OIL SPILL EATER INTERNATIONAL, CORP.

P.O. Box 515429
Dallas, Texas 75075
Ph: (972) 669-3390
Fax: (469) 241-0896
Email: oseicorp@msn.com
Web: http://www.osei.us

SUMMARY

SECOND U.S. EPA/NETAC (Bioremediation Test)
Using OIL SPILL EATER II
February 28, 2001

The second U.S. EPA/NETAC Test was more thorough with different days for testing the amount of bioremediation occurring. EPA/NETAC wanted to determine if there was a statistical difference between the control (doing nothing at all), the nutrient control (EPA – Dr. Venosa's nutrients) and the test product, **OIL SPILL EATER II.**

Table 2 shows the raw data where on day 0 the control, nutrient control and OSE II started at approximately 8,000 ppm (parts per million). In seven (7) days, OSE II had remediated the oil to an average of 6,529 ppm. The control and nutrient control were still around 8,000 ppm. On day twenty eight (28), OSE II had remediated the oil to 3,658 ppm. While the control was where it started and the nutrient control showed only minimal reduction of the oil.

In fact, OSE II remediated more of the oil in seven (7) days than the nutrient or nutrient control remediated in twenty eight (28) days.

EPA/NETAC through scientifically valid testing wanted to determine through an Anova Table if there was significant statistical difference between the nutrient, nutrient control, and the test product, OSE II.

In this very limited closed system, OSE II reduced the oil over 50%, while very little reduction occurred in the control or nutrient control. In fact, on Page 3, in the last paragraph, EPA/NETAC explains that for OSE II (Group 3) "at day 7 and day 28 are significantly different from (Group 1) and (Group 2)."

This test is reproduced as the example in the U.S. Code of Federal Regulations under Bioremediation Efficacy Test.

EPA/NETAC conclude, "Therefore in terms of total aromatic degradation, the test indicates the desired statistically significant difference between the mean of the product (OSE II) and the mean of the non-nutrient control.

EPA/NETAC's scientifically valid Bioremediation Test proves that OSE II is a very significant oil spill cleanup product.

By: Steven R. Pedigo
Chairman

SRP/AJL

National Environmental Technology Applications Center
UNIVERSITY OF PITTSBURGH APPLIED RESEARCH CENTER
615 William Pitt Way · Pittsburgh, PA 15238
Facsimile (412) 826-5552
(412) 826-5511

NETAC

OIL SPILL RESPONSE BIOREMEDIATION AGENTS EVALUATION METHODS VALIDATION TESTING DISCUSSION OF RESULTS

The following data are provided for the oil spill response bioremediation agent producer as a means to begin to assess how this bioremediation agent may behave in response to an oil spill in the environment. The data we are providing are limited to the gas chromatographic/mass spectrometer (GC/MS) results.

Note that a total of 69 analytes (components naturally occurring in oil) were measured in these experiments. These analytes constitute a small but highly representative fraction of the toxic and biodegradable portion of oil. We are providing you with a summary of the ultimate results and a summary of the most germane analytes to facilitate our reporting of this information and to reduce confusion in reporting caused by the modification of the selected test results.

The following table of GC/MS results indicate the percent reduction of analyte(s) versus the same analyte(s) present in the control (i.e., product results/control results x 100). For example, if 100 percent of an analyte is present at Day 21 after mixing oil, seawater and product as compared to the control (oil and seawater only) then the product did not stimulate the decomposition of hydrocarbons in oil. Note, that the greater the number of analytes with a low percentage the more capable the product of enhancing the biodegradation of oil.

From this experiment, the results indicated that there was sufficient comparability of the data between the laboratories conducting this experiment. The resultant data presented for this bioremediation agent and the comparative nutrient treatment did not show a significant statistical difference between the product mean and the control mean at the $p \leq 0.05$ level of significance. That is, biodegradation was occurring but not significantly faster than the control. We note that even though these treatments did not produce statistical significant degradation of the test oil, several of the products in this research did achieve this standard.

An analysis of the total aromatic data (in ppm) was conducted for the following three groups:

GROUP 1: Non-nutrient Control
GROUP 2: Nutrient Control
GROUP 3: Test Product – OSE II

The raw data is shown in Table 2 below. Note the three replications for each group-time combination.

TABLE 2

PRODUCT TEST DATA
TOTAL AROMATICS (PPM)

	GROUP 1	GROUP 2	GROUP 3
DAY 0	8153	7912	7711
	8299	8309	8311
	8088	8111	8200
DAY 7	8100	7950	6900
	8078	8200	6702
	7999	8019	5987
DAY 28	8259	8102	4000
	8111	7754	3875
	8344	7659	3100

Table 3 gives the summary statistics (number of observations, means, and standard deviations) for each group-time combination.

TABLE 3

SUMMARY STATISTICS FOR PRODUCT TEST DATA
TOTAL AROMATICS (PPM)

	GROUP 1	GROUP 2	GROUP 3
DAY 0	8153	7912	7711
	8299	8309	8311
	8088	8111	8200
DAY 7	8100	7950	6900
	8078	8200	6702
	7999	8019	5987
DAY 28	8259	8102	4000
	8111	7754	3875
	8344	7659	3100

Table 4 shows the results of the two-way ANOVA.

TABLE 4

TWO-WAY ANOVA TABLE

Source	df	Sum of Squares	Mean Square	F-Statistic	p-Value
GROUP	2	23944857.41	11972428.70	151.94	0.0001
TIME	2	10954731.19	5477365.59	69.51	0.0001
INTERACTION	4	19347589.04	4836897.26	61.39	0.0001
ERROR	18	1418303.33	78794.63		
TOTAL	26	55665480.96			

From the ANOVA table, we see that the F-statistic for INTERACTION is significant (F=61.39, p=0.0001). This indicates that group differences exist for one or more days. Protected LSD mean separations were then conducted for each day to determine which group differences exist. The results are summarized in Table 5. Note that means with the same letter (T grouping) are not significantly different.

TABLE 5

PAIRWISE PROTECTED LSD MEAN SEPARATION

T Grouping	Mean	n	Interaction
A	8238.0	3	Group 1, Day 28
A	8180.0	3	Group 1, Day 0
A	8110.7	3	Group 2, Day 0
A	8074.0	3	Group 3, Day 0
A	8059.0	3	Group 1, Day 7
A	8056.3	3	Group 2, Day 7
A	7838.3	3	Group 2, Day 28
B	6529.7	3	Group 3, Day 7
C	3658.3	3	Group 3, Day 28

Significance Level = 0.05
Degrees of Freedom = 18
Mean Square Error = 78794.63
Critical Value = 2.10
Least Significant Difference = 481.52

The grouping letters indicate that the product mean values (group 3) at day 7 and day 28 are significantly different from those of both the nutrient control (group 2) and the non-nutrient control (group 1) for those days. No other significant differences are shown. Therefore, in terms of total aromatic degradation, the test indicates the desired statistically significant difference between the mean of the product and the mean of the non-nutrient control.

EXPERIMENTAL DESIGN

The shaker flask evaluation conducted in Tier II is an experiment designed to determine the product's ability to degrade crude oil components at a rate or extent greater than a natural seawater microbial population. The experimental design includes a control, nutrient treatment, and the product treatment. The resultant data are compared and tested statistically using a two-way analysis of variance to determine data trends. The experimental design for Tier II testing is known as a factorial experiment with two factors. The first factor is product/control group; the second factor is time (as measured in days). For example, if two groups (product A and a non-nutrient control) are tested at each of three points in time (day 0, 7, and 28), the experiment is called a 2x3 factorial experiment. There were three replications (replicated shaker flasks) of each group-time combination.

DATA ANALYSIS METHODS

For each analyte and each product used in Tier II, a product is deemed a success by the demonstration of a statistically significant difference between the mean analyte degradation by the product and the mean analyte degradation by the non-nutrient control. Such a determination will be made by performing a two-way analysis of variance (ANOVA) on the sample data. The technical aspects of this procedure are outlined in Snedecor and Cochran (1980). Most statistical software packages support the use of two-way ANOVA. However, the format required for the input data differs among the various commercial packages. Whichever package is used, the following ANOVA table will be provided as part of the output.

TABLE 1
TWO WAY ANOVA TABLE

Source	df	Sum of Squares	Mean Square	F-statistic	p-value
Group	p-1	SSG	MSG = MSG/MSE	MSG/MSE	*
Time	t-1	SST	MST = MST/MSE	MST/MSE	*
Interaction	(p-1)(t-1)	SSI	MSI = MSI/MSE	MSI/MSE	*
Error	pt(n-1)	SSE	MSE = SSE		
TOTAL	npt-1	SSTOT			

* To be determined from the value of the F-statistic

In the degrees of freedom column (df) of Table 1, p denotes the number of product/ control groups, t denotes the number of days at which each group is analyzed and n denotes the number of replications. For the example of the 2x3 factorial experiment discussed in the previous section, p=2, t=3, and n=3. The significance of the F-statistics (as indicated by their corresponding p-value) are used to interpret the analysis.

INTERPRETATION

If the F-statistic for the INTERACTION is significant at the 0.05 level (i.e. the p-value is less than 0.05), the data indicate that the mean response of at least two groups being tested differ for at least one point in time. In order to find out which groups and at which points in time the difference occurs, pairwise comparisons between the group means should be conducted for all time points. These comparisons can be made using protected least squared difference (LSD) or Dunnett mean separation techniques. The protected LSD procedure is detailed in Snedecor and Cochran (1980); the Dunnett procedure is outlined in Montgomery (1991). For both methods, the mean square error (MSE) from the two-way ANOVA table should be used to compute the separation values.

If the F-statistic for the INTERACTION is not significant at the 0.05 level (i.e. the p-value is not less than 0.05), but the F-statistic for the GROUP is significant (i.e. the p-value is less than 0.05), but data indicate that any differences which exist among the group means are consistent across time. To find out which group means differ, a pairwise comparison of the group means should be carried out by pooling data across all points in time. Again, the mean square error (MSE) from the two-way ANOVA table should be used to compute the separation values.

If the F-statistic corresponding to both INTERACTION and GROUP are not significant at the 0.05 level, the data indicate no difference between the group means at any point in time. In this case, no further analysis is necessary.

Finally, Snedecor and Cochran (1980) caution about the use of multiple comparisons. If many such comparisons are being conducted, then about 5 percent of the tested differences will erroneously be concluded as significant. The researcher must guard against such differences causing undue attention.

REQUIRED DOCUMENTATION

The following documents should be included to summarize findings from a product test.

- Data listings for each analyte that was analyzed. These should show all raw data.
- A table of summary statistics for each analyte. The table should include the mean, standard deviation and sample size for each group at each day.
- An ANOVA table for each analyte. The table should be of the same format as Table 1.
- A clear summary of the mean separations (if mean separations were necessary). The mean separation methods (LSD or Dunnett), the significance level, the minimum significant difference value and the significant differences should be clearly marked on each output page.
- All computer outputs should be included. No programming alterations are necessary. The specific computer package used to analyze the data should be included in the report.