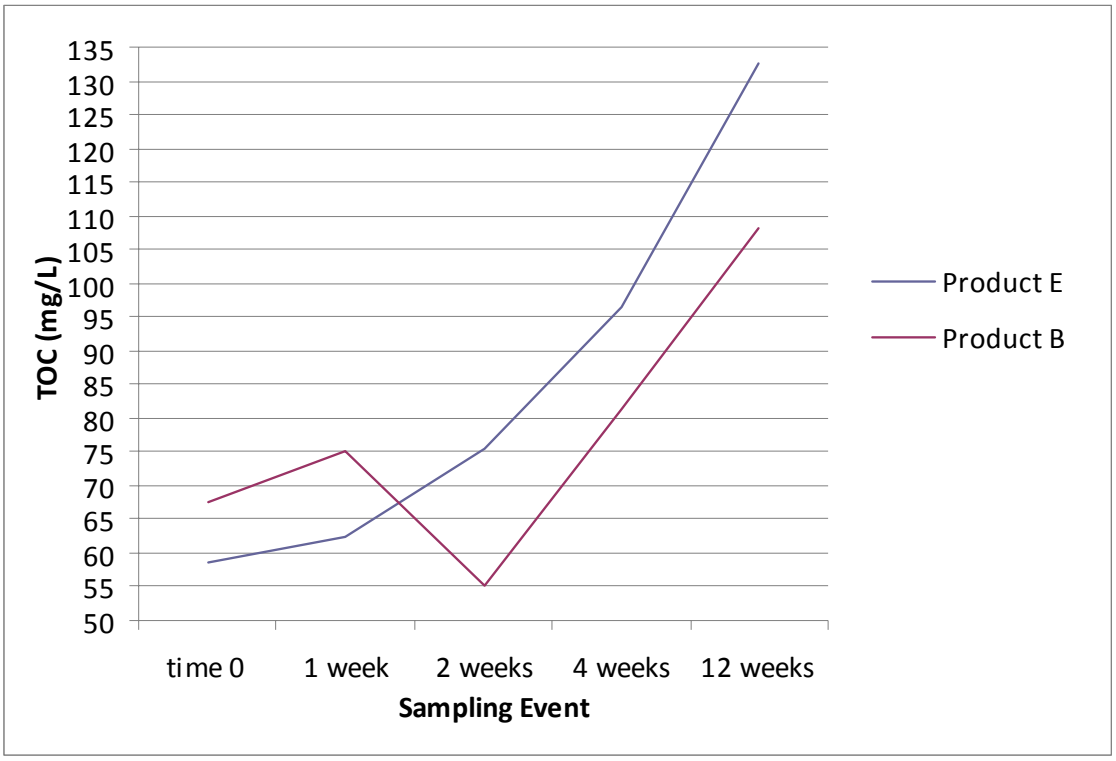


Figure 3. Treatment flasks with TOC concentrations ranging from approximately 6 mg/L to 115 mg/L. Positive Control 2, Product I, Product D, Product C and Product H each demonstrated typical increases in TOC concentration corresponding to the conversion of hydrocarbons to cellular carbon or biomass.



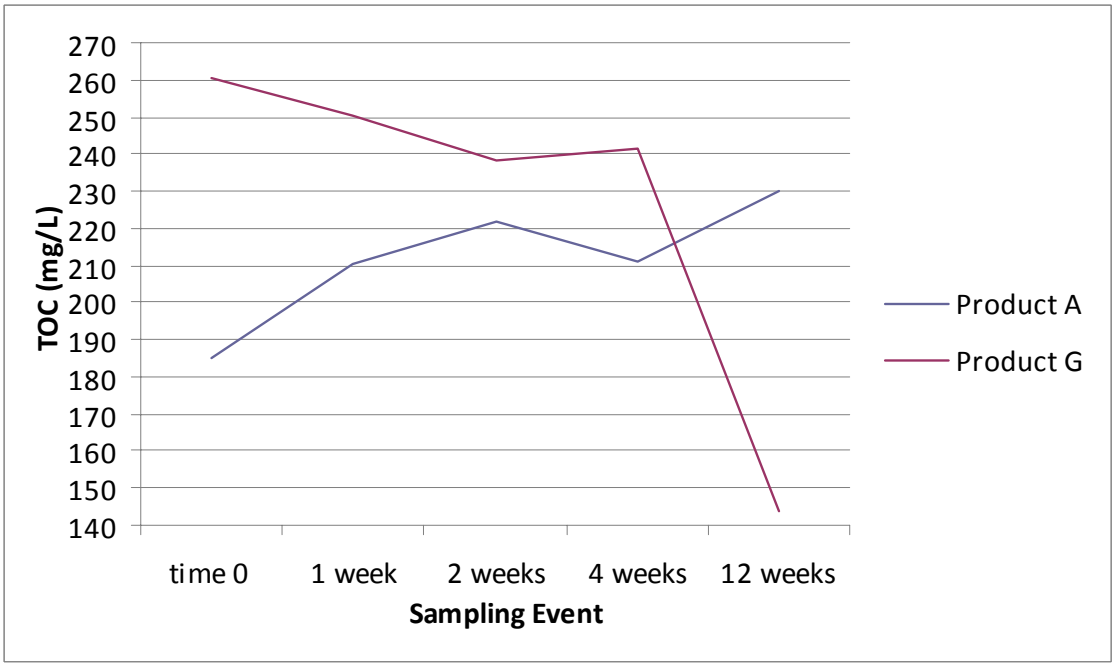


Figure 4a & 4b: Treatments ranging from approximately 50 mg/L to 135 mg/L and 140 mg/L and 260 mg/L respectively. TOC concentration of both Product E and Product A demonstrated the conversion of inorganic carbon to biomass over the 82 treatment days. Product B demonstrated a similar curve, but the product contained additional surfactant, the increase in TOC did not necessarily correspond to an increase in biomass.

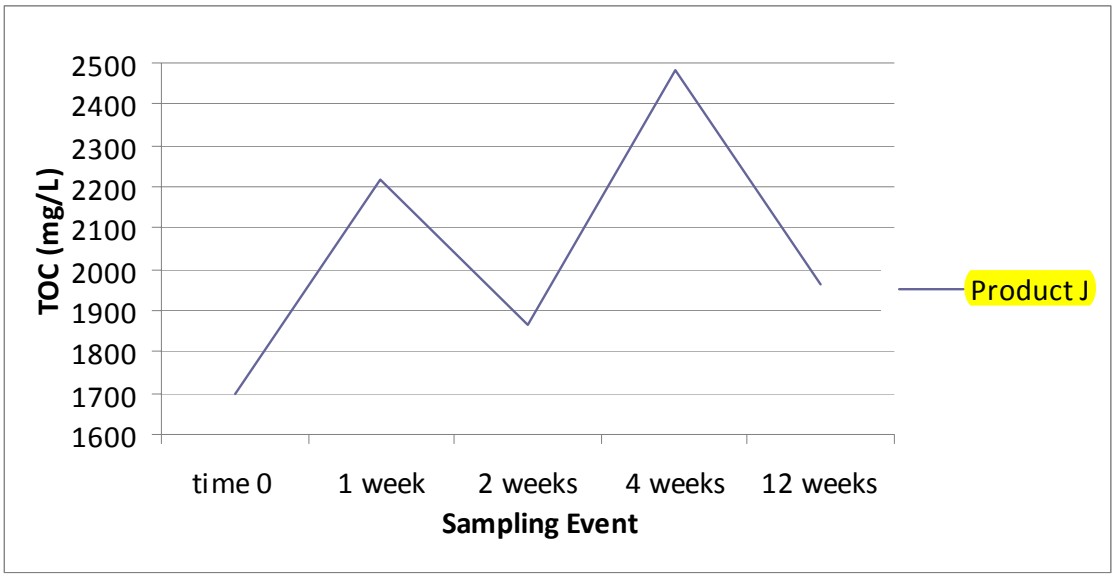


Figure 5: Product J exhibited the highest concentration of TOC of all products, ranging from approximately 1,700 mg/L to 2,500 mg/L. The curve does not indicate microbial degradation of weathered crude oil.

Hydrocarbons differ in their susceptibility to microbial attack with *n*-alkanes and branched alkanes being more susceptible than low molecular weight aromatics and high molecular weight aromatics and cyclic alkanes. As expected, the shorter-chain alkanes including *n*-C10 through *n*-C14 were most often thoroughly degraded by the end of 12 weeks, while the heavier chains were left in greater concentrations. Generally, the PAH groups including Phenanthrenes C1-C3, Pyrenes C2-C4 as well as Fluorenes C2 and C3 were left intact by the end of 12 weeks. The PAHs of toxicological concern including the Benzo constituents were degraded in every treatment flask. Data indicates that the most effective time frame of product performance was approximately 4 weeks; most products did not demonstrate significant hydrocarbon degradation after this period of time. The degradation rates demonstrated in the laboratory study is consistent with the anticipated time frame for field application.

The TOC concentrations curves for Positive Control 2, Product A, Product C, Product D, Product E, Product H and Product I each demonstrate the conversion of inorganic carbon in the form of weathered crude oil to cellular, organic carbon. This was to be expected as each of the products contained hydrocarbon degrading microbial blends or enzymatic additives promoting the growth of such microfauna. Product B demonstrated a similar increase in TOC concentration along with the reduction of weathered oil constituents, but as the product contained surfactant, it could not be determined that the increase in organic carbon was due to the conversion of hydrocarbons to biomass. Three products contained additional proprietary ingredients including surfactant, Product G, Product J and Product F. Each demonstrated fluctuations in TOC concentration along

with the reduction of hydrocarbons over the 12 week test period. It is unclear as to why TOC did not increase as weathered oil components were degraded.

The current laboratory study showed that the NCP products can promote the conversion of oil to CO_2, biomass and water. The study also demonstrates that nitrogen and phosphorous amendments also work to enhance in the degradation of oil under controlled closed systems. This supports earlier EPA research into remediation of spilled oil where interpretation of those data sets argued that the limiting factor for biodegradation/mineralization is dependent upon the availability of nitrogen and phosphorus (Boufadel *et al.* 1999; Boufadel *et al.* 2010; Venosa *et al.* 2010) While these results were generated under ideal laboratory conditions with controlled closed systems, field demonstration trials would be needed to document the efficacy of bioremediation products on weathered oil and to determine their net contribution to biodegradation/mineralization under the specific environmental, climate and ecological conditions of the spill. Factors such as fluctuating temperatures, salinities and dissolved oxygen levels may affect not only nitrogen and phosphorus nutrient availability but also the performance of microflora to acclimate to the field conditions (Portier 2006 & Igbal *et al.* 2007).

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Appendix A. Data sets from shaker flask studies

Data sets for the 10 products with controls are presented for the 12 week screening period.

	Time = 0 11/10/2010										
	NO_3^--N mg/L	PO_4^{3-} mg/L	TOC mg/L	Alkanes mg/kg	PAHs mg/kg	TPH mg/kg	DRO mg/kg	ORO mg/kg	pH	DO mg/L	Temp °C
Negative Control											
A	2.2	0.0	7.29	27400	394	27794	21774.20	20847.00	7.96	9.5	25.0
B	1.9	0.0	8.20	28200	415	28615	22344.30	21071.00	7.96	9.5	25.0
C	2.3	14.6	7.56	28200	415	28615	22398.60	21053.00	7.96	9.5	25.0
Standard Deviation				461.88	12.12	474.00					
Mean			*7.68*	*27933*	*408*	*28341*					
% relative standard deviation				*1.65*	*2.97*	*1.67*					
Positive Control 1											
A	1.4	21.8	6.60	21300	452	21752	17761.50	15252.00	7.96	9.5	25.0
B	3.5	7.4	7.56	19900	437	20337	17029.80	14010.00	7.96	9.5	25.0
C	1.6	0.0	7.00	22400	423	22823	18203.30	16471.00	7.96	9.5	25.0
Standard Deviation				1253.00	14.50	1246.96					
Mean			*7.05*	*21200*	*437*	*21637*					
% relative standard deviation				*5.91*	*3.32*	*5.76*					
Positive Control 2											
A	72.0	1730.0	7.20	23400	427	23827	19149.20	16940.00	7.96	9.5	25.0
B	145.0	2010.0	8.44	22600	435	23035	18851.20	16198.00	7.96	9.5	25.0
C	165.0	1970.0	7.92	19700	374	20074	15333.10	14090.00	7.96	9.5	25.0
Standard Deviation				1946.79	33.15	1978.21					
Mean			*7.85*	*21900*	*412*	*22312*					
% relative standard deviation				*8.89*	*8.05*	*8.87*					
Positive Control 3											
A	1.7	0.0	8.77	222000	96300	318300	222000	0.00	7.96	9.5	25.0
B	1.0	0.0	7.96	217000	96700	313700	217000	0.00	7.96	9.5	25.0
C	1.7	0.0	8.54	239000	97000	336000	239000	0.00	7.96	9.5	25.0
Standard Deviation				11532.56	351.19	11773.84					
Mean			*8.42*	*226000*	*96667*	*322667*					
% relative standard deviation				*5.10*	*0.36*	*3.65*					
Product A											
A	1670.0	1690.0	153.80	24500	308	24808	18893.00	18603.00	7.96	9.5	25.0
B	1630.0	1710.0	197.80	23000	312	23312	17100.80	16954.00	7.96	9.5	25.0
C	1790.0	1810.0	202.60	24300	234	24534	18562.90	18456.00	7.96	9.5	25.0
Standard Deviation				814.45	43.92	796.49					
Mean			*184.73*	*23933*	*285*	*24218*					
% relative standard deviation				*3.40*	*15.43*	*3.29*					
Product B											
A	2.6	80.1	70.60	10500	457	10957	7256.70	8015.00	7.96	9.5	25.0
B	3.5	65.8	66.36	8100	317	8417	5439.20	6195.00	7.96	9.5	25.0
C	2.7	63.8	65.96	7790	371	8161	5385.10	5838.00	7.96	9.5	25.0
Standard Deviation				1483.25	70.61	1545.68					
Mean			*67.64*	*8797*	*382*	*9178*					
% relative standard deviation				*16.86*	*18.50*	*16.84*					
Product C											
A	2.4	4.7	20.96	16500	337	16837	11999.70	12036.00	7.96	9.5	25.0
B	1.8	6.8	20.73	18700	502	19202	14178.68	13760.00	7.96	9.5	25.0
C	1.8	5.4	28.82	17000	444	17444	12195.80	12571.00	7.96	9.5	25.0
Standard Deviation				1153.26	83.70	1228.29					
Mean			*23.50*	*17400*	*428*	*17828*					
% relative standard deviation				*6.63*	*19.57*	*6.89*					

	Time = 0 11/10/2010										
	NO3--N mg/L	PO43- mg/L	TOC mg/L	Alkanes mg/kg	PAHs mg/kg	TPH mg/kg	DRO mg/kg	ORO mg/kg	pH	DO mg/L	Temp °C
Product D											
A	1.3	9.0	7.90	14900	593	15493	10397.90	10492.00	7.96	9.5	25.0
B	1.4	8.7	7.52	12700	626	13326	8028.40	9108.00	7.96	9.5	25.0
C	1.1	14.0	8.09	13400	540	13940	9253.80	9105.00	7.96	9.5	25.0
Standard Deviation				1123.98	43.39	1116.89					
Mean			*7.84*	*13667*	*586*	*14253*					
% relative standard deviation				*8.22*	*7.40*	*7.84*					
Product E											
A	77.0	1450.0	69.49	22200	290	22490	17777.30	16436.00	7.96	9.5	25.0
B	59.0	1590.0	37.21	22100	314	22414	18077.40	16163.00	7.96	9.5	25.0
C	195.0	1670.0	69.16	25800	361	26161	20302.20	19371.00	7.96	9.5	25.0
Standard Deviation				2107.92	36.12	2141.73					
Mean			*58.62*	*23367*	*322*	*23688*					
% relative standard deviation				*9.02*	*11.23*	*9.04*					
Product F											
A	1.0	2.9	12.46	17000	486	17486	13348.20	11992.00	7.96	9.5	25.0
B	0.8	2.0	11.61	18600	533	19133	14018.30	13275.00	7.96	9.5	25.0
C	1.4	1.2	12.58	20100	500	20600	14681.40	14694.00	7.96	9.5	25.0
Standard Deviation				1550.27	24.13	1557.87					
Mean			*12.22*	*18567*	*506*	*19073*					
% relative standard deviation				*8.35*	*4.77*	*8.17*					
Product G											
A	1.2	55.7	234.60	27000	405	27405	22294.10	18784.00	7.96	9.5	25.0
B	1.6	56.8	275.10	27700	359	28059	22707.20	19652.00	7.96	9.5	25.0
C	1.2	53.8	271.70	29700	500	30200	24629.80	20867.00	7.96	9.5	25.0
Standard Deviation				1401.19	71.91	1461.94					
Mean			*260.47*	*28133*	*421*	*28555*					
% relative standard deviation				*4.98*	*17.07*	*5.12*					
Product H											
A	22.1	1540.0	35.16	23500	330	23830	18487.00	17576.00	7.96	9.5	25.0
B	18.0	1630.0	36.01	23300	306	23606	18345.00	17483.00	7.96	9.5	25.0
C	23.1	1630.0	30.99	22100	313	22413	18389.00	15975.00	7.96	9.5	25.0
Standard Deviation				757.19	12.34	761.72					
Mean			*34.05*	*22967*	*316*	*23283*					
% relative standard deviation				*3.30*	*3.90*	*3.27*					
Product I	20-Jan-2011										
A	500.0	2000.0	6.77	29200	482	29682	23637.25	21404.00	7.96	9.5	25.0
B	790.0	1790.0	6.42	28500	442	28942	23119.51	20888.00	7.96	9.5	25.0
C	310.0	1790.0	5.58	27800	458	28258	22944.87	20118.00	7.96	9.5	25.0
Standard Deviation				700.00	20.13	712.18					
Mean			*6.26*	*28500*	*461*	*28961*					
% relative standard deviation				*2.46*	*4.37*	*2.46*					
Product J											
A	1890.0	1550.0	1915.00	27900	522	28422	22744.29	20006.00	7.96	9.5	25.0
B	1890.0	1890.0	1525.00	29000	585	29585	23512.56	20974.00	7.96	9.5	25.0
C	3110.0	2030.0	1655.00	26300	455	26755	19232.32	19416.00	7.96	9.5	25.0
Standard Deviation				1357.69	65.01	1422.46					
Mean			*1698.33*	*27733*	*521*	*28254*					
% relative standard deviation				4.90	12.49	5.03					

	Time = 1 Week 11/17/2010												
	NO_3^--N mg/L	PO_4^{3-} mg/L	TOC mg/L	Alkanes mg/kg	PAHs mg/kg	TPH mg/kg	DRO mg/kg	ORO mg/kg	% Red. Alkanes	% Red. PAHs	pH	DO mg/L	Temp °C
Negative Control													
A	1.1	0.0	9.64	20500	390	20890	17503.10	14058.00			8.01	5.75	23.4
B	1.1	0.0	9.95	21800	414	22214	18380.83	14827.00			8.06	5.10	23.4
C	1.2	0.8	10.51	28000	551	28551	23080.70	19424.00			8.06	4.88	23.4
Standard Deviation				4007.91	86.86	4094.74							
Mean			*10.03*	*23433*	*452*	*23885*			*16.1%*	*-10.7%*			
% relative standard deviation				*17.10*	*19.23*	*17.14*							
Positive Control 1													
A	1.1	0.0	7.56	19200	368	19568	16191.86	12975.00			7.88	4.38	24.0
B	0.8	0.0	7.50	19900	376	20276	16667.86	13459.00			7.93	4.44	24.3
C	0.9	0.0	7.50	17700	301	18001	15151.63	11922.00			7.96	4.39	24.0
Standard Deviation				1123.98	41.19	1164.21							
Mean			*7.52*	*18933*	*348*	*19282*			*10.7%*	*20.4%*			
% relative standard deviation				*5.94*	*11.82*	*6.04*							
Positive Control 2													
A	242.0	1710.0	24.80	6620	331	6951	4736.98	4247.00			5.27	3.53	23.8
B	360.0	1810.0	24.47	5960	401	6361	3992.48	3685.00			5.25	2.98	23.8
C	570.0	1690.0	21.65	4370	297	4667	3018.36	2578.50			5.22	3.42	24.0
Standard Deviation				1156.59	53.03	1185.64							
Mean			*23.64*	*5650*	*343*	*5993*			*74.2%*	*16.7%*			
% relative standard deviation				*20.47*	*15.46*	*19.78*							
Positive Control 3													
A	0.5	0.0	5.63	45800	32300	78100	45800	0.00			7.95	4.32	24.0
B	1.6	0.0	4.91	50000	28100	78100	50000	0.00			7.97	4.61	24.0
C	0.9	0.1	7.29	47900	24100	72000	47900	0.00			7.98	4.61	24.0
Standard Deviation				2100.00	4100.41	3521.84							
Mean			*5.94*	*47900*	*28167*	*76067*			*78.8%*	*70.9%*			
% relative standard deviation				*4.38*	*14.56*	*4.63*							
Product A													
A	2380.0	2230.0	234.40	9560	315	9875	7593.77	6460.00			5.21	2.31	24.0
B	1760.0	2050.0	187.80	11600	390	11990	9209.73	8316.00			5.25	1.81	24.6
C	2050.0	2030.0	208.10	11200	238	11438	8970.68	7406.00			5.26	2.02	24.4
Standard Deviation				1080.99	76.00	1097.03							
Mean			*210.10*	*10787*	*314*	*11101*			*54.9%*	*-10.4%*			
% relative standard deviation				*10.02*	*24.18*	*9.88*							
Product B													
A	1.2	62.6	68.80	2440	312	2752	1913.78	1197.00			7.56	2.25	23.8
B	1.8	57.5	84.25	1620	344	1964	1386.61	615.17			7.58	2.61	23.8
C	1.0	48.5	72.35	1470	324	1794	1206.09	625.91			7.65	2.68	23.9
Standard Deviation				522.14	16.17	511.14							
Mean			*75.13*	*1843*	*327*	*2170*			*79.0%*	*14.4%*			
% relative standard deviation				*28.33*	*4.95*	*23.56*							
Product C													
A	1.6	3.7	36.67	7900	412	8312	5835.60	4952.00			7.50	3.17	24.5
B	1.7	6.2	42.58	8440	260	8700	6437.60	5335.00			7.59	3.18	25.0
C	1.4	3.4	33.82	9140	346	9486	6647.60	6066.00			7.67	3.20	24.8
Standard Deviation				621.72	76.22	598.14							
Mean			*37.69*	*8493*	*339*	*8833*			*51.2%*	*20.7%*			
% relative standard deviation				*7.32*	*22.46*	*6.77*							

	Time = 1 Week 11/17/2010												
	NO3--N mg/L	PO43- mg/L	TOC mg/L	Alkanes mg/kg	PAHs mg/kg	TPH mg/kg	DRO mg/kg	ORO mg/kg	% Red. Alkanes	% Red. PAHs	pH	DO mg/L	Temp °C
Product D													
A	0.7	2.5	28.15	2450	423	2873	1996.89	1015.19			7.32	2.75	24.4
B	1.1	1.2	37.07	2010	329	2339	1552.74	945.22			7.29	3.03	24.4
C	0.8	1.1	35.15	2590	399	2989	1748.70	1240.10			7.29	2.60	24.3
Standard Deviation				302.65	48.84	346.68							
Mean			*33.46*	*2350*	*384*	*2734*			*82.8%*	*34.6%*			
% relative standard deviation				*12.88*	*12.73*	*12.68*							
Product E													
A	1330.0	1290.0	87.40	7560	366	7926	5947.96	4972.00			6.04	3.04	24.4
B	920.0	1340.0	55.27	7780	232	8012	6058.78	5230.00			6.08	2.94	24.4
C	1180.0	1290.0	44.98	8600	299	8899	7033.37	5742.00			6.05	3.09	24.4
Standard Deviation				548.09	67.00	538.65							
Mean			*62.55*	*7980*	*299*	*8279*			*65.8%*	*7.0%*			
% relative standard deviation				*6.87*	*22.41*	*6.51*							
Product F													
A	0.6	0.0	28.56	7570	306	7876	5836.80	4702.00			7.49	3.23	24.4
B	1.3	0.0	28.97	8940	328	9268	6829.20	5726.00			7.42	3.17	24.2
C	1.1	0.0	32.83	9790	387	10177	7673.20	6209.00			7.40	3.16	24.2
Standard Deviation				1120.10	41.88	1158.92							
Mean			*30.12*	*8767*	*340*	*9107*			*52.8%*	*32.8%*			
% relative standard deviation				*12.78*	*12.31*	*12.73*							
Product G													
A	1.1	33.8	236.90	20900	320	21220	17479.80	14684.00			7.24	2.81	24.5
B	1.0	40.8	248.60	18900	159	19059	15707.10	13289.00			7.32	3.08	24.4
C	1.2	36.5	265.40	21600	257	21857	17496.90	15413.00			7.34	3.10	24.3
Standard Deviation				1401.19	81.13	1466.54							
Mean			*250.30*	*20467*	*245*	*20712*			*27.3%*	*41.8%*			
% relative standard deviation				*6.85*	*33.07*	*7.08*							
Product H													
A	21.7	1780.0	32.02	5330	312	5642	4393.51	3056.00			5.36	2.75	24.5
B	17.6	2070.0	34.35	10600	382	10982	7920.55	7119.00			5.35	2.55	24.5
C	21.1	2300.0	40.86	6340	329	6669	5096.15	3679.00			5.27	3.01	24.7
Standard Deviation				2797.04	36.51	2833.50							
Mean			*35.74*	*7423*	*341*	*7764*			*67.7%*	*-7.8%*			
% relative standard deviation				*37.68*	*10.71*	*36.49*							
Product I	27-Jan-2011												
A	620.0	1530.0	43.05	14300	494	14794	11043.63	9764.00			5.26	3.80	26.3
B	1030.0	1940.0	45.60	11300	479	11779	7940.14	7543.00			5.21	3.64	26.2
C	340.0	1670.0	30.01	13400	476	13876	9974.75	9251.00			5.24	3.55	26.5
Standard Deviation				1539.48	9.64	1545.44							
Mean			*39.55*	*13000*	*483*	*13483*			*54.4%*	*-4.8%*			
% relative standard deviation				*11.84*	*2.00*	*11.46*							
Product J													
A	1640.0	1710.0	2089.00	10100	457	10557	7341.07	7219.00			5.51	2.56	25.1
B	1130.0	2020.0	2506.00	10500	458	10958	7588.55	7466.00			5.46	2.05	25.6
C	2140.0	2240.0	2056.00	9960	450	10410	7091.58	7084.00			5.42	3.12	25.8
Standard Deviation				280.24	4.36	283.64							
Mean			*2217.00*	*10187*	*455*	*10642*			*63.3%*	*12.6%*			
% relative standard deviation				*2.75*	*0.96*	*2.67*							

	TIME = 2 WEEKS 11/24/2010												
	NO_3^--N mg/L	PO_4^{3-} mg/L	TOC mg/L	Alkanes mg/kg	PAHs mg/kg	TPH mg/kg	DRO mg/kg	ORO mg/kg	% Red. Alkanes	% Red. PAHs	pH	DO mg/L	Temp °C
Negative Control													
A	1.0	0.0	9.30	10400	521	10921	9466.43	6678.10			7.82	4.80	23.5
B	0.6	0.2	9.87	10500	499	10999	9511.80	6507.20			7.89	4.74	24.1
C	1.1	0.0	9.85	9110	390	9500	8344.90	5649.80			7.92	4.72	24.2
Standard Deviation				775.26	70.15	843.83							
Mean			*9.67*	*10003*	*470*	*10473*			*64.2%*	*-15.2%*			
% relative standard deviation				*7.75*	*14.93*	*8.06*							
Positive Control 1													
A	1.2	0.0	9.29	10500	487	10987	9490.20	6721.00			7.90	4.53	24.3
B	1.1	0.0	6.41	4990	215	5205	4535.20	3128.80			7.95	4.37	24.3
C	0.6	1.0	8.15	9630	572	10202	8690.80	6076.20			7.96	4.57	24.4
Standard Deviation				2962.17	186.48	3136.29							
Mean			*7.95*	*8373*	*425*	*8798*			*60.5%*	*2.9%*			
% relative standard deviation				*35.38*	*43.91*	*35.65*							
Positive Control 2													
A	190.0	2540.0	51.40	1030	281	1311	817.91	391.13			5.16	3.59	25.2
B	331.0	2710.0	58.39	1100	471	1571	922.61	394.01			5.21	3.64	25.2
C	210.0	2360.0	51.16	1320	523	1843	1103.29	494.03			5.14	3.54	24.8
Standard Deviation				151.33	127.39	266.02							
Mean			*53.65*	*1150*	*425*	*1575*			*94.7%*	*-3.2%*			
% relative standard deviation				*13.16*	*29.97*	*16.89*							
Positive Control 3													
A	0.9	0.6	10.54	55600	24400	80000	55600	0.00			7.91	4.56	24.5
B	1.6	0.0	9.23	54500	25700	80200	54500	0.00			7.96	4.20	24.5
C	1.2	0.0	10.54	56400	24900	81300	56400	0.00			7.96	4.49	24.8
Standard Deviation				953.94	655.74	700.00							
Mean			*10.10*	*55500*	*25000*	*80500*			*75.4%*	*74.1%*			
% relative standard deviation				*1.72*	*2.62*	*0.87*							
Product A													
A	2490.0	2470.0	232.80	2980	334	3314	2710.20	1539.20			5.36	3.34	25.9
B	2300.0	2290.0	210.40	3840	385	4225	3473.53	2062.30			5.16	3.39	25.9
C	1800.0	2410.0	223.10	2550	340	2890	2249.30	1300.30			5.31	3.30	25.8
Standard Deviation				656.84	27.87	682.14							
Mean			*222.10*	*3123*	*353*	*3476*			*86.9%*	*-24.0%*			
% relative standard deviation				*21.03*	*7.90*	*19.62*							
Product B													
A	1.7	47.8	55.49	413	398	811	409.49	91.59			7.71	3.28	25.9
B	1.6	48.6	56.49	62.6	320	383	62.62	13.98			7.76	2.89	26.0
C	1.5	42.2	53.59	154	459	613	142.20	59.90			7.85	3.17	25.9
Standard Deviation				181.76	69.67	214.40							
Mean			*55.19*	*210*	*392*	*602*			*97.6%*	*-2.8%*			
% relative standard deviation				*86.61*	*17.76*	*35.60*							
Product C													
A	1.5	4.7	50.72	1720	666	2386	1557.18	412.30			7.51	3.82	25.4
B	1.5	4.7	39.63	3290	500	3790	3025.10	1506.10			7.64	3.77	25.2
C	2.6	4.8	34.64	3050	755	3805	2838.70	1380.80			7.64	3.97	25.2
Standard Deviation				845.71	129.42	814.96							
Mean			*41.66*	*2687*	*640*	*3327*			*84.6%*	*-49.7%*			
% relative standard deviation				*31.48*	*20.21*	*24.50*							

	TIME = 2 WEEKS 11/24/2010												
	NO3--N mg/L	PO43- mg/L	TOC mg/L	Alkanes mg/kg	PAHs mg/kg	TPH mg/kg	DRO mg/kg	ORO mg/kg	% Red. Alkanes	% Red. PAHs	pH	DO mg/L	Temp °C
Product D													
A	1.2	2.7	33.01	968	813	1781	967.40	155.80			7.42	3.70	25.8
B	1.1	4.0	36.90	914	878	1792	806.37	200.60			7.32	3.40	25.5
C	1.3	3.2	30.95	533	708	1241	533.60	74.70			7.42	3.47	25.7
Standard Deviation				237.10	85.78	314.99							
Mean			*33.62*	*805*	*800*	*1605*			*94.1%*	*-36.4%*			
% relative standard deviation				*29.45*	*10.73*	*19.63*							
Product E													
A	1520.0	1640.0	73.70	1690	499	2189	1465.81	782.80			6.00	3.34	25.2
B	1080.0	1970.0	64.16	2110	305	2415	1845.13	980.90			6.05	3.73	25.3
C	2020.0	2100.0	88.56	1120	421	1541	953.80	423.60			5.97	3.33	25.2
Standard Deviation				496.89	97.62	453.66							
Mean			*75.47*	*1640*	*408*	*2048*			*93.0%*	*-26.9%*			
% relative standard deviation				*30.30*	*23.91*	*22.15*							
Product F													
A	1.0	0.0	32.49	4050	914	4964	3721.00	1937.20			7.70	3.84	25.8
B	1.6	0.5	33.57	3190	981	4171	2962.00	1375.10			7.70	3.70	25.7
C	1.4	0.0	30.81	4280	940	5220	4025.00	2015.70			7.70	3.73	25.9
Standard Deviation				574.54	33.78	546.93							
Mean			*32.29*	*3840*	*945*	*4785*			*79.3%*	*-86.6%*			
% relative standard deviation				*14.96*	*3.57*	*11.43*							
Product G													
A	1.0	29.2	250.30	11990	1050	13040	1252.00	7373.70			7.21	3.86	25.9
B	1.1	22.5	223.60	8400	601	9001	7954.90	5175.90			7.16	3.30	25.9
C	1.9	26.7	241.30	9990	622	10612	9410.56	6169.10			7.24	0.46	25.9
Standard Deviation				1798.90	253.39	2033.23							
Mean			*238.40*	*10127*	*758*	*10884*			*64.0%*	*-79.8%*			
% relative standard deviation				*17.76*	*33.44*	*18.68*							
Product H													
A	18.8	2310.0	61.91	2840	432	3272	2373.20	1412.70			5.21	3.57	25.6
B	16.7	2500.0	58.88	1680	594	2274	1359.50	645.30			5.15	3.29	25.5
C	18.9	2650.0	56.50	1180	304	1484	930.30	501.40			5.10	3.30	25.4
Standard Deviation				851.59	145.33	896.01							
Mean			*59.10*	*1900*	*443*	*2343*			*91.7%*	*-40.1%*			
% relative standard deviation				*44.82*	*32.78*	*38.24*							
Product I	3-Feb-2011												
A	930.0	1530.00	49.95	4490	317	4807	2878.52	2578.00			5.36	4.86	20.5
B	710.0	1940.00	38.57	5820	456	6276	3614.75	3492.30			5.31	4.77	20.7
C	900.0	1670.00	43.67	4540	365	4905	2971.95	2538.30			5.28	4.76	20.8
Standard Deviation				753.86	70.60	821.30							
Mean			*44.06*	*4950*	*379*	*5329*			*82.6%*	*17.7%*			
% relative standard deviation				*15.23*	*18.61*	*15.41*							
Product J													
A	1720.0	1710.00	2278.00	1610	298	1908	1256.17	1004.79			5.30	3.93	21.1
B	2470.0	2020.00	2304.00	2470	348	2818	1585.17	1398.00			5.49	4.69	21.5
C	1930.0	2240.00	1019.00	1700	342	2042	1308.83	949.44			5.39	4.64	21.4
Standard Deviation				472.69	27.30	491.30							
Mean			*1867.00*	*1927*	*329*	*2256*			*93.1%*	*36.7%*			
% relative standard deviation				*24.53*	*8.29*	*21.78*							

	TIME = 4 WEEKS 12/8/2010												
	NO_3^--N mg/L	PO_4^{3-} mg/L	TOC mg/L	Alkanes mg/kg	PAHs mg/kg	TPH mg/kg	DRO mg/kg	ORO mg/kg	% Red. Alkanes	% Red. PAHs	pH	DO mg/L	Temp °C
Negative Control													
A	0.6	0.0	12.80	13900	267	14167	11681.60	9281.00			7.87	4.61	24.2
B	0.6	0.1	13.49	14200	254	14454	11924.20	10048.00			7.93	3.99	24.1
C	0.9	0.0	11.72	14300	269	14569	11884.30	9615.00			7.97	4.57	24.4
Standard Deviation				208.17	8.14	207.04							
Mean			*12.67*	*14133*	*263*	*14397*			*49.4%*	*35.5%*			
% relative standard deviation				*1.47*	*3.09*	*1.44*							
Positive Control 1													
A	0.8	0.0	9.95	11500	67.7	11568	8795.81	7923.00			7.93	4.47	25.1
B	1.1	0.0	13.04	13300	99.5	13400	9749.81	8816.00			7.97	4.56	25.2
C	0.8	1.0	11.61	11800	73.1	11873	9400.17	10077.00			7.98	4.01	25.4
Standard Deviation				964.37	17.02	981.38							
Mean			*11.53*	*12200*	*80*	*12280*			*42.5%*	*81.7%*			
% relative standard deviation				*7.90*	*21.24*	*7.99*							
Positive Control 2													
A	300.0	2070.0	73.39	1030	91.4	1121	664.75	672.10			5.28	4.04	25.4
B	290.0	1770.0	90.00	723	116	839	496.55	492.90			5.19	4.11	25.4
C	920.0	1730.0	84.61	703	111	814	466.13	504.61			5.19	4.14	25.5
Standard Deviation				183.3	13.0	170.7							
Mean			*82.67*	*819*	*106*	*925*			*96.3%*	*74.2%*			
% relative standard deviation				*22.39*	*12.25*	*18.46*							
Positive Control 3													
A	0.6	0.4	10.68	55500	28100	83600	55500	0.00			7.86	4.28	25.4
B	0.9	0.0	10.45	53100	27600	80700	53100	0.00			7.90	4.15	25.7
C	0.8	0.0	10.47	59100	32800	91900	59100	0.00			7.92	4.22	DNR
Standard Deviation				3019.93	2868.80	5812.92							
Mean			*10.53*	*55900*	*29500*	*85400*			*75.3%*	*69.5%*			
% relative standard deviation				*5.40*	*9.72*	*6.81*							
Product A													
A	1280.0	1920.0	213.90	1600	23.4	1623	975.88	1063.25			5.65	3.54	25.3
B	1870.0	1960.0	199.20	2940	70.4	3010	1739.52	2084.20			5.55	3.77	25.3
C	1900.0	1650.0	220.00	2640	56.9	2697	1545.00	1940.50			5.44	3.69	25.3
Standard Deviation				703.23	24.20	727.38							
Mean			*211.03*	*2393*	*50*	*2444*			*90.0%*	*82.4%*			
% relative standard deviation				*29.38*	*48.17*	*29.77*							
Product B													
A	2.0	44.3	84.98	0	82.8	83	0.00	0.00			7.81	3.20	26.1
B	2.3	42.3	84.81	2.05	58.4	60	2.05	0.00			7.39	3.00	25.9
C	DNR	35.5	74.07	0	29.2	29	0.00	0.00			7.12	3.25	25.8
Standard Deviation				1.18	26.84	26.92							
Mean			*81.29*	*1*	*57*	*57*			*100.0%*	*85.1%*			
% relative standard deviation				*173.21*	*47.25*	*46.84*							
Product C													
A	1.5	5.0	31.02	1760	227	1987	1252.71	829.17			7.49	3.65	25.2
B	1.5	2.1	40.29	2250	182	2432	1586.76	1177.50			7.64	3.45	25.4
C	2.4	7.9	33.83	3520	246	3766	2568.10	2040.20			7.72	3.25	25.5
Standard Deviation				908.35	32.87	925.78							
Mean			*35.05*	*2510*	*218*	*2728*			*85.6%*	*48.9%*			
% relative standard deviation				*36.19*	*15.05*	*33.93*							

	TIME = 4 WEEKS 12/8/2010												
	NO3--N mg/L	PO43- mg/L	TOC mg/L	Alkanes mg/kg	PAHs mg/kg	TPH mg/kg	DRO mg/kg	ORO mg/kg	% Red. Alkanes	% Red. PAHs	pH	DO mg/L	Temp °C
Product D													
A	0.8	1.7	36.24	495	234	729	291.91	329.82			7.35	3.39	25.6
B	0.9	2.4	35.19	642	133	775	476.65	348.07			7.41	3.61	25.6
C	0.7	2.1	28.13	773	180	953	700.62	289.69			7.46	3.41	25.6
Standard Deviation				139.08	50.54	118.30							
Mean			*33.19*	*637*	*182*	*819*			*95.3%*	*68.9%*			
% relative standard deviation				*21.84*	*27.72*	*14.45*							
Product E													
A	340.0	960.0	91.35	1090	149	1239	814.94	635.10			6.19	3.78	25.5
B	990.0	1050.0	91.82	1210	84.4	1294	832.23	759.43			6.17	3.69	25.5
C	780.0	1070.0	105.90	2170	143	2313	1454.42	1462.20			6.13	3.77	25.4
Standard Deviation				591.95	35.69	604.72							
Mean			*96.36*	*1490*	*125*	*1615*			*93.6%*	*61.0%*			
% relative standard deviation				*39.73*	*28.45*	*37.43*							
Product F													
A	1.1	0.0	36.43	3230	219	3449	2418.70	1770.30			7.68	3.37	25.2
B	1.1	0.4	39.11	4070	308	4378	3114.46	2314.80			7.66	3.94	25.4
C	0.9	0.0	26.02	4490	310	4800	3308.50	2769.20			7.73	4.30	25.3
Standard Deviation				641.56	51.97	691.17							
Mean			*33.85*	*3930*	*279*	*4209*			*78.8%*	*44.9%*			
% relative standard deviation				*16.32*	*18.63*	*16.42*							
Product G													
A	0.6	27.0	192.30	8610	203	8813	7000.85	5842.00			7.12	3.65	26.2
B	0.9	28.6	292.90	10500	192	10692	8669.79	7139.00			7.10	3.45	26.1
C	1.1	30.2	239.90	12600	266	12866	10302.53	8633.00			6.82	3.75	26.1
Standard Deviation				1995.92	39.93	2028.29							
Mean			*241.70*	*10570*	*220*	*10790*			*62.4%*	*47.7%*			
% relative standard deviation				*18.88*	*18.12*	*18.80*							
Product H													
A	17.2	1860.0	105.70	718	206	924	525.80	420.16			5.13	4.03	25.3
B	19.6	1660.0	70.77	1610	190	1800	1127.82	908.50			5.33	4.05	25.3
C	15.9	1730.0	120.30	1110	204	1314	829.00	567.89			5.15	3.80	25.3
Standard Deviation				447.09	8.72	438.88							
Mean			*98.92*	*1146*	*200*	*1346*			*95.0%*	*36.8%*			
% relative standard deviation				*39.01*	*4.36*	*32.61*							
Product I	17-Feb-2011												
A	12300.0	15400.00	81.44	1670	372	2042	1521.85	1025.51			5.36	4.91	22.2
B	6000.0	15500.00	70.28	1370	350	1720	1284.42	938.05			5.34	4.96	22.2
C	10800.0	16700.00	70.80	1060	305	1365	1100.87	776.10			5.30	4.61	22.4
Standard Deviation				305.01	34.15	338.63							
Mean			*74.17*	*1367*	*342*	*1709*			*95.2%*	*25.7%*			
% relative standard deviation				*22.32*	*9.98*	*19.81*							
Product J													
A	13400.0	16900.00	2466.00	1600	331	1931	1329.81	822.64			5.58	4.58	22.9
B	15600.0	18700.00	2330.00	1770	364	2134	1495.82	887.09			5.56	3.88	23.0
C	10100.0	17700.00	2657.00	1930	381	2311	1521.85	1025.51			5.76	3.78	22.9
Standard Deviation				165.03	25.42	190.15							
Mean			*2484.33*	*1767*	*359*	*2125*			*93.6%*	*31.1%*			
% relative standard deviation				9.34	7.09	8.95							

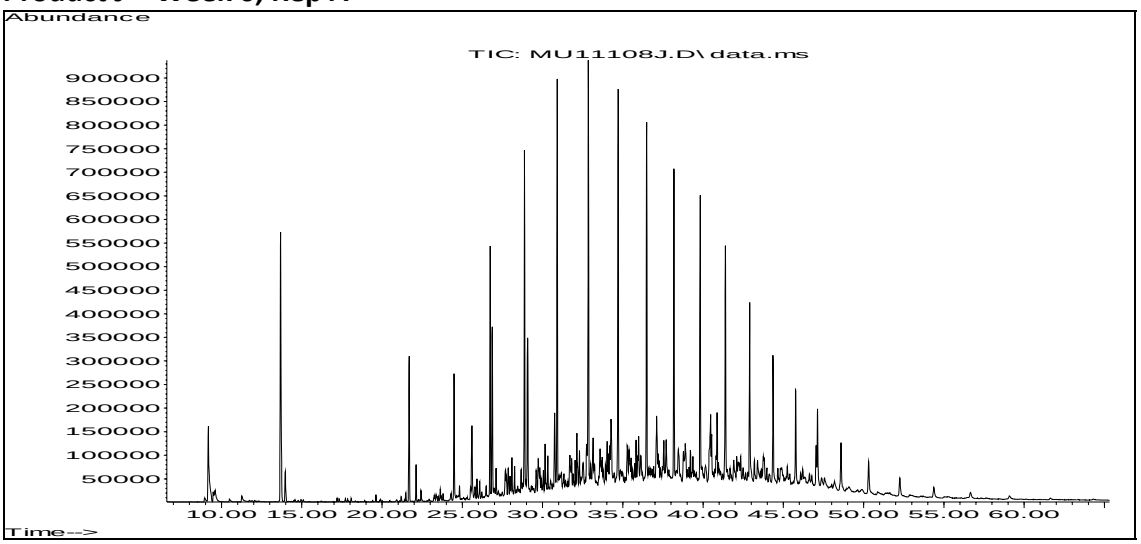
	TIME = 12 WEEKS 2/3/2011												
	NO_3^--N mg/L	PO_4^{3-} mg/L	TOC mg/L	Alkanes mg/kg	PAHs mg/kg	TPH mg/kg	DRO mg/kg	ORO mg/kg	% Red. Alkanes	% Red. PAHs	pH	DO mg/L	Temp °C
Negative Control													
A	0.7	0.0	16.03	25100	401	25501	21010.30	18418.00			7.81	5.58	22.1
B	0.6	0.0	19.40	23400	309	23709	16284.50	14137.00			7.89	4.81	22.1
C	0.9	0.0	13.86	23400	341	23741	19448.00	17173.00			7.96	5.02	22.3
Standard Deviation				981.50	46.70	1,025.50							
Mean			*16.43*	*23967*	*350*	*24317*			*14.2%*	*14.1%*			
% relative standard deviation				*4.10*	*13.33*	*4.22*							
Positive Control 1													
A	0.5	0.0	16.18	25100	341	25441	21188.92	18417.00			7.77	4.90	21.9
B	0.7	0.0	13.84	23000	291	23291	19625.70	16540.00			7.78	4.61	21.9
C	0.4	0.0	21.56	24100	303	24403	20087.90	17605.00			7.91	5.03	22.4
Standard Deviation				1050.40	26.10	1075.21							
Mean			*17.19*	*24067*	*312*	*24378*			*-13.5%*	*28.7%*			
% relative standard deviation				*4.36*	*8.38*	*4.41*							
Positive Control 2													
A	7600.0	DNR	99.63	900	340	1240	596.94	691.51			5.24	5.04	21.9
B	16400.0	15800.0	95.00	1020	359	1379	685.62	800.09			5.23	4.95	22.1
C	11400.0	16300.0	101.40	957	333	1290	590.10	763.46			5.25	5.01	23.3
Standard Deviation				60.02	13.45	70.41							
Mean			*98.68*	*959*	*344*	*1303*			*95.6%*	*16.5%*			
% relative standard deviation				*6.26*	*3.91*	*5.40*							
Positive Control 3													
A	0.3	0.0	5.21	48700	24800	73500	48700	0.00			7.88	4.70	22.1
B	0.5	0.0	5.25	51700	23400	75100	51700	0.00			7.93	4.72	22.1
C	0.4	0.0	5.45	48300	24500	72800	48300	0.00			7.96	4.64	22.3
Standard Deviation				1858.31	737.11	1178.98							
Mean			*5.30*	*49567*	*24233*	*73800*			*78.1%*	*74.9%*			
% relative standard deviation				*3.75*	*3.04*	*1.60*							
Product A													
A	11000.0	15800.0	254.50	743	216	959	548.75	544.09			5.75	4.43	21.1
B	17000.0	15800.0	236.50	982	268	1250	698.22	688.54			5.76	5.50	21.3
C	16300.0	17300.0	199.40	1240	274	1514	964.44	783.30			5.76	5.27	21.3
Standard Deviation				248.56	31.90	277.61							
Mean			*230.13*	*988*	*253*	*1241*			*95.9%*	*11.2%*			
% relative standard deviation				*25.15*	*12.62*	*22.37*							
Product B													
A	0.9	62.0	88.00	30.7	228	259	30.75	5.25			6.27	4.54	22.2
B	1.2	64.4	112.40	324	358	682	224.12	223.18			5.62	4.30	22.1
C	1.3	60.3	123.90	7.95	114	122	7.95	0.00			5.83	4.98	22.0
Standard Deviation				176.27	122.09	291.99							
Mean			*108.10*	*121*	*233*	*354*			*98.6%*	*38.9%*			
% relative standard deviation				*145.82*	*52.32*	*82.43*							
Product C													
A	1.0	0.0	54.57	1030	49	1079	963.17	474.74			7.60	4.92	20.4
B	1.0	0.4	46.04	2950	122	3072	2162.80	1714.10			7.73	4.88	21.0
C	1.4	0.9	49.70	1950	104	2054	1421.21	1155.37			7.74	4.78	21.2
Standard Deviation				960.28	38.03	996.58							
Mean			*50.10*	*1977*	*92*	*2068*			*88.6%*	*78.6%*			
% relative standard deviation				*48.58*	*41.49*	*48.18*							

	TIME = 12 WEEKS 2/3/2011												
	NO3--N mg/L	PO43- mg/L	TOC mg/L	Alkanes mg/kg	PAHs mg/kg	TPH mg/kg	DRO mg/kg	ORO mg/kg	% Red. Alkanes	% Red. PAHs	pH	DO mg/L	Temp °C
Product D													
A	1.1	3.2	66.65	3.05	7.52	11	3.05	0.00			7.42	4.64	20.6
B	1.4	2.6	69.27	36.6	19.2	56	36.62	3.48			7.45	4.22	20.7
C	1.5	5.4	49.59	10.9	0	11	10.89	4.40			7.55	4.47	20.8
Standard Deviation				17.55	9.67	26.02							
Mean			*61.84*	*17*	*9*	*26*			*99.9%*	*98.5%*			
% relative standard deviation				*104.15*	*108.62*	*101.02*							
Product E													
A	17500.0	7700.0	139.90	325	322	647	149.80	269.17			6.13	5.01	21.0
B	12900.0	9000.0	133.20	321	378	699	112.21	289.16			6.12	4.92	21.1
C	16200.0	8800.0	125.00	419	248	667	219.78	322.99			6.09	4.91	21.0
Standard Deviation				55.46	65.21	26.23							
Mean			*132.70*	*355*	*316*	*671*			*98.5%*	*1.8%*			
% relative standard deviation				*15.62*	*20.64*	*3.91*							
Product F													
A	0.6	0.0	33.24	4050	47.6	4098	2937.30	2443.10			7.71	4.90	21.1
B	0.6	0.0	31.43	5560	143	5703	3850.73	3809.80			7.74	4.70	21.2
C	0.9	0.0	33.15	1450	124	1574	1299.10	797.52			7.82	4.58	21.4
Standard Deviation				2078.95	50.50	2081.45							
Mean			*32.61*	*3687*	*105*	*3792*			*80.1%*	*79.3%*			
% relative standard deviation				*56.39*	*48.15*	*54.90*							
Product G													
A	1.5	25.3	85.27	5180	218	5398	3529.00	3383.90			7.56	4.84	20.5
B	0.6	26.7	166.90	5450	215	5665	3663.00	3622.10			7.65	4.52	20.8
C	1.6	25.3	179.50	5270	222	5492	3724.10	3362.80			7.58	4.79	20.8
Standard Deviation				137.48	3.51	135.43							
Mean			*143.89*	*5300*	*218*	*5518*			*81.2%*	*48.2%*			
% relative standard deviation				*2.59*	*1.61*	*2.45*							
Product H													
A	420.0	16500.0	107.90	1640	279	1919	1283.49	988.05			5.35	4.52	21.2
B	1830.0	16700.0	99.66	887	191	1078	567.46	640.79			5.39	4.75	21.4
C	1310.0	17500.0	93.04	796	212	1008	442.49	601.07			5.32	5.01	21.5
Standard Deviation				463.25	45.96	506.97							
Mean			*100.20*	*1108*	*227*	*1335*			*95.2%*	*28.1%*			
% relative standard deviation				*41.82*	*20.22*	*37.98*							
Product I	14-Apr-2011												
A	8900.0	14900.00	124.40	425	378	803	171.69	359.89			5.21	4.90	22.6
B	7900.0	16500.00	115.80	450	375	825	137.51	398.43			5.22	4.95	22.4
C	10200.0	13700.00	101.50	440	353	793	193.01	391.03			5.22	4.80	22.3
Standard Deviation				12.58	13.65	16.37							
Mean			*113.90*	*438*	*369*	*807*			*98.5%*	*20.0%*			
% relative standard deviation				*2.87*	*3.70*	*2.03*							
Product J													
A	12000.0	17800.00	2229.00	318	362	680	141.06	269.23			5.82	4.82	22.4
B	10500.0	15700.00	2178.00	340	350	690	163.99	295.63			5.76	4.63	22.7
C	8500.0	16900.00	1489.00	337	303	640	203.55	305.25			5.84	4.62	22.7
Standard Deviation				11.93	31.18	26.46							
Mean			*1965.33*	*332*	*338*	*670*			*98.8%*	*35.0%*			
% relative standard deviation				*3.60*	*9.22*	*3.95*							

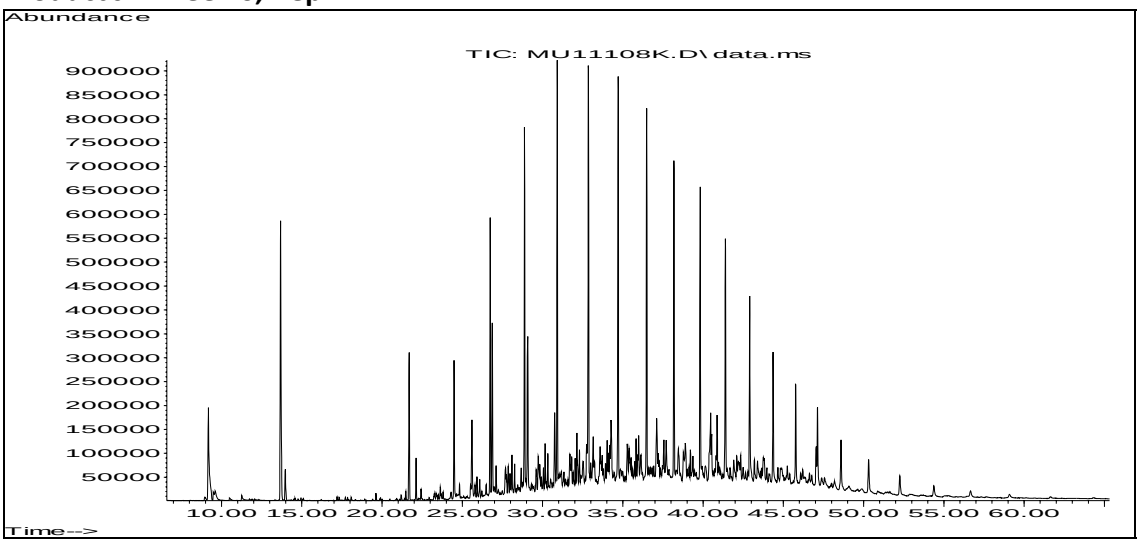
Appendix B. Chromatographs of Extracted Flasks Over Time

Data sets presented are for total alkanes and PAHs (noted on each figure as TIC, Total Ion Chromatogram) from Time 0 to Time 12.

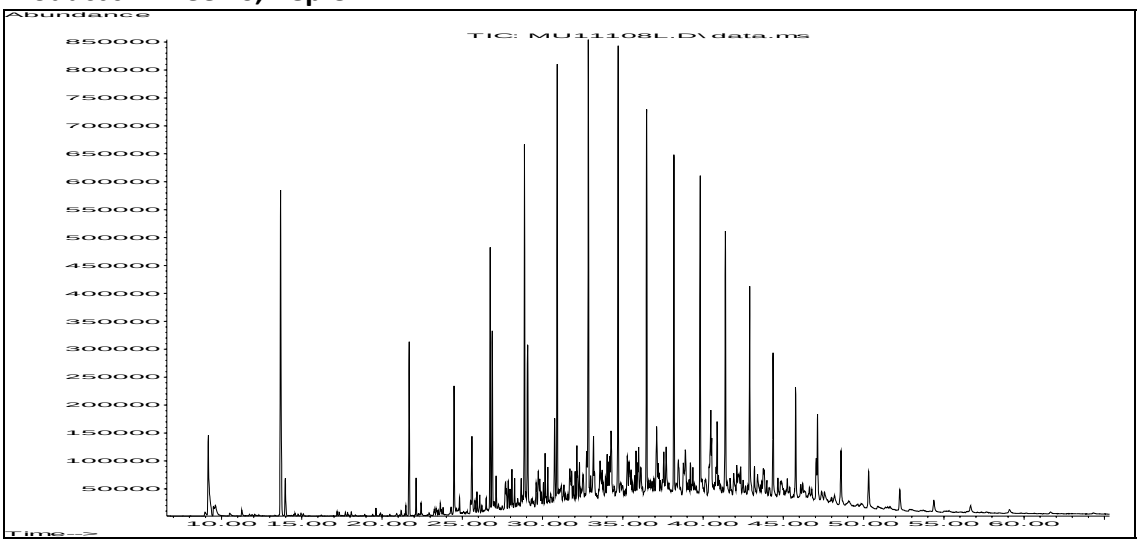
Product J – Week 0, Rep A



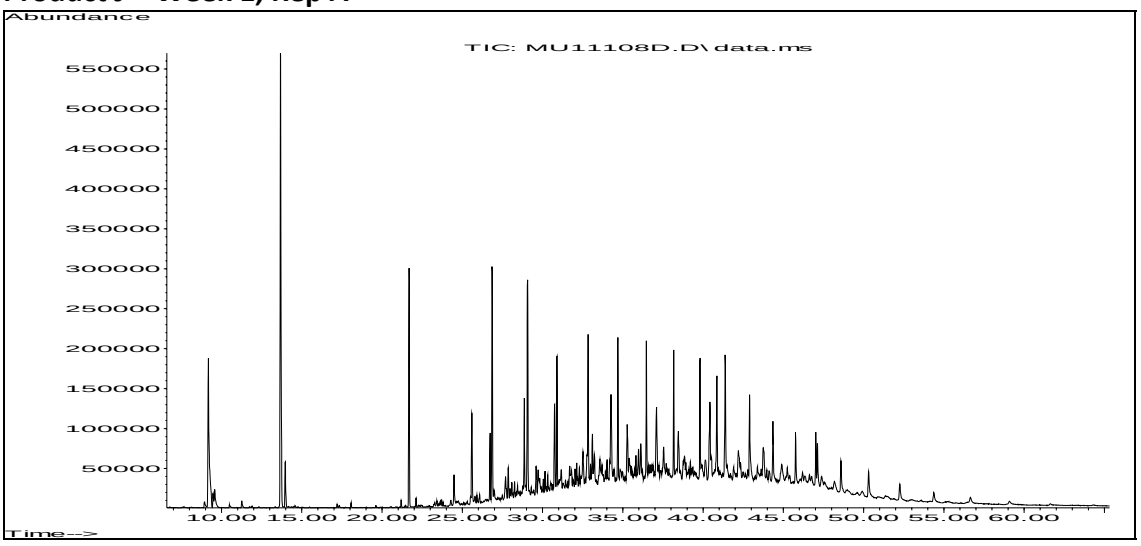
Product J – Week 0, Rep B



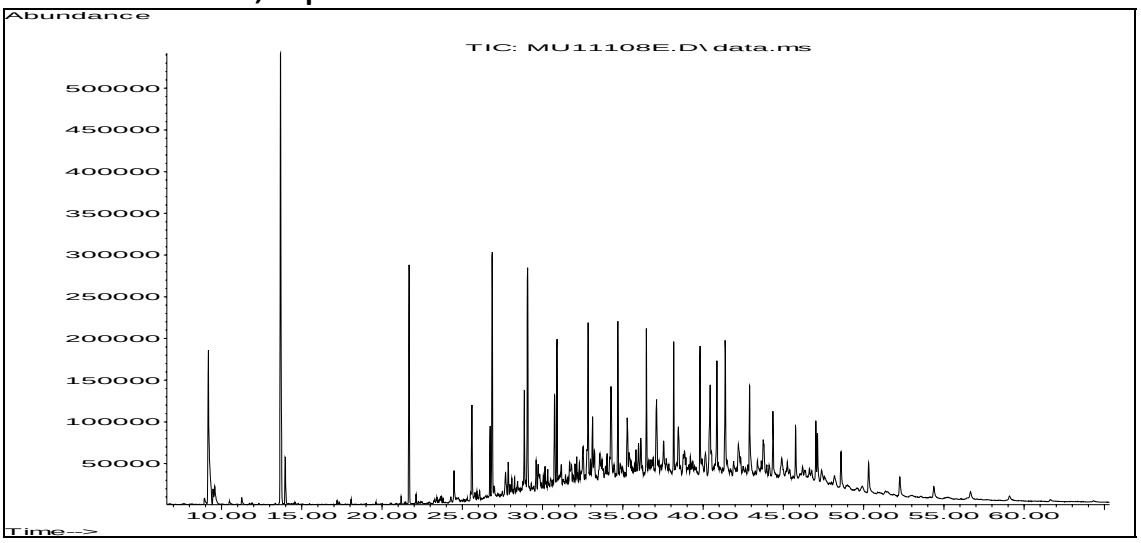
Product J – Week 0, Rep C



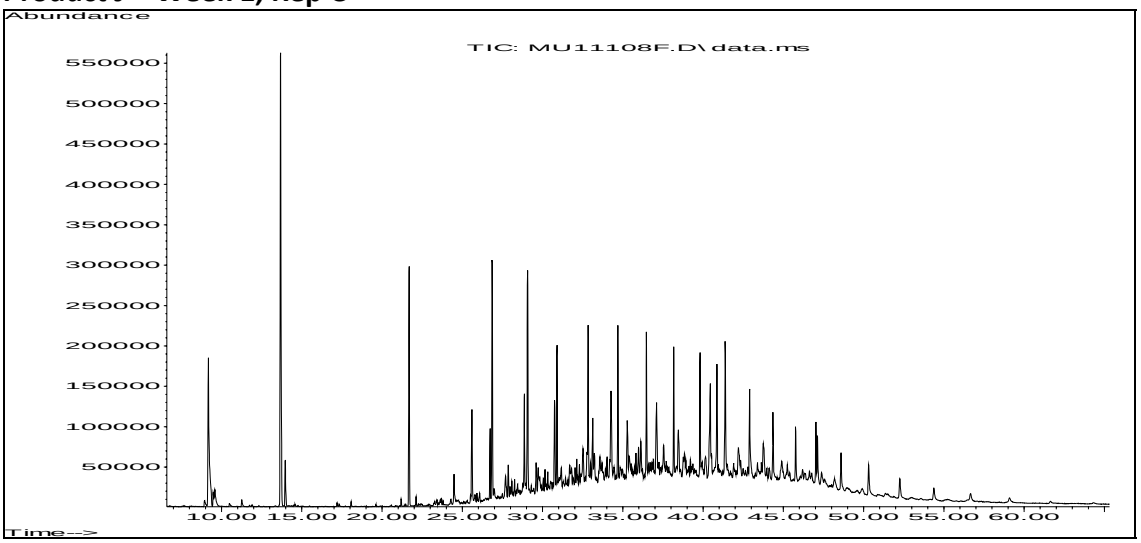
Product J – Week 1, Rep A



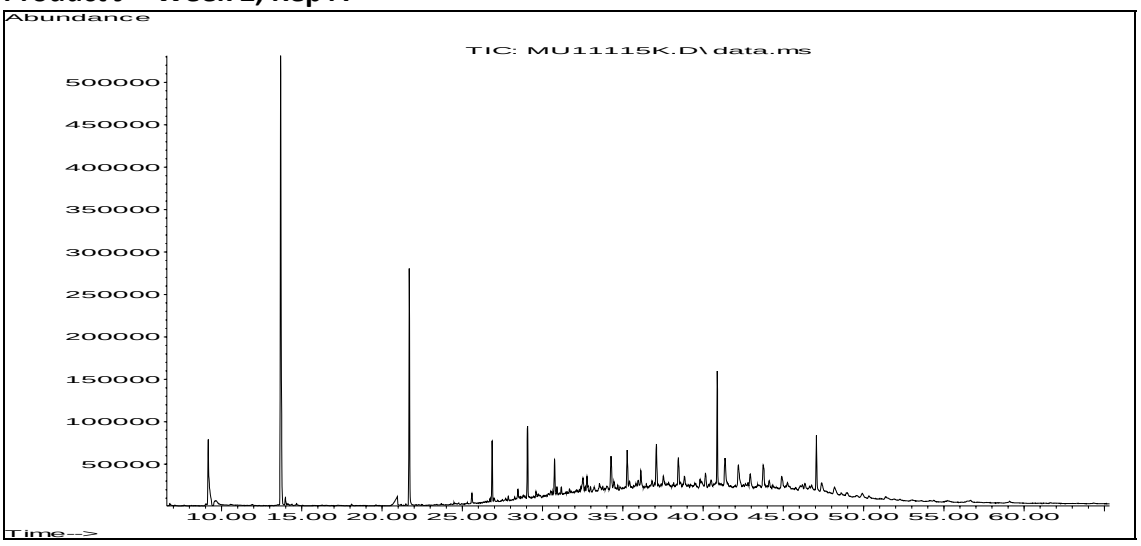
Product J – Week 1, Rep B



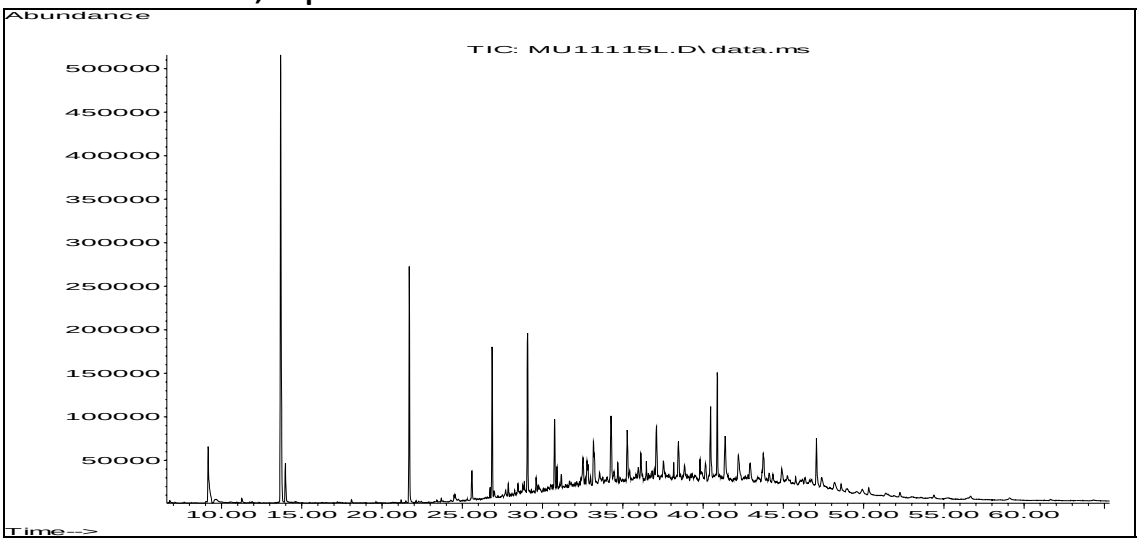
Product J – Week 1, Rep C



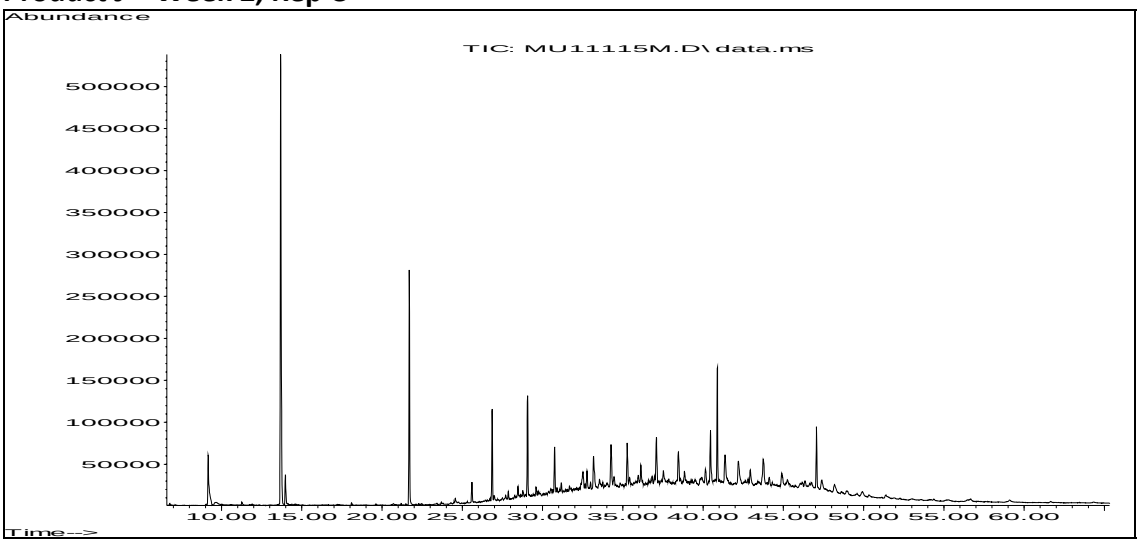
Product J – Week 2, Rep A



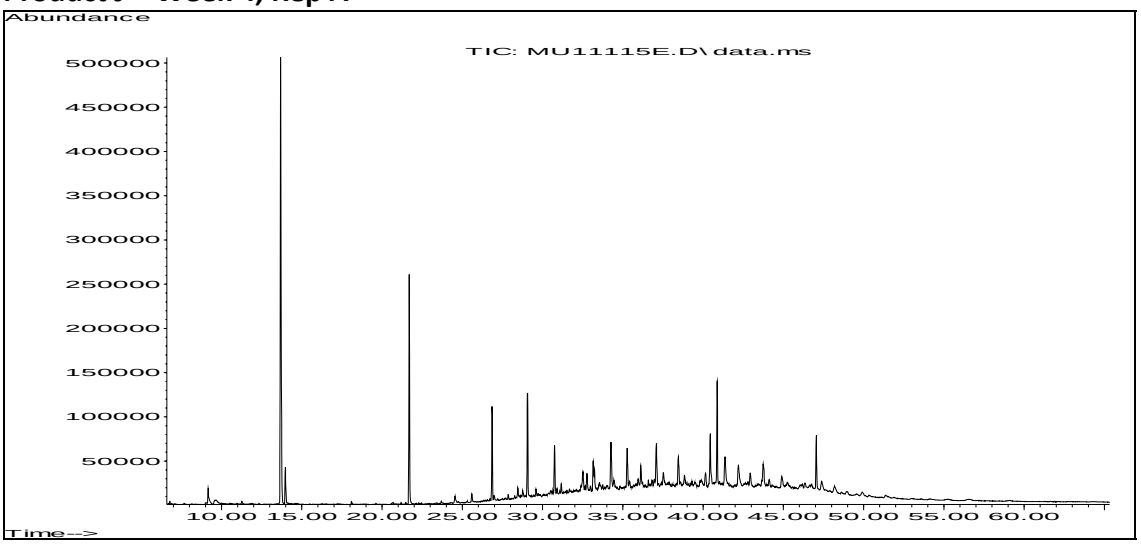
Product J – Week 2, Rep B



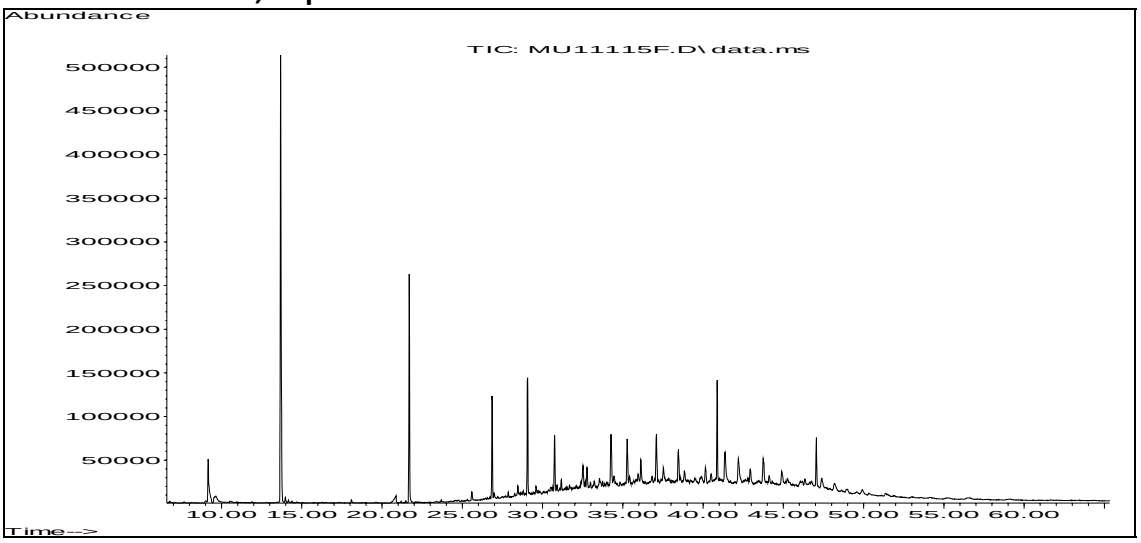
Product J – Week 2, Rep C



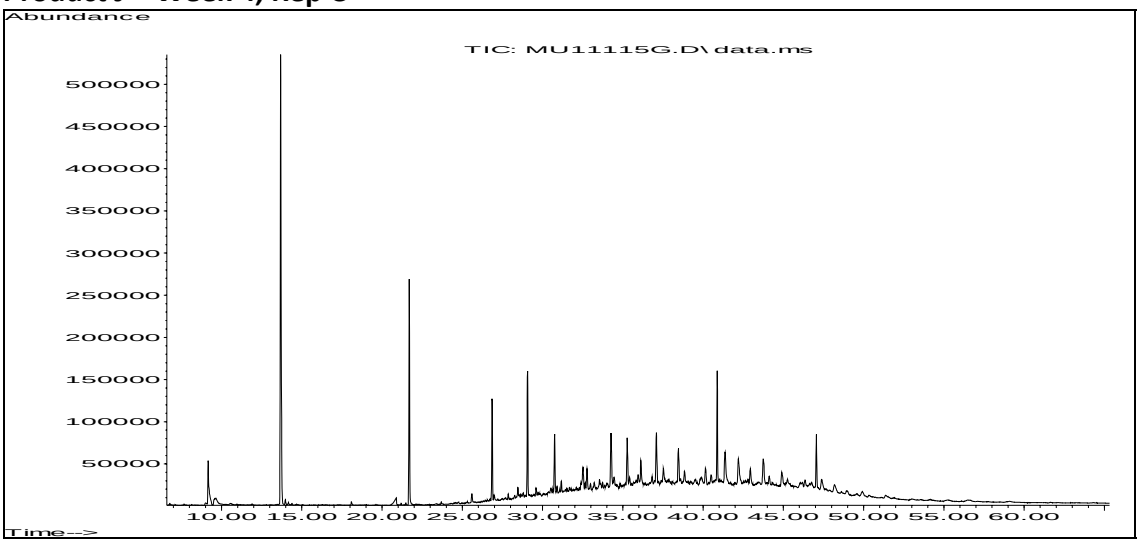
Product J – Week 4, Rep A



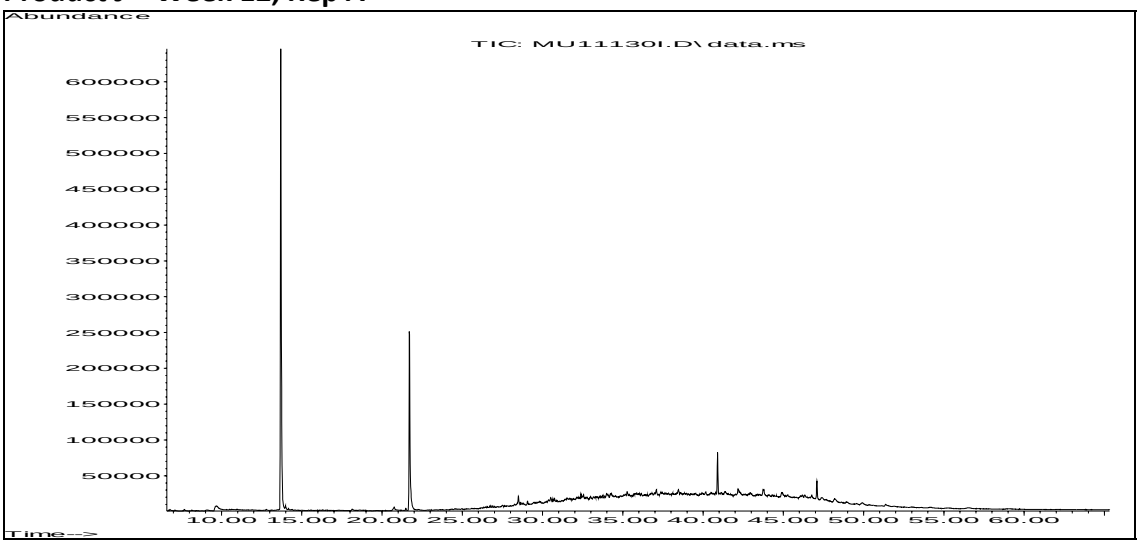
Product J – Week 4, Rep B



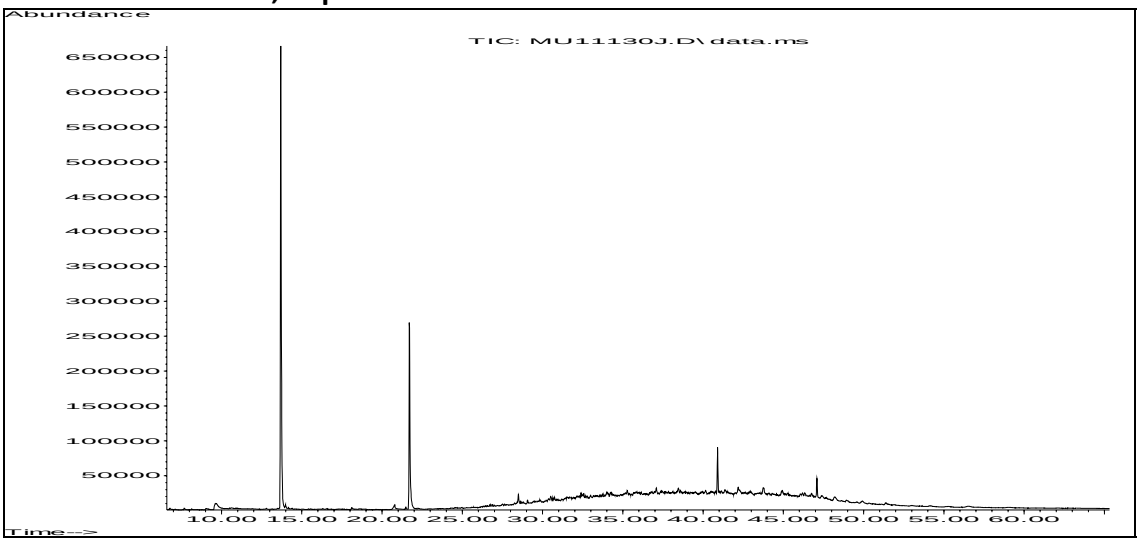
Product J – Week 4, Rep C



Product J – Week 12, Rep A



Product J – Week 12, Rep B



Product J – Week 12, Rep C