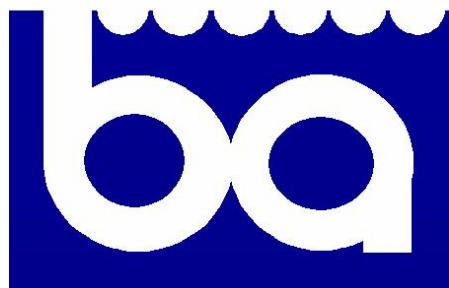




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Bio-Aquatic Testing

2501 Mayes Rd
Suite 100
Carrollton, TX 75006
(972) 242-7750

Bioremediation Agent Effectiveness Test

Oil Spill Eater II

Oil Spill Eater International, Corp.

June 25, 2009

Prepared by:

A handwritten signature in black ink, appearing to read "R. J. Flatt".

Vice President

6/26/2009

Date

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EXECUTIVE SUMMARY

Bio-Aquatic Testing, Inc. located at 2501 Mayes Rd. Suite 100 Carrollton, Texas 75006 was contracted by Oil Spill Eater International, Corp. (OSEI) to test effectiveness of their bioremediation product, Oil Spill Eater II, using Environmental Protection Agency (EPA) protocol listed in 40 CFR Chapter 1 (7-1-99) Pt. 300 Appendix C, Item 4.0. The test protocol calls for application of products onto ANS 521 oil. The product was applied to test flasks according to manufacturer's specifications. Samples were sacrificed on Day 0, Day 7, and Day 28 of the test period. Day 0 and Day 7 samples were sampled for microbiological analysis and then frozen at -10° C until GC/MS results were known for the Day 28 samples. Each replicate of product and control were tested for continued microbiological viability over time, reduction in weight via gravimetric analysis, and reduction in alkane and/or aromatic constituents via Gas Chromatography/Mass Spectroscopy (GC/MS). The product was deemed effective if the data showed the GC/MS product results for Day 28 treatments to be statistically less than the Day 28 controls and Day 28 treatments to be statistically less than Day 0 treatments.

GC/MS data for Days 0, 7, and 28, were consolidated and analyzed with the Minitab Statistical program 13.3. Data was analyzed for a significant difference between controls and treatments (products) using a General Linear ANOVA Model with Dunnett's and/or Tukey's means comparison test. GC/MS analysis showed significant reduction of both alkane and aromatic constituents of the test oil as indicated by the statistically significant difference between the Day 28 controls and Day 28 treatments as well as between Day 0 control and Day 28 treatments. Day 7 results also showed a statistically significant reduction of treatments as compared to controls.

The surrogate compounds, d-10 phenanthrene and 5- α androstane showed recovery percentages which indicates the test meets acceptability criteria and is considered valid.

Microbiological results showed continued viability of the oil-eating microorganisms over time. Day 7 and Day 28 gravimetric analysis showed a statistically significant reduction from the controls to the treatments.

Based on the parameters of this test, the product should be included on the NCP list of approved bio-remediation products.

BIOREMEDIATION AGENT EFFECTIVENESS TEST USING OSEI CORP.
PRODUCT "Oil Spill Eater II"

Introduction

The bioremediation agent effectiveness testing protocol is designed to determine a product's ability to biodegrade oil by quantifying changes in the oil composition resulting from biodegradation. The protocol quantifies the disappearance of saturated hydrocarbons and polynuclear aromatic hydrocarbons (PAHs) as well as weight loss. The protocol also tests for microbial activity over time to ascertain continued viability of oil degrading microorganisms.

Summary of Method

The protocol calls for gas chromatography/mass spectrophotometry and gravimetric analyses to quantify saturated hydrocarbons and PAHs, and determine weight loss respectively. The sample preparation procedure extracts the oil phase into dichloromethane (DCM), with a subsequent distillation to 1-3-mL using a K-D apparatus and Snyder column. To effectively accomplish the goals of the testing protocol, it is necessary to normalize the concentration of the various analytes in oil to a non-biodegradable marker, either C₂- or C₃ – phenanthrene, C₂–chrysene, or hopane. The test method targets the relatively easy to degrade normal alkanes and the more resistant and toxic PAHs. It normalizes their concentrations to C₂ or C₃ phenanthrene, C₂–chrysene, or C₃₀17α(H), 21β(H)-hopane on an oil weight basis (mg marker/kg oil, mg target analyte/kg). The analytical technique uses a high-resolution gas chromatography/mass spectrophotometer (GC/MS) because of its high degree of chemical separation and spectral resolution. GC/MS has long been used to study the weathering and fate of oil spilled into the environment. For quantitative analyses, the instrument is operated in the selective ion detection mode (SIM) at a scan rate of greater than 1.5 scans per second to maximize the linear quantitative range and precision of the instrument. The sample preparation method does not exclude analysis of selected samples by GC/MS in the full scanning mode of operation to qualitatively assess changes in the oil not accounted for by the SIM approach. Gravimetric analysis is used to support the GC/MS analysis by measuring weight loss of samples over time as compared to controls by drying the extracted samples using nitrogen a blowdown technique.

Performed concurrently with the chemical analysis described above is a microbiological analysis. The microbiological analysis is performed to determine and monitor the viability of relative concentrations of the microbial cultures being studied. Using this method, continued viability is measured over time by comparing serial dilutions of microorganisms, to determine statistical significance between treatments and controls.

MATERIALS AND METHODS

The following methods* were obtained from 40 CFR Chapter 1 (7-1-99) Pt. 300 Appendix C, item 4.0 Bioremediation Effectiveness test, as submitted by the Environmental Protection Agency. Some modifications were made to these methods as discussed below.

The procedure consists of an experimental orbital shaker flask setup using 250-mL Erlenmeyer flasks labeled with unique identifiers using the following treatment design:

Table 1.

*Details from these methods can be found in the aforementioned 40 CFR Chapter 1 (7-1-99) Pt. 300 Appendix C, item 4.0. A copy is available upon request.

Treatment	Number of samples at sampling times			Total number of analytical determinations		
	Day 0	Day 7	Day 28	ANALYSES		
				Microbial counts	Gravimetric	GC/MS
Control	3	3	3	9	9	9
Nutrient	3	3	3	9	9	9
Oil Spill Eater II	3	3	3	9	9	9

Number of replicates per treatment or control per sampling event - 3

Number of replicates per treatment or control - 9

Total replicates - 27

Control - Oil + Seawater

Nutrient – Oil + Seawater + EPA Nutrient

Oil Spill Eater II – Oil + Seawater + Product

Using sterile technique, each appropriately labeled replicate flask has 100-mL of seawater added. The seawater obtained was from the Gulf of Mexico by faculty at LSU. Each flask is placed on a balance and the weight recorded. Approximately one half-gram (0.5 g) of artificially weathered oil (Alaska North Slope 521)* is then added to each flask while still on the balance and the weight recorded again.

*The ANS 521 oil was obtained from John Haines of the Environmental Protection Agency's Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, 45268

The control flasks were prepared by adding oil to the natural seawater.

The nutrient flasks were prepared as instructed in 40 CFR Chapter 1 (7-1-99) Pt. 300 Appendix C.

The product mix was prepared according to the manufacturer's instructions. The product was applied to each oil + product flask at a ratio of 10:1 (V/V).

After preparing all treatments and controls, three replicates of each treatment and control were shaken on an orbital shaker at 190 – 200 rpm and incubated at 20° C until sacrificed for the Day 0, 7, and 28 analyses. At each sampling (sacrifice) day, a 0.5-mL aliquot was set aside for microbiological analysis and the remaining solution is prepared for chemical analysis.

A phosphate buffer solution was made from a recipe obtained from Jan Kurtz of the Microbial Ecology Branch of the Environmental Protection Agency's Gulf Breeze Ecology Division. A 0.5-mL aliquot from each replicate was added to a test tube containing 4.5-mL of a sterile phosphate buffer for the microbiological analysis. Aseptic technique was then used to make serial dilutions down to a 10^{-8} dilution. Microtiter plates were prepared by adding 1.75-mL of Bushnell-Haas broth into to each well. Six replicates per dilution are used per treatment or control giving a total of forty-eight wells, (48) per treatment or control. Each of the wells was inoculated with 0.1-mL of solution from each of the serial dilutions made from the original aliquot of 0.5-mL of sample. 20 μ L of sterile No. 2 fuel oil was then carefully placed on top of the solution in each well. Each microtiter plate was then incubated for fourteen (14) days at 20° C. At the conclusion of the fourteen-day incubation period, 100 μ L of p-iodotetrazolium violet dye was added to each well and the results were recorded after at least 45 minutes to 2 hours of reaction time. Appearance of a pink to purple color constituted a positive test (continued microbial viability).

Each replicate sacrificed was extracted with an initial volume of 50-mL dichloromethane (DCM) for the chemical analysis. The sample was first extracted three times with 10-mL aliquots of the DCM. The remaining 20-mL was used to rinse the separatory funnel and added to the first 30-mL of extract. Just prior to the initial extraction, each replicate is spiked with 100 μ L of a surrogate-recovery standards stock solution. This stock solution was made up of 500 mg/L 5 α -androstane and d₁₀-phenanthrene. The separatory funnel was then capped and shaken vigorously for approximately thirty seconds to insure good mixing between phases. After mixing, the separatory funnel was allowed to sit for up to three hours to insure the greatest amount of separation between phases. This was done because of the presence of thick emulsions caused by microbiological activity. After a period of up to three hours, a 10-mL aliquot of the extract is poured into a 40-mL amber vial with a Teflon™ lined cap, and taped with Teflon™ tape. The samples were then stored in a 4° C walk-in refrigerator until retrieval for gravimetric analysis. The extraction was completed by filtering the remaining 40ml of DCM through a glass filter containing 20 grams of anhydrous sodium sulfate (Na₂SO₄) and into a 250-ml flat-bottom distillation flask. The Na₂SO₄ was rinsed with DCM until all traces of oil were removed from the funnel. The 250-ml flat-bottom distillation flask was placed on a Rotovap distillation unit until a volume of 10-ml was attained. Approximately 50-ml of hexane was added to the DCM extract and distilled to a volume of 10-ml. Another 50-ml of hexane was added to the hexane extract and distilled down to a final volume of 10-ml. A 1-ml aliquot of the final extract was removed and prepared for analysis on the GC/MS.

The gravimetric analysis was accomplished by first weighing an empty 40-mL vial and recording the weight. The 10-mL aliquot of extract was then placed in the vial, weighed and concentrated to dryness using a nitrogen gas blowdown technique. The remaining sample was then weighed and subjected to nitrogen blowdown for another ten to fifteen minutes. This was repeated once more to insure that the weight had changed no more than 5% weight difference between the second and third blowdown. If there was greater than a 5% difference, the sample was subjected a final blowdown to insure complete dryness. Weights were recorded after each blowdown, and then subjected to statistical analysis discussed below.

*The GC/MS analysis was subcontracted to Louisiana State University-IES, 42 Atkinson Hall, Baton Rouge, Louisiana, 70803.

STATISTICAL METHODS

GC/MS Data

Surrogate-adjusted data or rank-transformed surrogate adjusted data were analyzed using the Minitab™ 13.3 program. The computer program, unlike many others, is powerful enough to analyze unbalanced sets (uneven replication) of data using a general linear multiple factor ANOVA model. The probability of a type I error (α) was set apriori to 0.05.

Data sets were first analyzed for normality using the Anderson-Darling Goodness of Fit test. This test compares plot points with the normal theoretical distribution. Minitab calculates the statistic, above which there is a danger of non-normality. This is then compared to the chosen (preset by program), alpha (α) level of 0.01. For least-squares estimation, Minitab calculates a Pearson correlation coefficient. If the distribution fits the data well, then the plot points on a probability plot will fall on a straight line. The correlation measures the strength of the linear relationship between the X and Y variables on a probability plot. The correlation will range between 0 and 1, with higher values indicating a better fitting distribution.

Data passing a formal test for normality may not, strictly speaking, come from a normal distribution. Data that has sufficient linearity as shown by the passing results of a formal test for normality, may have attributes that weaken the ANOVA and Dunnett's test's ability to detect statistically significant differences between treatments (Zar, 1984).

Routine transformations were not amenable to non-normal data so an acceptable procedure for multiple-comparison ANOVA was found by using the rank-transformation test (Helsel, 1993). This technique first rank transforms the data and subjects it to the same multiple factor ANOVA test. This allows for an acceptable multiple comparison non-parametric test. After the program calculated the "F" and "P" statistics, the data were automatically subjected to Dunnett's means comparison test for comparison between treatments and controls.

Tables below give the final adjusted P-Values. Values of less than 0.05 (chosen α) indicate statistical significance. The T-Value is a ratio of the Difference of Means and Standard Error of Difference and indicates the degree and direction of the difference.

Microbiological Data

Microbiological data was analyzed with the Environmental Protection Agency's Most Probable Number Calculator, designed by the Risk Reduction Engineering Laboratory, Cincinnati, Ohio. This program calculates the most probable number (mpn) per mL with Salama correction for bias, and a Spearman-Karber Estimate. The program is based on the number of positive reactions in each of six replicates per serial dilution made. Confidence limits are included in the output of the program.

Gravimetric Data

Gravimetric data were analyzed with a simple two sample t-test available on the Minitab™ 13.3 program which compares the Day 0, 7, or 28 control means with their respective treatment means for statistical significance. The calculated p-Value is then compared to the chosen alpha (α) level of 0.05, as in the ANOVA analysis above. If the calculated value exceeds the 0.05, there is no statistical significance.

RESULTS AND DISCUSSION

GC/MS Data

Results of the statistical analysis for the surrogate-adjusted data are reported and discussed below. Results for transformed data, if transformations were necessary, are discussed last, preceded by the non-transformed data. Actual data (raw followed by surrogate-adjusted) are presented in the Appendices. GC/MS spectra appear in APPENDIX I along with computer printouts of the Minitab™ ANOVA analysis discussed below, which appear in APPENDIX II.

OSEI CORP. “OIL SPILL EATER II” Product Solution

Surrogate-Adjusted Alkane Data

Preliminary analysis of surrogate-adjusted alkane data for normality (fig.1) showed the raw data to be non-normal with an Anderson-Darling P-statistic of 0.000. This is below the selected α -level of 0.01 and indicates the data are not normally distributed. Further visual evidence of the data’s non-linearity can be seen in the probability plot for residuals of the data (fig.2). The data were rank-transformed and reanalyzed for normality (fig.3) giving an Anderson-Darling statistic of 0.585, well above the chosen α -level of 0.01. The probability plot for the residuals (fig. 4) of the data still show a small degree of non-linearity which can slightly lower the ANOVA and Dunnett’s test ability to detect a statistical difference between treatments and controls. More on this subject is discussed in the conclusions.

Normality Test for Non-Transformed Alkane Data

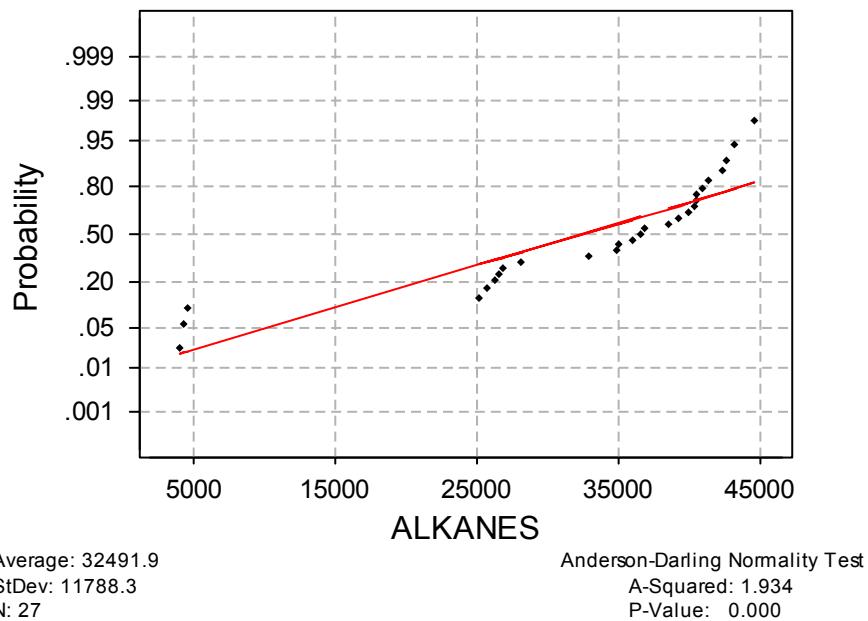


Figure 1. – Anderson-Darling test for normality showing non-linearity of surrogate adjusted alkane data.

Normal Probability Plot for Non-Transformed Alkane Data

LSXY Estimates - 95% CI

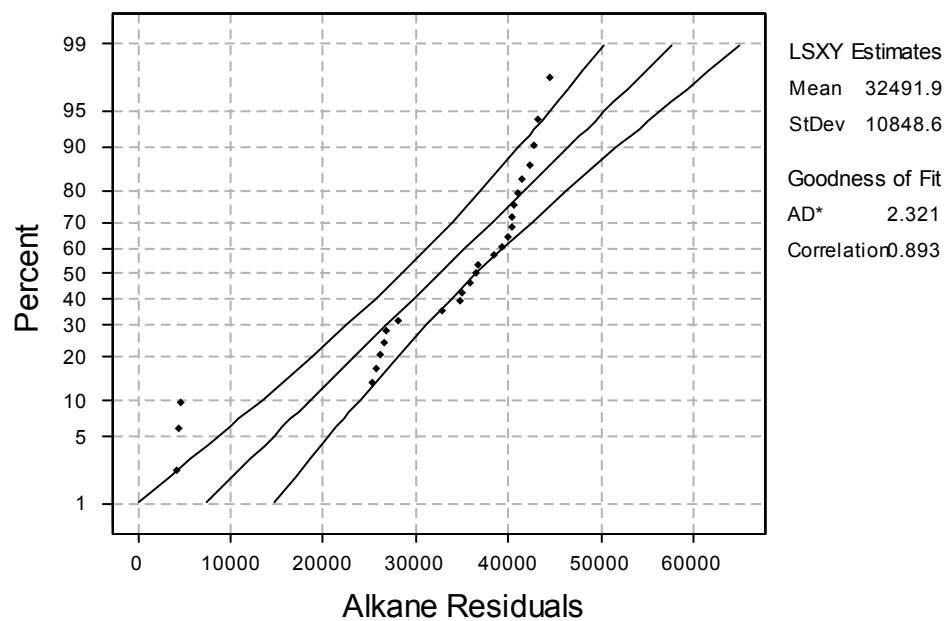


Figure 2. – Probability plot of the surrogate-adjusted alkane residuals showing further evidence of non-linearity.

Normality Test for Rank-Transformed Alkane Data

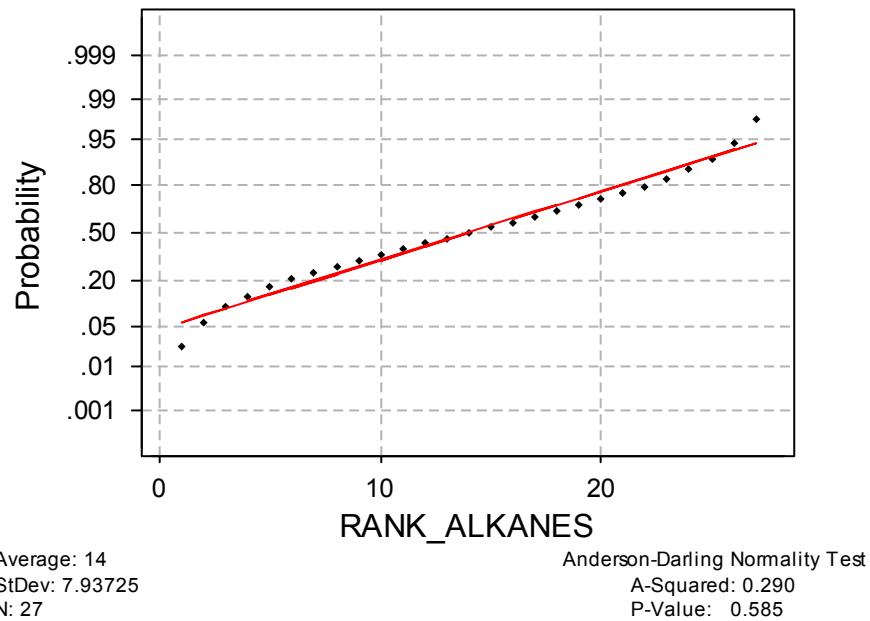


Figure 3. - Anderson-Darling test for normality showing improved linearity of the rank transformed surrogate-adjusted alkane data.

Normal Probability Plot for Rank-Trans Alkane Data

LSXY Estimates - 95% CI

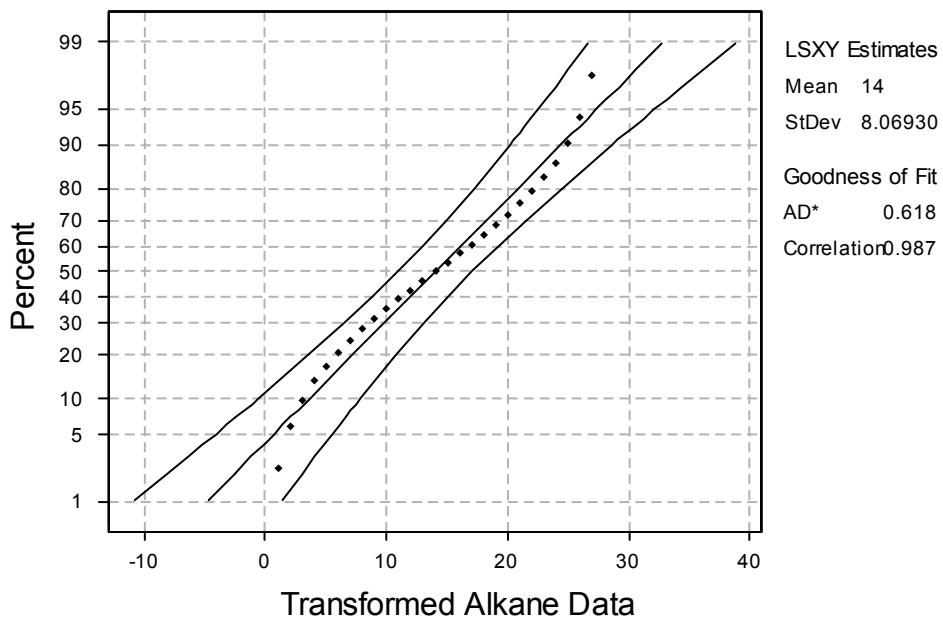


Figure 4. – Probability plot of the rank-transformed surrogate-adjusted alkane residuals showing improved linearity.

Non-transformed and rank-transformed surrogate-adjusted alkane data were analyzed with the General Linear ANOVA Model and Dunnett's multiple comparison tests between treatments and controls. P-statistics calculated for the F-test in the ANOVA table for non-transformed and transformed treatment main effects, and treatment/day interactions are all under the chosen alpha (α) level of 0.05 indicating at least one significant difference between one or more treatments over one or more days.

Adjusted P-values for non-transformed and transformed data Oil Spill Eater II Days 7 and 28 are shown to be significantly less than the Day 0 controls (Table 3). Adjusted P-values for non-transformed and transformed Oil Spill Eater II data, Days 7 and 28 are shown to be significantly less than the Day 7 controls (Table 4). Both transformed and non-transformed product data on Day 28 statistically demonstrated significantly more reduction than the Day 28 control (Table 5).

The Nutrient control behaved in the same manner as the product, showing the same significant differences between the Days 7 and 28 results from the Day 0, Day 7, and Day 28 controls. However, using Tukey's pairwise means comparison method on non-transformed data, the Day 28 Oil Spill Eater II product is also significantly less than the Nutrient alone (Table 6).

Table 5. Dunnett's test results using the Day 28 control as the control level vs. all other treatments and controls (all interactions). Note - non = non-transformed data, trans = transformed data

Treatment	Day	Difference of Means		T-Value		Adjusted P-Value	
		NON	TRANS	NON	TRANS	NON	TRANS
Control	0	7373	12.33	8.07	8.415	1.0000	1.0000
Nutrient	0	4773	7.33	5.22	5.003	1.0000	1.0000
Oil Spill Eater II	0	5934	10.33	6.50	7.050	1.0000	1.0000
Control	7	3453	4.00	3.78	2.729	1.0000	1.0000
Nutrient	7	-981	-1.00	-1.07	-0.682	0.4720	0.6528
Oil Spill Eater II	7	-9481	-7.00	-10.38	-4.776	0.0000	0.0005
Nutrient	28	-9290	-6.00	-10.17	-4.094	0.0000	0.0022
Oil Spill Eater II	28	-31523	-11.00	-34.51	-7.505	0.0000	0.0000

Table 6. Tukey's pairwise means comparison results between the Day 28 Nutrient and the Day 28 OIL SPILL EATER II non-transformed alkane data.

Treatment	Day	Difference of Means	T-Value	Adjusted P-Value
Oil Spill Eater II	28	-22234	-24.34	0.0000

Surrogate-adjusted Aromatic Data

Preliminary analysis of surrogate-adjusted aromatic data for normality (fig.5) showed the raw data to be non-normal with an Anderson-Darling P-statistic of 0.000. This is below the selected α -level of 0.01 and indicates the data are not normally distributed. Further visual evidence of the data's non-linearity can be seen in the probability plot for residuals of the data (fig.6). The data were rank-transformed and reanalyzed for normality (fig.7) giving an Anderson-Darling statistic of 0.585, well above the chosen α -level of 0.01. The probability plot for the residuals (fig. 6) of the data still show a small degree of non-linearity which can slightly lower the ANOVA and Dunnett's test ability to detect a statistical difference between treatments and controls. More on this subject is discussed in the conclusions.

Normality Test for Non-Transformed Aromatic Data

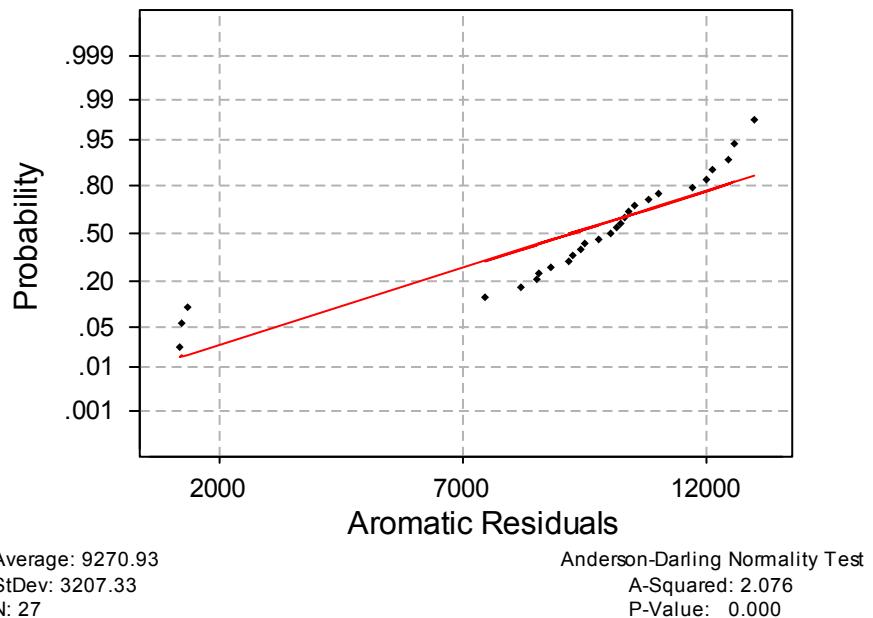


Figure 5. - Anderson-Darling test for normality showing non-linearity of the surrogate adjusted aromatic data.

Normal Probability Plot for Non-Trans Aromatic Data

LSXY Estimates - 95% CI

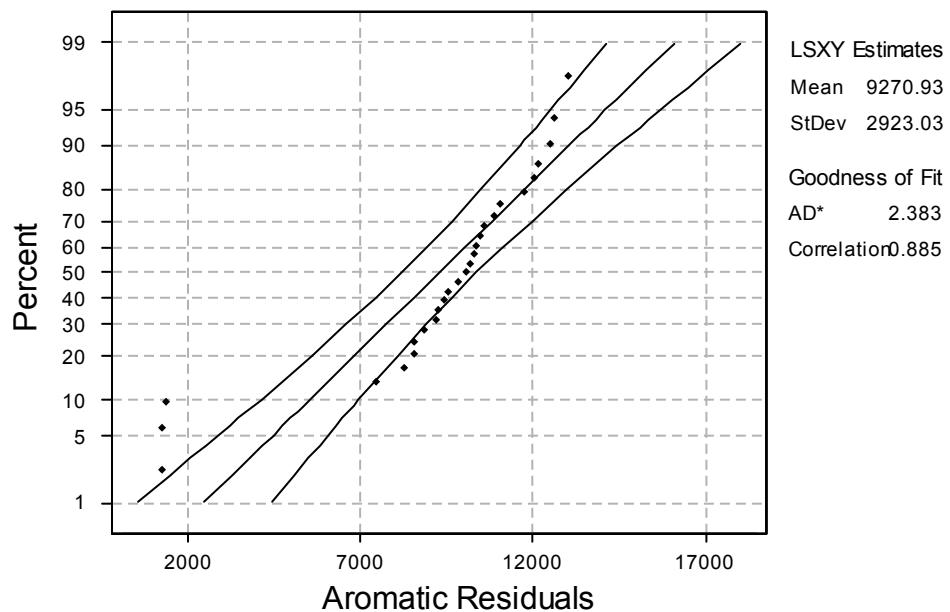
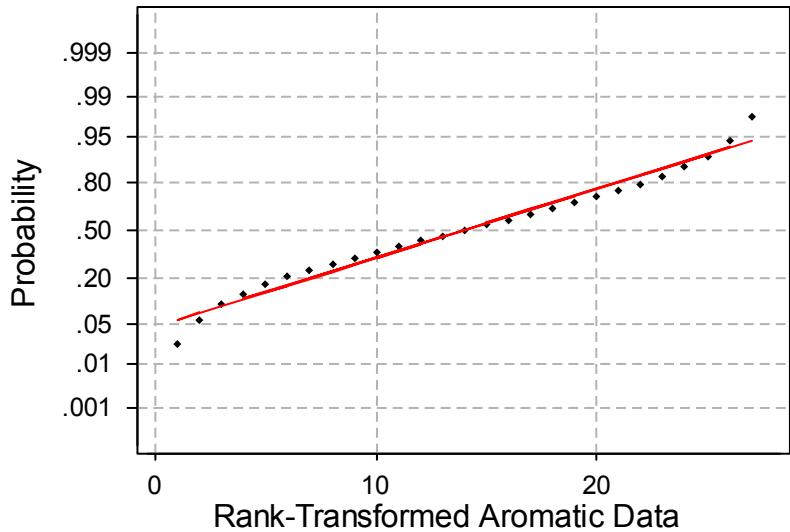


Figure 6. – Probability plot of the surrogate-adjusted aromatic residuals showing further evidence of non-linearity.

Normality Test for Rank-Transformed Aromatic Data



Average: 14
StDev: 7.93725
N: 27

Anderson-Darling Normality Test
A-Squared: 0.290
P-Value: 0.585

Figure 7. - Anderson-Darling test for normality showing improved linearity of the rank transformed surrogate-adjusted aromatic data.

Normal Probability Plot for Rank-Transformed Aromatic Data

LSXY Estimates - 95% CI

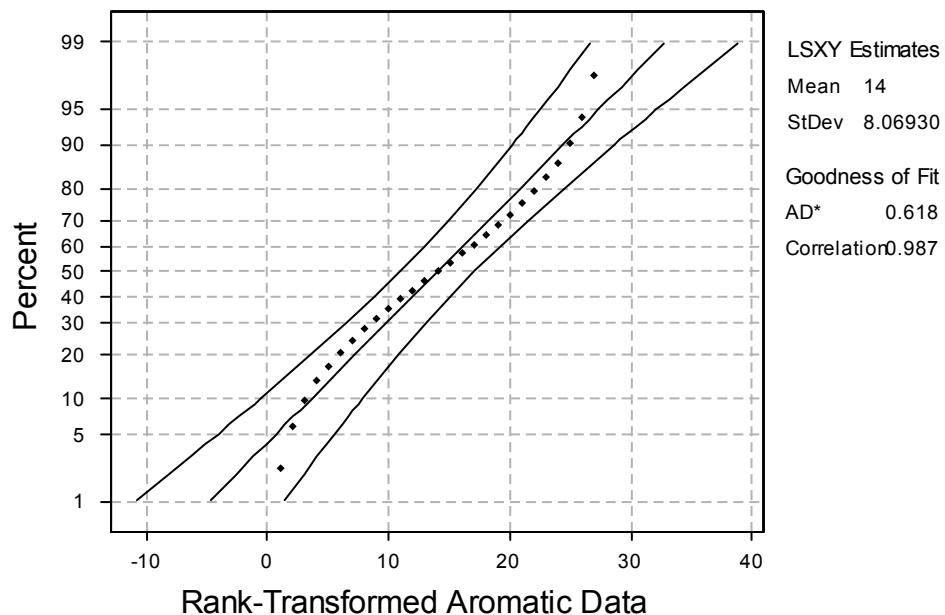


Figure 8. – Probability plot of the rank-transformed surrogate-adjusted aromatic residuals showing improved linearity.

Non-transformed and rank-transformed surrogate-adjusted aromatic data were analyzed with the General Linear ANOVA Model and Dunnett's multiple comparison tests between treatments and controls. P-statistics calculated for the F-test in the ANOVA table for non-transformed and transformed treatment main effects, and treatment/day interactions are all under the chosen alpha (α) level of 0.05 indicating at least one significant difference between one or more treatments over one or more days.

Adjusted P-values for non-transformed and transformed data Oil Spill Eater II Days 7 and 28 are shown to be significantly less than the Day 0 controls (Table 8). Adjusted P-values for non-transformed and transformed product data, Days 7 and 28 are shown to be significantly less than the Day 7 controls (Table 9). Both transformed and non-transformed product data on Day 28 statistically demonstrated significantly more reduction than the Day 28 control (Table 10).

The Nutrient control behaved in a similar manner as the product up to Day 28, showing the same significant differences between the Day 7 and 28 results from Day 0 and Day 7, but not the Day 28 controls. This indicates that nutrient alone is not as effective as the product in reducing aromatics. Using Tukey's pairwise means comparison method on non-transformed data; the Day 28 Oil Spill Eater II product is also significantly less than the Nutrient alone, reinforcing the previous statement (Table 11).

Table 10. Dunnett's test using Day 28 control as the control level vs. all other treatments and controls (all interactions). Note - non = non-transformed data, trans = transformed data

Treatment	Day	Difference of Means		T-Value		Adjusted P-Value	
		NON	TRANS	NON	TRANS	NON	TRANS
Control	0	1902	9.67	3.54	4.138	1.0000	1.0000
Nutrient	0	2251	11.00	4.20	4.709	1.0000	1.0000
Oil Spill Eater II	0	2651	12.33	4.88	5.280	1.0000	1.0000
Control	7	821	5.33	4.53	2.283	0.9985	0.9999
Nutrient	7	364	2.00	0.68	0.856	0.9788	0.9872
Oil Spill Eater II	7	-1462	-6.67	-2.73	-2.854	0.0379	0.0294
Nutrient	28	-596	-3.00	-1.11	-1.284	0.4554	0.3778
Oil Spill Eater II	28	-8266	-9.67	-15.41	-4.138	0.0000	0.0020

Table 11. Tukey's pairwise means comparison results between the Day 28 Nutrient and the Day 28 OIL SPILL EATER II non-transformed aromatic data.

Treatment	Day	Difference of Means	T-Value	Adjusted P-Value
Oil Spill Eater II	28	-7671	-14.30	0.0000

Microbiological Analysis Data

The following tables show the most probable number calculated by EPA's most probable number calculator Version 4.04. The data show the continued viability of the organisms through 28 days.

Table 12. Micro Results, MPN (per mL)

Treatments	Day 0	Day 7	Day 28
Control Rep# 1	7,968	8,406	9,843
Control Rep #2	8,179	8,072	10,136
Control Rep #3	7,647	8,724	9,549
Nutrient Rep #1	8,493	1,832,536	7,274,655
Nutrient Rep #2	7,647	2,015,665	7,967,738
Nutrient Rep #3	7,852	2,115,255	7,646,602
Oil Spill Eater II Rep# 1	8,724	7,274,655	182,054,230
Oil Spill Eater II Rep# 2	8,406	7,967,738	175,038,856
Oil Spill Eater II Rep# 3	8,972	7,646,602	197,910,169

Gravimetric Data

The following tables show the P-Values calculated by the two-sample t-test of the Minitab™ program. Table 13 shows the calculated values for Day 28 controls the Day 28 product, and the p-value of the comparison is lower than the chosen alpha (α) level of 0.05 and therefore indicate statistical significance. A computer printout of the analyses can be seen in APPENDIX III. Table 14 shows that the calculated values for the Day 28 controls and both the Day 7 and Day 28 nutrient are both statistically significant.

Table 13. P-Values calculated by the two-sample t-test for product (OIL SPILL EATER II) and the controls

Treatments	Day	Treatment Weight Means (mg)	T-test Scores	p-value
Controls	0	0.099	-2.79	0.966
Oil Spill Eater II	0	0.100		
Controls	7	0.093	1.04	0.187
Oil Spill Eater II	7	0.077		
Controls	28	0.082	42.25	0.000
Oil Spill Eater II	28	0.015		

Table 14. P-Values calculated by the two-sample t-test for the nutrient and the controls

Treatments	Day	Treatment Weight Means (mg)	T-test Scores	p-value
Controls	0	0.099	1.36	0.154
NUTRIENT	0	0.101		
Controls	7	0.093	10.07	0.005
NUTRIENT	7	0.079		
Controls	28	0.082	33.84	0.000
NUTRIENT	28	0.048		

Conclusions

Our conclusions will begin with a discussion of the GC/MS due to its relative importance in judging the tested product effective. A discussion of the microbiological results and gravimetric results will follow.

GC/MS Data

OSEI Corp. Product (Oil Spill Eater II)

Surrogate-adjusted Alkane Data

Surrogate-adjusted alkane Oil Spill Eater II data was shown to be non-normal and had to be rank-transformed to attain an acceptable degree of linearity. Analysis of the surrogate-adjusted data with ANOVA and Dunnett's test did however show the product treatments at Day 7 and 28 to be significantly less than Day 0, 7, and 28 controls. The extreme non-linearity of the non-transformed data makes the results of the ANOVA and Dunnett's test less reliable. The data, upon rank-transformation, achieved the desired linearity showing Day 7 and 28 product results to be significantly less than the respective Day 0, Day 7 and Day 28 controls. Based on this parameter the product appears to be effective.

Surrogate-adjusted alkane nutrient data was shown to be non-normal and had to be rank-transformed to attain an acceptable degree of linearity. Analysis of the surrogate-adjusted data with ANOVA and Dunnett's test did however show the nutrient treatments at Day 7 and 28 to be significantly less than their respective controls. The non-linearity of the non-transformed data may make the results of the ANOVA and Dunnett's test less reliable, however. The data, upon rank-transformation, achieved the desired linearity showing Day 7 and Day 28 nutrient results to be significantly less than the respective Day 0, 7, and 28 controls. Based on this parameter the nutrient treatment alone appears to be effective.

Tukey's test on untransformed alkane data showed a significant difference between the Day 28 Oil Spill Eater II results and Day 28 Nutrient results, indicating that the product seems more effective than nutrient treatment by itself.

Surrogate-adjusted Aromatic Data

Surrogate-adjusted aromatic Oil Spill Eater II data was shown to be non-normal and had to be rank-transformed to attain an acceptable degree of linearity. Analysis of the surrogate-adjusted data with ANOVA and Dunnett's test did however show the product treatments at Day 7 and 28 to be significantly less than Day 0, 7, and 28 controls. The extreme non-linearity of the non-transformed data makes the results of the ANOVA and Dunnett's test less reliable. The data, upon rank-transformation, achieved the desired linearity showing Day 7 and 28 product results to be significantly less than the respective Day 0, Day 7 and Day 28 controls. Based on this parameter the product appears to be effective.

Surrogate-adjusted aromatic nutrient data was shown to be non-normal and had to be rank-transformed to attain an acceptable degree of linearity. Analysis of the surrogate-adjusted data with ANOVA and Dunnett's test did however show the nutrient treatments at Day 7 to be significantly less than the Day 0 and Day 7 controls. The non-linearity of the non-transformed data may make the results of the ANOVA and Dunnett's test less reliable, however. The data, upon rank-transformation, achieved the desired linearity showing Day 7 nutrient results to be significantly less than the respective Day 0, and Day 7, but not the Day 28 controls. Based on this parameter the nutrient treatment alone is not as effective as the product after 28 days and is not significantly than the control alone.

Tukey's test on the aromatic data also showed a significant difference between the Day 28 Oil Spill Eater II results and Day 28 Nutrient results, indicating that the product seems to be more effective than nutrient treatment.

Microbiological Results

OSEI Corp. Product (Oil Spill Eater II)

The microbiological results speak for themselves. They show a definite continued microbiological viability over time for the product treatments. Similar to the product treatment, the nutrient treatments show a definite continued microbiological viability over time also.

Gravimetric Results

OSEI Corp. Product (Oil Spill Eater II)

Gravimetric results showed statistical significance between products and controls by Day 28. This tends to support the bulk of the data seen in both GC/MS analysis and microbiological analysis. Gravimetric results showed statistical significance between the Nutrient and the control on Day 7 and Day 28. This data tends to support the bulk of the data in both GC/MS analysis and microbiological analysis.

Discussion on Surrogate Recovery – QA/QC

The purpose of incorporating surrogate recovery percentages into the raw data is to check the efficiency of extraction techniques and in most cases is a valid quality control check. The acceptable range of surrogate recovery percentages is given in the cited Federal Register document titled Environmental Protection Agency, (EPA) Pt. 300, Appendix C, page 237, as 70%-120%. Percentage recoveries for product and controls for Day 0, Day 7 and Day 28 are given in Table 15 below.

Table 15. Surrogate recovery percentages.

Treatment		Day 0	Day 7	Day 28
Control Rep #1	5-Alpha Andorstane	0.90	0.82	0.82
	Phenanthrene-d10	0.94	0.77	0.79
Control Rep #2	5-Alpha Andorstane	0.86	0.90	0.76
	Phenanthrene-d10	0.81	0.94	0.77
Control Rep #3	5-Alpha Andorstane	0.78	0.87	0.87
	Phenanthrene-d10	0.78	0.90	0.83
NUT Rep #1	5-Alpha Andorstane	0.86	0.99	0.85
	Phenanthrene-d10	0.88	0.74	0.96
NUT Rep# 2	5-Alpha Andorstane	0.90	0.96	0.77
	Phenanthrene-d10	0.94	0.71	0.95
NUT Rep# 3	5-Alpha Andorstane	0.88	0.89	0.84
	Phenanthrene-d10	0.96	0.72	0.95
Oil Spill Eater II Rep #1	5-Alpha Andorstane	0.85	0.90	0.90
	Phenanthrene-d10	0.89	0.77	0.71
Oil Spill Eater II Rep# 2	5-Alpha Andorstane	0.83	0.87	0.92
	Phenanthrene-d10	0.85	0.76	0.73
Oil Spill Eater II Rep# 3	5-Alpha Andorstane	0.91	0.86	0.90
	Phenanthrene-d10	0.94	0.79	0.71

Statistical Analysis

Lastly, we feel that the nature of the data may reduce the ANOVA and Dunnett's means comparison test to detect a legitimate statistical effect between treatments and controls. Before the data can be subjected to the ANOVA analysis, it must pass a "normality" test where a calculated P-value is compared to a chosen alpha (α) level (usually 0.01). ANOVA has reduced power to detect a significant statistical difference when analyzing non-normal data (Zar, 1984). However, data that passes a formal test for normality is not necessarily from a "normal distribution" strictly speaking. A test for normality looks for linearity, which is only one aspect of the assumptions of normality. The data may also be skewed to the left or right as indicated by measurement of the median, may have 'heavy tails' in the distribution or may contain outliers. Normality after all, is usually a matter of degrees and not just whether the data are, or are not normally distributed. If data are not normal in the strictest sense, we feel the test's ability to detect subtle but significant statistical differences may be compromised to some degree.

Literature References

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APPENDIX I

	Day 0 (g)	Day 7 (g)	Day 28 (g)	% Reduction	Avg % Red.
Ctrl. #1	0.097	0.093	0.082	15.5	
Ctrl. #2	0.099	0.093	0.084	15.2	16.5
Ctrl. #3	0.100	0.094	0.081	19.0	
Mean	0.099	0.093	0.082		
Nutrient #1	0.097	0.081	0.044	54.6	
Nutrient #2	0.101	0.077	0.049	51.5	52.0
Nutrient #3	0.104	0.079	0.052	50.0	
Mean	0.101	0.079	0.048		
Product #1	0.099	0.077	0.018	81.8	
Product #2	0.101	0.078	0.014	86.1	85.4
Product #3	0.101	0.075	0.012	88.1	
Mean	0.100	0.077	0.015		

Vial + DCM +				
	Vial wt. (g)	Oil (g)	Vial + Oil (g)	Oil (g)
D0-C-1	13.473	26.985	13.570	0.097
D0-C-2	14.015	27.530	14.114	0.099
D0-C-3	13.865	26.751	13.965	0.100
D0-N-1	14.249	27.189	14.346	0.097
D0-N-2	13.785	27.087	13.886	0.101
D0-N-3	13.591	27.025	13.695	0.104
D0-P-1	13.687	27.176	13.786	0.099
D0-P-2	13.798	27.115	13.899	0.101
D0-P-3	13.981	27.125	14.082	0.101
D7-C-1	13.976	27.043	14.069	0.093
D7-C-2	14.151	27.148	14.244	0.093
D7-C-3	13.591	26.887	13.689	0.098
D7-N-1	13.687	26.964	13.768	0.081
D7-N-2	13.798	27.195	13.875	0.077
D7-N-3	13.981	27.045	14.060	0.079
D7-P-1	14.211	27.193	14.288	0.077
D7-P-2	14.323	27.187	14.401	0.078
D7-P-3	14.063	27.131	14.138	0.075
D28-C-1	13.976	26.864	14.058	0.082
D28-C-2	14.151	27.112	14.235	0.084
D28-C-3	13.591	27.058	13.672	0.081
D28-N-1	13.687	27.283	13.731	0.044
D28-N-2	14.111	27.217	14.160	0.049
D28-N-3	13.981	27.156	14.033	0.052
D28-P-1	14.211	26.947	14.229	0.018
D28-P-2	14.323	26.852	14.337	0.014
D28-P-3	14.063	27.099	14.075	0.012

	Day 0 (MPN, per ml)	Day 7 (MPN, per ml)	Day 28 (MPN, per ml)
Ctrl. #1	7,968	8,406	9,843
Ctrl. #2	8,179	8,072	10,136
Ctrl. #3	7,647	8,724	9,549
Nutrient #1	8,493	1,832,536	7,274,655
Nutrient #2	7,647	2,015,665	7,967,738
Nutrient #3	7,852	2,115,255	7,646,602
Product #1	8,724	7,274,655	182,054,230
Product #2	8,406	7,967,738	175,038,856
Product #3	8,972	7,646,602	197,910,169

APPENDIX II

General Linear Model: ALKANES versus DAY, TREATMENT

Factor Type Levels Values
DAY fixed 3 0 7 28
TREATMEN fixed 3 Control Nutrient OSI

Analysis of Variance for ALKANES, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	2	1746813937	1746813937	873406968	697.73	0.000
TREATMEN	2	1082517417	1082517417	541258708	432.39	0.000
DAY*TREATMEN	4	761225884	761225884	190306471	152.03	0.000
Error	18	22531957	22531957	1251775		
Total	26	3613089194				

Dunnett Simultaneous Tests

Response Variable ALKANES

Comparisons with Control Level

DAY = 0

TREATMEN = Control subtracted from:

Level	Difference	SE of		Adjusted
DAY*TREATMEN	of Means	Difference	T-Value	P-Value
0 Nutrient	-2600	913.5	-2.85	0.0597
0 OSI	-1439	913.5	-1.58	0.5103
7 Control	-3920	913.5	-4.29	0.0029
7 Nutrient	-8354	913.5	-9.15	0.0000
7 OSI	-16854	913.5	-18.45	0.0000
28 Control	-7373	913.5	-8.07	0.0000
28 Nutrient	-16663	913.5	-18.24	0.0000
28 OSI	-38896	913.5	-42.58	0.0000

General Linear Model: ALKANES versus DAY, TREATMENT

Factor Type Levels Values
DAY fixed 3 0 7 28
TREATMEN fixed 3 Control Nutrient OSI

Analysis of Variance for ALKANES, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	2	1746813937	1746813937	873406968	697.73	0.000
TREATMEN	2	1082517417	1082517417	541258708	432.39	0.000
DAY*TREATMEN	4	761225884	761225884	190306471	152.03	0.000
Error	18	22531957	22531957	1251775		
Total	26	3613089194				

Dunnett Simultaneous Tests

Response Variable ALKANES

Comparisons with Control Level

DAY = 7

TREATMEN = Control subtracted from:

Level	Difference	SE of		Adjusted
DAY*TREATMEN	of Means	Difference	T-Value	P-Value
0 Control	3920	913.5	4.29	0.0029
0 Nutrient	1319	913.5	1.44	0.5977
0 OSI	2480	913.5	2.72	0.0772
7 Nutrient	-4435	913.5	-4.85	0.0009
7 OSI	-12934	913.5	-14.16	0.0000
28 Control	-3453	913.5	-3.78	0.0086
28 Nutrient	-12743	913.5	-13.95	0.0000
28 OSI	-34977	913.5	-38.29	0.0000

General Linear Model: ALKANES versus DAY, TREATMENT

Factor Type Levels Values
 DAY fixed 3 0 7 28
 TREATMEN fixed 3 Control Nutrient OSI

Analysis of Variance for ALKANES, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	2	1746813937	1746813937	873406968	697.73	0.000
TREATMEN	2	1082517417	1082517417	541258708	432.39	0.000
DAY*TREATMEN	4	761225884	761225884	190306471	152.03	0.000
Error	18	22531957	22531957	1251775		
Total	26	3613089194				

Dunnett Simultaneous Tests

Response Variable ALKANES

Comparisons with Control Level

DAY = 28

TREATMEN = Control subtracted from:

Level	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
DAY*TREATMEN				
0 Control	7373	913.5	8.07	1.0000
0 Nutrient	4773	913.5	5.22	1.0000
0 OSI	5934	913.5	6.50	1.0000
7 Control	3453	913.5	3.78	1.0000
7 Nutrient	-981	913.5	-1.07	0.4720
7 OSI	-9481	913.5	-10.38	0.0000
28 Nutrient	-9290	913.5	-10.17	0.0000
28 OSI	-31523	913.5	-34.51	0.0000

General Linear Model: RANK_ALKANES versus DAY, TREATMENT

Factor Type Levels Values
 DAY fixed 3 0 7 28
 TREATMEN fixed 3 Control Nutrient OSI

Analysis of Variance for RANK_ALK, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	2	1178.00	1178.00	589.00	182.79	0.000
TREATMEN	2	298.67	298.67	149.33	46.34	0.000
DAY*TREATMEN	4	103.33	103.33	25.83	8.02	0.001
Error	18	58.00	58.00	3.22		
Total	26	1638.00				

Dunnett Simultaneous Tests

Response Variable RANK_ALK

Comparisons with Control Level

DAY = 0

TREATMEN = Control subtracted from:

Level	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
DAY*TREATMEN				
0 Nutrient	-5.00	1.466	-3.41	0.0094
0 OSI	-2.00	1.466	-1.36	0.3439
7 Control	-8.33	1.466	-5.69	0.0001
7 Nutrient	-13.33	1.466	-9.10	0.0000
7 OSI	-19.33	1.466	-13.19	0.0000
28 Control	-12.33	1.466	-8.41	0.0000
28 Nutrient	-18.33	1.466	-12.51	0.0000
28 OSI	-23.33	1.466	-15.92	0.0000

General Linear Model: RANK_ALKANES versus DAY, TREATMENT

Factor Type Levels Values
DAY fixed 3 0 7 28
TREATMEN fixed 3 Control Nutrient OSI

Analysis of Variance for RANK_ALK, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	2	1178.00	1178.00	589.00	182.79	0.000
TREATMEN	2	298.67	298.67	149.33	46.34	0.000
DAY*TREATMEN	4	103.33	103.33	25.83	8.02	0.001
Error	18	58.00	58.00	3.22		
Total	26	1638.00				

Dunnett Simultaneous Tests

Response Variable RANK_ALK

Comparisons with Control Level

DAY = 7

TREATMEN = Control subtracted from:

Level	Difference	SE of	Adjusted	
DAY*TREATMEN	of Means	Difference	T-Value	P-Value
0 Control	8.33	1.466	5.69	1.0000
0 Nutrient	3.33	1.466	2.27	0.9999
0 OSI	6.33	1.466	4.32	1.0000
7 Nutrient	-5.00	1.466	-3.41	0.0094
7 OSI	-11.00	1.466	-7.51	0.0000
28 Control	-4.00	1.466	-2.73	0.0376
28 Nutrient	-10.00	1.466	-6.82	0.0000
28 OSI	-15.00	1.466	-10.23	0.0000

General Linear Model: RANK_ALKANES versus DAY, TREATMENT

Factor Type Levels Values
DAY fixed 3 0 7 28
TREATMEN fixed 3 Control Nutrient OSI

Analysis of Variance for RANK_ALK, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	2	1178.00	1178.00	589.00	182.79	0.000
TREATMEN	2	298.67	298.67	149.33	46.34	0.000
DAY*TREATMEN	4	103.33	103.33	25.83	8.02	0.001
Error	18	58.00	58.00	3.22		
Total	26	1638.00				

Dunnett Simultaneous Tests

Response Variable RANK_ALK

Comparisons with Control Level

DAY = 28

TREATMEN = Control subtracted from:

Level	Difference	SE of	Adjusted	
DAY*TREATMEN	of Means	Difference	T-Value	P-Value
0 Control	12.33	1.466	8.415	1.0000
0 Nutrient	7.33	1.466	5.003	1.0000
0 OSI	10.33	1.466	7.050	1.0000
7 Control	4.00	1.466	2.729	1.0000
7 Nutrient	-1.00	1.466	-0.682	0.6528
7 OSI	-7.00	1.466	-4.776	0.0005
28 Nutrient	-6.00	1.466	-4.094	0.0022
28 OSI	-11.00	1.466	-7.505	0.0000

General Linear Model: AROMATICS versus DAY, TREATMENT

Factor Type Levels Values
 DAY fixed 3 0 7 28
 TREATMEN fixed 3 Control Nutrient OSI

Analysis of Variance for AROMATIC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	2	122630081	122630081	61315041	142.02	0.000
TREATMEN	2	60150172	60150172	30075086	69.66	0.000
DAY*TREATMEN	4	76909629	76909629	19227407	44.54	0.000
Error	18	7770989	7770989	431722		
Total	26	267460872				

Dunnett Simultaneous Tests
 Response Variable AROMATIC
 Comparisons with Control Level
 DAY = 0
 TREATMEN = Control subtracted from:

Level	Difference		Adjusted	
DAY*TREATMEN	of Means	Difference	T-Value	P-Value
0 Nutrient	350	536.5	0.65	0.9772
0 OSI	719	536.5	1.34	0.9971
7 Control	-1080	536.5	-2.01	0.1364
7 Nutrient	-1537	536.5	-2.87	0.0288
7 OSI	-3364	536.5	-6.27	0.0000
28 Control	-1902	536.5	-3.54	0.0071
28 Nutrient	-2497	536.5	-4.66	0.0007
28 OSI	-10168	536.5	-18.95	0.0000

General Linear Model: AROMATICS versus DAY, TREATMENT

Factor Type Levels Values
 DAY fixed 3 0 7 28
 TREATMEN fixed 3 Control Nutrient OSI

Analysis of Variance for AROMATIC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	2	122630081	122630081	61315041	142.02	0.000
TREATMEN	2	60150172	60150172	30075086	69.66	0.000
DAY*TREATMEN	4	76909629	76909629	19227407	44.54	0.000
Error	18	7770989	7770989	431722		
Total	26	267460872				

Dunnett Simultaneous Tests
 Response Variable AROMATIC
 Comparisons with Control Level
 DAY = 7
 TREATMEN = Control subtracted from:

Level	Difference		Adjusted	
DAY*TREATMEN	of Means	Difference	T-Value	P-Value
0 Control	1080	536.5	2.01	0.9997
0 Nutrient	1430	536.5	2.67	1.0000
0 OSI	1799	536.5	3.35	1.0000
7 Nutrient	-457	536.5	-0.85	0.5756
7 OSI	-2283	536.5	-4.26	0.0016
28 Control	-821	536.5	-1.53	0.2788
28 Nutrient	-1417	536.5	-2.64	0.0445
28 OSI	-9088	536.5	-16.94	0.0000

General Linear Model: AROMATICS versus DAY, TREATMENT

Factor Type Levels Values
 DAY fixed 3 0 7 28
 TREATMEN fixed 3 Control Nutrient OSI

Analysis of Variance for AROMATIC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	2	122630081	122630081	61315041	142.02	0.000
TREATMEN	2	60150172	60150172	30075086	69.66	0.000
DAY*TREATMEN	4	76909629	76909629	19227407	44.54	0.000
Error	18	7770989	7770989	431722		
Total	26	267460872				

Dunnett Simultaneous Tests

Response Variable AROMATIC

Comparisons with Control Level

DAY = 28

TREATMEN = Control subtracted from:

Level	Difference	SE of	Adjusted	
DAY*TREATMEN	of Means	Difference	T-Value	P-Value
0 Control	1902	536.5	3.54	1.0000
0 Nutrient	2251	536.5	4.20	1.0000
0 OSI	2621	536.5	4.88	1.0000
7 Control	821	536.5	1.53	0.9985
7 Nutrient	364	536.5	0.68	0.9788
7 OSI	-1462	536.5	-2.73	0.0379
28 Nutrient	-596	536.5	-1.11	0.4554
28 OSI	-8266	536.5	-15.41	0.0000

General Linear Model: Rank_aromatics versus DAY, TREATMENT

Factor Type Levels Values
 DAY fixed 3 0 7 28
 TREATMEN fixed 3 Control Nutrient OSI

Analysis of Variance for Rank_aromatics, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	2	1102.89	1102.89	551.44	67.37	0.000
TREATMEN	2	194.00	194.00	97.00	11.85	0.001
DAY*TREATMEN	4	193.78	193.78	48.44	5.92	0.003
Error	18	147.33	147.33	8.19		
Total	26	1638.00				

Dunnett Simultaneous Tests

Response Variable Rank_aromatics

Comparisons with Control Level

DAY = 0

TREATMEN = Control subtracted from:

Level	Difference	SE of	Adjusted	
DAY*TREATMEN	of Means	Difference	T-Value	P-Value
0 Nutrient	1.33	2.336	0.571	0.9716
0 OSI	2.67	2.336	1.142	0.9946
7 Control	-4.33	2.336	-1.855	0.1753
7 Nutrient	-7.67	2.336	-3.282	0.0123
7 OSI	-16.33	2.336	-6.992	0.0000
28 Control	-9.67	2.336	-4.138	0.0020
28 Nutrient	-12.67	2.336	-5.422	0.0001
28 OSI	-19.33	2.336	-8.276	0.0000

General Linear Model: Rank_aromatics versus DAY, TREATMENT

Factor Type Levels Values
DAY fixed 3 0 7 28
TREATMEN fixed 3 Control Nutrient OSI

Analysis of Variance for Rank_aro, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	2	1102.89	1102.89	551.44	67.37	0.000
TREATMEN	2	194.00	194.00	97.00	11.85	0.001
DAY*TREATMEN	4	193.78	193.78	48.44	5.92	0.003
Error	18	147.33	147.33	8.19		
Total	26	1638.00				

Dunnett Simultaneous Tests

Response Variable Rank_aro

Comparisons with Control Level

DAY = 7

TREATMEN = Control subtracted from:

Level	Difference	SE of	Adjusted	
DAY*TREATMEN	of Means	Difference	T-Value	P-Value
0 Control	4.33	2.336	1.855	0.9995
0 Nutrient	5.67	2.336	2.426	0.9999
0 OSI	7.00	2.336	2.997	1.0000
7 Nutrient	-3.33	2.336	-1.427	0.3186
7 OSI	-12.00	2.336	-5.137	0.0002
28 Control	-5.33	2.336	-2.283	0.0862
28 Nutrient	-8.33	2.336	-3.567	0.0068
28 OSI	-15.00	2.336	-6.421	0.0000

General Linear Model: Rank_aromatics versus DAY, TREATMENT

Factor Type Levels Values
DAY fixed 3 0 7 28
TREATMEN fixed 3 Control Nutrient OSI

Analysis of Variance for Rank_aro, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	2	1102.89	1102.89	551.44	67.37	0.000
TREATMEN	2	194.00	194.00	97.00	11.85	0.001
DAY*TREATMEN	4	193.78	193.78	48.44	5.92	0.003
Error	18	147.33	147.33	8.19		
Total	26	1638.00				

Dunnett Simultaneous Tests

Response Variable Rank_aro

Comparisons with Control Level

DAY = 28

TREATMEN = Control subtracted from:

Level	Difference	SE of	Adjusted	
DAY*TREATMEN	of Means	Difference	T-Value	P-Value
0 Control	9.667	2.336	4.138	1.0000
0 Nutrient	11.000	2.336	4.709	1.0000
0 OSI	12.333	2.336	5.280	1.0000
7 Control	5.333	2.336	2.283	0.9999
7 Nutrient	2.000	2.336	0.856	0.9872
7 OSI	-6.667	2.336	-2.854	0.0294
28 Nutrient	-3.000	2.336	-1.284	0.3778
28 OSI	-9.667	2.336	-4.138	0.0020

28	Control	-3453	913.5	-3.78	0.0289
28	Nutrient	-12743	913.5	-13.95	0.0000
28	OSI	-34977	913.5	-38.29	0.0000

DAY = 7

TREATMEN = Nutrient subtracted from:

Level	Difference	SE of		Adjusted
DAY*TREATMEN	of Means	Difference	T-Value	P-Value
7 OSI	-8500	913.5	-9.30	0.0000
28 Control	981	913.5	1.07	0.9710
28 Nutrient	-8308	913.5	-9.09	0.0000
28 OSI	-30542	913.5	-33.43	0.0000

DAY = 7

TREATMEN = OSI subtracted from:

Level	Difference	SE of		Adjusted
DAY*TREATMEN	of Means	Difference	T-Value	P-Value
28 Control	9481	913.5	10.38	0.0000
28 Nutrient	191	913.5	0.21	1.0000
28 OSI	-22042	913.5	-24.13	0.0000

DAY = 28

TREATMEN = Control subtracted from:

Level	Difference	SE of		Adjusted
DAY*TREATMEN	of Means	Difference	T-Value	P-Value
28 Nutrient	-9290	913.5	-10.17	0.0000
28 OSI	-31523	913.5	-34.51	0.0000

DAY = 28

TREATMEN = Nutrient subtracted from:

Level	Difference	SE of		Adjusted
DAY*TREATMEN	of Means	Difference	T-Value	P-Value
28 OSI	-22234	913.5	-24.34	0.0000

28	Control	-821	536.5	-1.53	0.8273
28	Nutrient	-1417	536.5	-2.64	0.2362
28	OSI	-9088	536.5	-16.94	0.0000

DAY = 7

TREATMEN = Nutrient subtracted from:

Level	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
DAY*TREATMEN				
7 OSI	-1826	536.5	-3.40	0.0607
28 Control	-364	536.5	-0.68	0.9985
28 Nutrient	-960	536.5	-1.79	0.6881
28 OSI	-8631	536.5	-16.09	0.0000

DAY = 7

TREATMEN = OSI subtracted from:

Level	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
DAY*TREATMEN				
28 Control	1462	536.5	2.73	0.2063
28 Nutrient	866	536.5	1.61	0.7855
28 OSI	-6804	536.5	-12.68	0.0000

DAY = 28

TREATMEN = Control subtracted from:

Level	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
DAY*TREATMEN				
28 Nutrient	-596	536.5	-1.11	0.9650
28 OSI	-8266	536.5	-15.41	0.0000

DAY = 28

TREATMEN = Nutrient subtracted from:

Level	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
DAY*TREATMEN				
28 OSI	-7671	536.5	-14.30	0.0000

APPENDIX III

Two-Sample T-Test and CI: Con_0, OSEI_0

Two-sample T for Con_0 vs OSEI_0

	N	Mean	StDev	SE Mean
Con_0	3	0.10067	0.00379	0.0022
OSEI_0	3	0.10833	0.00289	0.0017

Difference = mu Con_0 - mu OSEI_0

Estimate for difference: -0.00767

95% lower bound for difference: -0.01414

T-Test of difference = 0 (vs >): T-Value = -2.79 P-Value = 0.966 DF = 3

Two-Sample T-Test and CI: Con_7, OSEI_7

Two-sample T for Con_7 vs OSEI_7

	N	Mean	StDev	SE Mean
Con_7	3	0.09800	0.00200	0.0012
OSEI_7	3	0.09600	0.00265	0.0015

Difference = mu Con_7 - mu OSEI_7

Estimate for difference: 0.00200

95% lower bound for difference: -0.00251

T-Test of difference = 0 (vs >): T-Value = 1.04 P-Value = 0.187 DF = 3

Two-Sample T-Test and CI: Con_28, OSEI_28

Two-sample T for Con_28 vs OSEI_28

	N	Mean	StDev	SE Mean
Con_28	3	0.09533	0.00321	0.0019
OSEI_28	3	0.015667	0.000577	0.00033

Difference = mu Con_28 - mu OSEI_28

Estimate for difference: 0.07967

95% lower bound for difference: 0.07416

T-Test of difference = 0 (vs >): T-Value = 42.25 P-Value = 0.000 DF = 2

Two-Sample T-Test and CI: Con_0, Nutr_0

Two-sample T for Con_0 vs Nutr_0

	N	Mean	StDev	SE Mean
Con_0	3	0.10067	0.00379	0.0022
Nutr_0	3	0.097667	0.000577	0.00033

Difference = mu Con_0 - mu Nutr_0

Estimate for difference: 0.00300

95% lower bound for difference: -0.00346

T-Test of difference = 0 (vs >): T-Value = 1.36 P-Value = 0.154 DF = 2

Two-Sample T-Test and CI: Con_7, Nutr_7

Two-sample T for Con_7 vs Nutr_7

	N	Mean	StDev	SE Mean
Con_7	3	0.09800	0.00200	0.0012
Nutr_7	3	0.08500	0.00100	0.00058

Difference = mu Con_7 - mu Nutr_7

Estimate for difference: 0.01300

95% lower bound for difference: 0.00923

T-Test of difference = 0 (vs >): T-Value = 10.07 P-Value = 0.005 DF = 2

Two-Sample T-Test and CI: Con_28, Nutr_28

Two-sample T for Con_28 vs Nutr_28

	N	Mean	StDev	SE Mean
Con_28	3	0.09533	0.00321	0.0019
Nutr_28	3	0.02400	0.00173	0.0010

Difference = mu Con_28 - mu Nutr_28

Estimate for difference: 0.07133

95% lower bound for difference: 0.06637

T-Test of difference = 0 (vs >): T-Value = 33.84 P-Value = 0.000 DF = 3