OIL SPILL EATER INTERNATIONAL, CORP.

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SUMMARY

U.S EPA and NETAC EFFICACY TESTING

The United States Environmental Protection Agency spent one and one-half years testing and evaluating protocols using OIL SPILL EATER II.

Mr. Tom Merski (August 18, 1993) explained the control (oil and seawater only) showed such an insignificant change (no reduction in TPH) that the control results were not even released.

NOTE - that OIL SPILL EATER II Biodegraded Alaskan Crude Oil 98% in 21 days in NETAC's Tier II Test. This test specifically shows the reduction of Polynuclear Aromatic Hydrocarbons that are the Hydrocarbons that are more persistent and difficult to Bioremediate!

This test proves that using OIL SPILL EATER II is beneficial over doing nothing, and that 98% of a spill can be mitigated as opposed to mechanical cleanups, which after 30 days or more can only blot up 20% of a spill. Using OIL SPILL EATER II can reduce the impact to marine organisms and ECO systems faster and more efficiently than mechanical cleanups. This means huge savings on the cleanup costs and environmental damage assessment fees.

By: Steven R. Pedigo
Chairman
OSEI, CORP.

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

July 22, 1993

Mr. George Lively
President
OSEI Corporation
Oil Spill Eater International
Suite 1116, 5545 Harvest Hill
Dallas, TX 75230

New address as of Oct. 1999
13127 Chandler Drive
Dallas, TX 75243

Dear Mr. Lively:

Subject: ***Oil Spill Eater II Methods Validation Data***

Per your request, enclosed is the efficacy data generated with "Oil Spill Eater II" from the development and validation of our oil spill response bioremediation evaluation methods. The Toxicity data from this process will be provided as soon as it is released from the EPA Office of Research and Development laboratories. We have included information on the experimental methods and objectives intended to assist you in understanding the meaning of the numbers generated for this report.

On behalf of NETAC and all the members of our Oil Spill Product Protocol Development Panel, we wish to express our appreciation for the contribution of your bioremediation agent for use in validating these methods and for your availability to answer questions about how this agent was intended to be used. Your patience and cooperation over the past two years has been commendable.

As you are aware, these experiments were conducted by the NETAC and EPA Office of Research and Development laboratories in Cincinnati, OH and in Gulf Breeze, FL. These data give you a general idea of how your product may behave in an open environment. Note that these data were obtained during the development of our methods. Numerous refinements have been made to increase the sensitivity of these tests; therefore, your product may provide different results in future tests due to this increased sensitivity as well as from the natural variability of the product and the constituent(s) used in the test sequence.

Please bear in mind that, although the Tier II methods have been finalized, we anticipate that all of the methods will be refined and updated periodically as we learn more about these systems. This means that data which was incidentally obtained for your product during the development of the protocols as it currently stands may change as the protocol is further refined. We must emphasize the research nature of the data we are providing to you today!

These data are provided to give you an indication of how your product behaved in this particular phase of the research. Different results may occur with the newly refined methods. We recommend that you evaluate this information as another set of intermediate data. We strongly suggest that you initiate additional testing applying the final Tier II method to develop a product performance baseline.

We also wish to emphasize that the participation of any bioremediation agent in the development of validation of the protocol does not constitute endorsement, approval or recommendation on the part of either NETAC or the EPA Office of Research and Development.

Enclosed for your convenience are the tabulated results of the Day 21 Shaker flask experiment for efficacy testing, and a Statistical Method Summary used to generate data about your product. This statistical method can be found in the July 1993 issue of the *Evaluation Methods Manual for Oil Spill Response Bioremediation Agents*. This document is currently being printed and a copy of the manual will be sent to you as soon as possible.

If you have questions about the data which we have provided, its potential use or application, or development of the protocol please call me at (412) 826-5511.

Sincerely,

A. Thomas Merski
Vice-Chairman,
Treatability Protocol Development Subcommittee
Bioremediation Action Committee

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310-2015-141

cc: W.M. Griffin

NETAC

RESULTS:

TIER II EFFICACY DATA

PERCENT REDUCTION

	OIL SPILL EATER II (DAY 21)
ANALYTE	**LAB A (n = 3) (%)**
PRISTANE	88
C18	66
PHYTANE	82
C30	83
TOTAL n-PARAFFINS	77
FLUORENE	92
PHENANTHRENE	97
CHRYSENE	165
TOTAL AROMATICS	98

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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

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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.

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OSEI CORPORATION'S SUMMARY
of
Texas A&M's
Microbial Petroleum Degradation Enhancement
By Oil Spill Bioremediation Products

The General Land Office for the state of Texas (USA) asked the University of Texas A&M to perform a study on 13 bioremediation products listed in the EPA National Contingency Plan for oil spills.

The efficacy tests were to be performed using the EPA / NETAC guidelines in their test protocol for bioremediation agents.

The test covered the total oil and grease (O&G), the aliphatic fraction of oil, the aromatic fraction of oil, and the plate counts on the numbers of hydrocarbon degraders grown or colonized during this test.

OIL SPILL EATER II IS PRODUCT 10.

Oil Spill Eater II was one of the best products at reducing the oil and grease. ***Oil Spill Eater II*** was the most effective product at reducing the aliphatic fraction of the oil.

Oil Spill Eater II was the most effective product at reducing the Polar-aromatic (PAH, more toxic) fraction of the oil.

Oil Spill Eater II grew the most hydrocarbon degraders, an acceptable product grew 10^{5} numbers of hydrocarbon degraders while ***OSE II*** outperformed them all at enhancing hydrocarbon degraders at $10^{7.5}$.

Oil Spill Eater II proved it was the most efficient product at biodegrading Alaskan North Slope crude oil out of the 13 EPA / NCP Listed products tested.

Steven R. Pedigo
Chairman

Microbial Petroleum Degradation Enhancement By Oil Spill Bioremediation Products

A Report Submitted to the Texas General Land Office

October 12, 1995

Principal Investigators:

James S. Bonner

Robin L. Autenrieth

Contributing Students:

Salvador Aldrett

Marc A. Mills

Frank Stephens

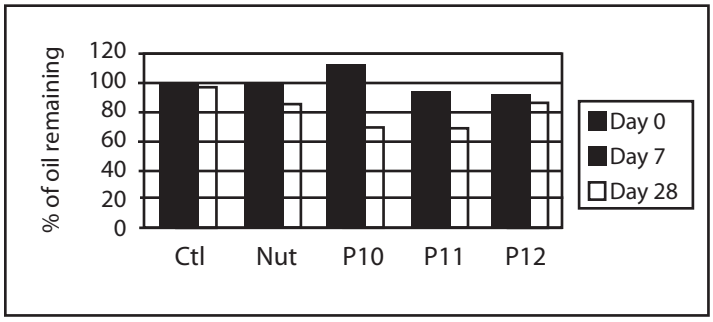
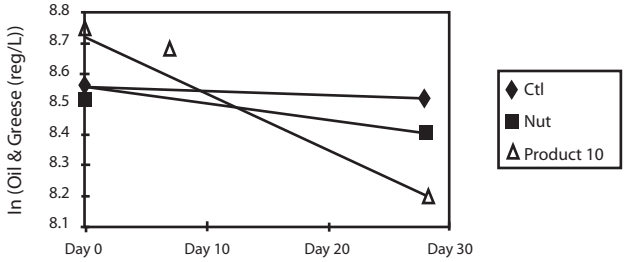


Figure 4 – Oil and Grease results (Batch D)
P10 is OSE II

High O&G numbers can be a result of a high production of extractable materials such as biomass or metabolites. As shown in Batch D, Product 10 is causing an increase in the O&G values at day 0 and 7, with an average value of 11% more of the initial weight. However, microbial counts indicate a high aliphatic degrader population through this period, as will be shown later Figure 16. After 28 days the oil was degraded more extensively by Product 10 than by the nutrient control. This suggests that the polar fraction is possibly being increased by the product's contents, on days 0 and 7, but does not imply that the oil is remaining undegraded. Microbial degradation of Product 10 could be producing metabolites that are being completely oxidized between day 7 and day 28.



Treatment	Slope	R square
Control	-0.0013	0.9505
Nutrient	-0.00563	0.8041
Product 10	-0.01859	0.9228

Figure 10 – Ln concentration change with time for product 10 (P10)
as compared with the nutrient and non-nutrient control

Figure 10 suggests a lag phase for Product 10 between day 0 and 7, after this period the microbial population shows a high degradation rate, achieving a final degradation extent higher than that of the nutrient and non-nutrient control.

The rate of oil removal is an important factor to consider when comparing the performance of each product. Table 7 presents a summary with the different rates of oil removal as well as the average.

	Product	Rate	Non-nutrient control	Nutrient control
	Product	0.007	0.00013	0.004
	Product	0.012	0.00013	0.004
	Product	0.014	0.002	0.005
	Product	0.017	0.0003	0.014
OSE II→	Product	0.018	0.00013	0.005
	Product	0.011	0.00013	0.005
	Average	0.013	0.0005	0.005

Table 7 - Rates of oil removal for the products passing the O7G criteria (mg of oil/L-Day)

OSE II had the highest rate of oil removal of the 13 EPA NCP Listed Products tested.

According to these results the average half-life of the petroleum mixture for this specific experiment is approximately 40 days. Prior studies suggest a half-life for petroleum mixtures of approximately 2 months (Stewart et. al., 1993).

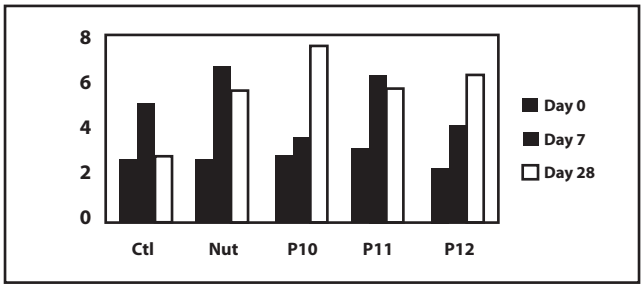


Figure 16 – MSN aliphatic degraders results (Batch D)

OSE II grew the highest number of oil degrading bacteria at $10^{7.5}$.

Products with a significant extent of oil removal show microbial counts in the order of 10^5 for the aliphatic degraders as presented in Figure 14, Figure 15, Figure 16, and Figure 17. Treatments with higher microbial populations, but similar degradation extents a compared with the control suggest the addition of an alternative carbon source other than the petroleum hydrocarbons.

Figures 32-34 show the composition of aliphatics, aromatics, and polars for batch D. As presented earlier for batches A and B, the aliphatic fraction is being degraded more severely than the aromatic fraction. The same results are found in the next two figures. Microbial counts for aliphatic degraders (Figure 16) show a higher number for Product 10, with a value of 4.06 E7 at day 28, as compared with the rest of the treatments in this batch, with values in the order of 10^6 at the most. This is reflected as a decrease in the aliphatic fraction composition from a 100% to 46% after 28 days.

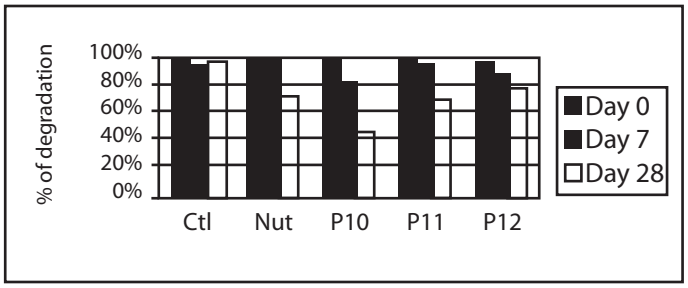


Figure 32 – Aliphatic fraction composition through time (% of degradation (Batch 1))

OSE II had the highest rate of degradation.

Products 10, 11, and 12 are decreasing in aliphatic and aromatic composition up to 50% for the aliphatic fraction and 25% for the aromatic. It is clear from these results that the oil is being degraded, and therefore, changing its composition. However, the aliphatic fraction is being degraded at a greater extent than the aromatic fraction, as mentioned before. Product 10 is showing a significant extent of hydrocarbons removal as presented in Figure 33 and Figure 34 for Product 10.

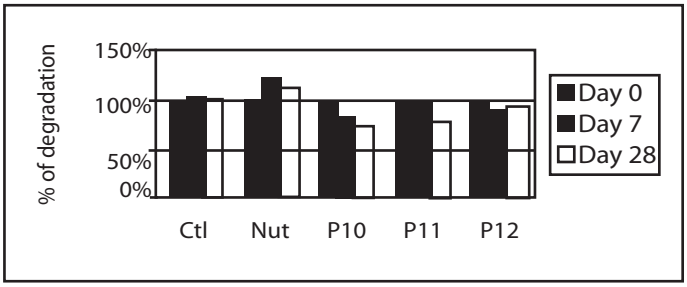


Figure 33 – Aromatic fraction composition through time (% of degradation (Batch D))

OSE II had the most (highest rate of) degradation of the aromatic fraction of the oil.

As presented in Figures 23 and 33 show the average of aliphatic fraction biodegraded was 34% (54% decrease for OSE II), while only 21% of the aromatic fraction showed to be biodegraded. The most degradation was by OSE II.

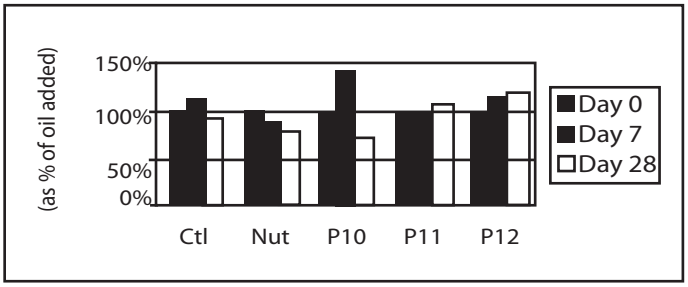


Figure 34 – Polar fraction composition through time as a percentage of the amount initially present (Batch D)

OSE II had the most or highest rate of (Polar) aromatic hydrocarbon degradation.

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OIL SPILL EATER II
EPA TEST – MARCH 1993
OIL SPILL EATER II – RESPIROCITY TEST - SUMMARY

This Respirocity Test was developed by NETAC and the Environmental Protection Agency to verify if a product could actually mitigate hydrocarbons to an end point of CO2 and water. The test was designed to measure the amount of oxygen-enhanced bacteria used. This would confirm the bacteria are in fact breaking the hydrocarbons down to CO2 and water.

At 100 parts Alaskan Gulf Seawater to 1 part OIL SPILL EATER II – applied at a 1 to 1 ratio to 1,000 parts per million Alaskan Prudhoe Bay Crude, the oxygen uptake is dramatic. This dramatic oxygen uptake proves a large amount of bacterial growth and decomposition of Prudhoe Bay Crude. The Chart on Page 2 shows an 86% decrease in heavy-end hydrocarbons and a 50% decrease in the aromatics. The test was stopped at 30 days; the test time prescribed by the EPA.

Our Standard Application Instructions for crude oil are 50 parts water to 1 part OIL SPILL EATER II applied at a 1 to 1 ratio to crude oil. The test results may be extrapolated to determine that with a 50 to 1 dilution, a 98% decrease in heavy-ends would occur in 24 days while an 85% decrease in aromatics would occur in 30 days. OIL SPILL EATER II can very effectively mitigate an oil spill.

After reviewing copies of the EPA Test on 10 other products, a comparison was initiated on the 2 products EPA claimed out-performed the other 9 products they tested. One product reduced the TPH approximately 158 parts per million and the other product reduced to 157 ppm of TPH. OIL SPILL EATER II reduced the TPH to 870 PPM. We feel this is a significant difference in efficacy.

March 1993
Respirocity Test

The Prudhoe Crude was supplied by the EPA, and was supposed to be the same crude used on the other two products. The crude sent to us for testing had a higher TPH (1,000 PPM) compared to the bacteria products tested by the EPA which only had a TPH of 168 ppm. Additionally, this crude did not have aromatics which the crude oil OSE II was tested on, did. The aromatics were reduced 50%.

It is our opinion that if you apply bacteria directly to a hydrocarbon with aromatics, that the toxicity of the aromatics will kill the bacteria. OIL SPILL EATER II first breaks the hydrocarbon walls, then grows bacteria so the toxicity is reduced first.

The accumulate oxygen uptake was also tested which shows bacterial activity. One of the products the EPA tested, they claim, performed well, had an uptake of 280 mg/L in 10 days and 460 mg/L in 30 days. The other product the EPA tested had 40 mg/L at 10 days and 440 mg/L at 30 days. OIL SPILL EATER II had an uptake of 520 mg/L at 10 days and 810 mg/L at 30 days. OSE II had more oxygen uptake at 10 days than the best bacterial products had at 30 days; on the 30 day comparison, OSE II had almost double the oxygen uptake any other product.

The EPA screened 31 products and tested 10. This test shows OIL SPILL EATER II reduced dramatically more TPH than these other products. OSE II produces more microbial activity than products with bacteria, and additionally, OSE II reduces aromatics. This test should help prove why we feel OSE II is the better product.

NOTE: In the summer of 2000 – Dr. Al Venosa (one of the EPA's top scientists at the time, on oil spills) reviewed this test. Dr. Venosa concluded that OSE II did, in fact biodegrade alkanes and aromatics. Dr. Venosa went on to explain that OSE II may be effective in degrading oil.

By: Steven R. Pedigo
Chairman
OSEI, Corp.

SRP/AJL

CHEMICAL ANALYSIS, INC.

Chemical ❄ Polymer ❄ Design

Research and Development
Consultation
Legal and Expert Witness

Failure Analysis
Formula Analysis
Engineering Design

July 3, 1990

Mr. Steve Pedigo
Sky Blue Chems
13355 Noel Road
1 Galleria Tower, Suite 500
Dallas, Texas 75240

NEW ADDRESS AS OF 10/96

OSEI, CORP.
13127Chandler Drive
Dallas, TX 75243

Subject: Oil Spill Eater Respirocity Evaluation
CAI Lab. No. 3265

Dear Mr. Pedigo:

Chemical Analysis, Inc. being an independent third party laboratory was employed to evaluate an oil spill additive for respirocity efficacy. The oil spill additive submitted to the laboratory was a product identified as Oil Spill Eater batch No. 124-E. The additive was evaluated at two different concentrations which included 1/100 and 1/500, additive parts to solution parts, respectively.

The concentration of the oil was 1000 parts per million (ppm). The oil and seawater was submitted to the laboratory to be similar to field material.

The results of our evaluation are attached to the report. Observing the results, it can be seen that the additive has a meaningful and significant effect on decreasing the oil concentration and increasing the oxygen take up.

The effect on decreasing the aliphatic content of the oil was in the range of 80 percent and the decrease of the aromatic content was in the range of 40 percent. An additive concentration of 1/500 appears to be effective. The concentration of the additive may have an adequate effect at even a lower concentration than 1/500.

The inherent effect of oxygen takeup was observed to be 178 mg/L for the additive (1/500), 12 for the seawater, and 8 for the oil. The net effect of the additive was 512 mg/L.

If there are any questions or if we may be of further assistance, please advise.

Sincerely yours,
CHEMICAL ANALYSIS, INC.

Galen Bartman
Laboratory Director
GWH:es

Oil Spill Eater (OSE) Respirocity Results

				Accumulated Oxygen Uptake				Aliphatic Content				Aromatic Content		Percent	Percent
Sample	Oil	Additive	Seawater	0 mg/L	10 mg/L	20 mg/L	30 days mg/L	0 ppm	10 ppm	20 ppm	30 days ppm	0 ppm	30 days ppm	Aliphatic Decrease	Aromatic Decrease
1	+	1/500	+	16	380	620	690	712	570	233	151	246	133	79	46
2	+	1/500	+	18	410	660	730	693	542	274	138	240	149	80	38
3	-	1/500	+	5	152	174	186	-	-	-	-	-	-	-	-
4	-	1/500	+	5	141	168	194	-	-	-	-	-	-	-	-
5	-	-	+	0	5	8	12	-	-	-	-	-	-	-	-
6	-	-	+	0	6	8	11	-	-	-	-	-	-	-	-
7	+	-	+	2	12	18	22	705	710	695	682	251	248	3	1
8	+	-	+	3	13	16	19	684	680	681	675	238	237	1	0
9	+	1/100	+	26	460	680	770	690	512	210	105	245	115	85	53
10	+	1/100	+	33	520	740	810	695	486	260	89	250	127	87	49
11	Spill Eater Batch No. 124-E														

OIL SPILL EATER INTERNATIONAL, CORP.

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March 23, 1990

OIL SPILL EATER II
BIODEGRADATION TESTS
CONCLUSIONS

These tests were conducted by the University of Alaska in Fairbanks, AK. The first test was on a heavy-end hydrocarbon (Hexadecane), which is left over once the light-ends volatize off. The mineral nutrients in nature refers to the use of Alaskan Sea Water used to perform the test. At 50 to 1, it shows good reduction and if the test would have continued another 48 hours, the results would have been substantially increased. The OIL SPILL EATER II has a good food source for bacteria and there was more food source than sea water ratio to grow a large colony quickly; therefore, the bacteria engulfed the food sources in the OSE II and slowly converted to hydrocarbons. Once all the OSE II food source runs out, then the only food source left are the hydrocarbons—so they switch over to stay alive. At 1 to 500 and 1 to 1000 absolute biodegradation was proven, the bacteria colonized quickly and ran out of food source because they started with less food source. The bacteria switched over quickly and a dramatic reduction in hexadecane was accomplished.

The second test was run on Naphthalene using minerals and nutrients (Alaskan Sea Water). Naphthalene is a polynuclear aeromatic hydrocarbon and are harder to break down than heavy-end hydrocarbons and they are the most toxic. These tests also show that OIL SPILL EATER II is a very effective means of mitigating naphthalene, a PAH which EPA's Dr. Al Venosa deems the hardest target compounds to Bioremediate!

By: Steven R. Pedigo
Chairman

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OIL SPILL EATER II

A PROTEIN POWER PACKAGE

The lack of knowledge about biological treatment of hydrocarbons has led to slow acceptance of proven methods of Bioremediation, particularly with respect to oil spills. However, following the EXXON VALDEZ incident, the U.S. Environmental Protection Agency undertook the first major governmental effort to use biological methods for site remediation. Although the early results are mixed, EPA is to be commended for its efforts which included application of a French Product (Inipol EPA 22) to enhance microbial degrading of weathered crude oil from beaches. Inipol has been described as "Popeye's Spinach" supplement to enhance the rate and extent of hydrocarbon degradation by naturally occurring microbial populations. The Inipol formulation probably does enhance the growth of hydrocarbon degradation bacteria (although this has not been clearly shown in the field portion of the EPA Study), but suffers in that it contains the potentially toxic solvent, 2-butoxyethanol.

There are many other agents which have potential to stimulate hydrocarbon removal from contaminated environments. These range from the solvent based cleaners and dispersants to simple water soluble inorganic fertilizers. One such product that has shown great potential for enhancing hydrocarbon biodegradation in standardized laboratory tests at the University of Alaska Fairbanks is OIL SPILL EATER II. If Inipol is a "Popeye's Spinach" formulation for hydrocarbon degrading micro-organisms, OIL SPILL EATER II is a "Protein Power Package" of mineral nutrients, enzymes and a carbon source concentrated in a non-toxic oleophilic surfactant. The surfactant base dissolves into hydrocarbon matrices with the aid of protease and amylase enzymes that act as micro-surface cleaners. The mineral nutrients enhance growth of natural hydrocarbon degrading micro-organisms with the pulse of easily metabolized carbon to quickly increase bio-mass. The high bio-mass, then begins to degrade hydrocarbon substrates and to product biosurfactants until the hydrocarbon substrate is depleted.

OIL SPILL EATER II
A PROTEIN POWER PACKAGE

In the aftermath of the EXXON VALDEZ Oil Spill, researchers from the University of Alaska evaluated the potential for naturally occurring micro-organisms to biodegrade oil contaminated beaches. Their studies showed that while natural micro-organisms have the potential to biodegrade both linear alkanes and aromatic hydrocarbons, their numbers and related metabolic activities can be substantially increased. In standard laboratory tests, these researchers showed that the marine formulation of OIL SPILL EATER II diluted into artificial seawater containing a consortium of micro-organisms and hydrocarbons from Prince William Sound, Alaska will degrade Hexadecane—300% faster than the same consortium amended with mineral nutrients and hydrocarbons without OIL SPILL EATER II.

By: Dr. Ed Brown
University of Alaska

DEB/AJL

OIL SPILL EATER CONCENTRATE
MINERALIZATION OF HEXADECANE BY A MICROBIAL CONSORTIUS FROM PRINCE WILLIAM SOUND, ALASKA (1)

Sample	Mineral Nutrients in nature HO OSE	Mineral Nutrients in nature 1/50 Dilution of Oil Spill Eater II	Mineral Nutrients in nature 1/500 Dilution of Oil Spill Eater II	Mineral Nutrients in nature 1/1000 Dilution of Oil Spill	Mineral Nutrients in nature 1/10 Dilution of Oil Spill Eater II
Hexadecane Transformation (I transformed to CO2) Mean of 3 trials	16	19.3	50	43.7	0
		Need more time so bacteria can use up molasses & convert to Hydrocarbon	300 increase proven efficacy Should totally eliminate Hydrocarbons		

1. Consortius was incubated for 70 hours with 100 mg of labeled hexadecane per sample.

Test Conducted at University of Alaska-Fairbanks

OIL SPILL EATER II CONCENTRATE
Mineralization of Naphthalene by a Microbial Consortius From
Prince William Sound, Alaska (1)
Alaskan Seawater

Sample	MINERAL Nutrients in nature No OSE	MINERAL Nutrients in nature 1/50 Dilution of Oil Spill Eater II	MINERAL Nutrients in nature 1/500 Dilution of Oil Spill Eater II	MINERAL Nutrients in nature 1/1000 Dilution of Oil Spill Eater II
N A P H T H A L E N E Transformation (% transformed To CO_2 Mean of 3 trials	3	29	46	27
		More time would have been allowed for the bacteria to completely use up the molasses and completely convert to hydrocarbon for its food source	1 5 3 3 % increase p r o v e n efficacy s h o u l d t o t a l l y eliminate raphthalene hydrocarbons	

1. Consortium (Alaska Sea Water) was incubated for 51 hours with 100 mg of labeled Naphthalene per 10 ML sample.

Test conducted at the University of Alaska
1/9/90

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August 13, 1990

MEGA BORG BIODEGRADATION TEST

Southwest Research Institute – one of the United States largest and most respected labs performed TPH reduction tests and residual weight tests using OIL SPILL EATER. This product, OSE, was applied to South African Crude Oil – spilled from the Mega Borg Tanker off the coast of Galveston, Texas. The sample of crude was supplied by the U.S. Coast Guard – Sky Blue Chems sent the sea water from Galveston to the Lab.

The initial TPH was 100,070 ppm; in 216 hours the TPH was reduced to 529 for a 99.5% reduction. This is a dramatic decrease and it proves Oil Spill Eater is a very viable Bioremediation product. This dramatic decrease shows how effective Oil Spill Eater is in reducing the chemical (toxic) constituent of the crude oil. The TPH was reduced approximately 90% in 48 hours rendering the crude oil virtually harmless quickly.

The physical reduction of the crude oil was also determined. In 216 hours, 94.7 of the residual weight of the South African Crude was remediated.

These tests prove "OIL SPILL EATER" is an extremely effective Bioremediation product that decreases not only the chemical components of crude oil, but it also Biodegrades the physical components as well.

Steven R. Pedigo
Chairman

SRP/AJL

SOUTHWEST RESEARCH INSTITUTE
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August 3, 1990

CHEMISTRY AND CHEMICAL ENGINEERING DIVISION
DEPARTMENT OF ENVIRONMENTAL SCIENCE

Attention: Mr. Steven R. Pedigo

Subject: Second Test for Sky Blue Chemical 01-3108-092

A sample of Megaborg oil and seawater was analyzed as per your instructions. The results of this initial test were inconclusive and a second test was requested. The second test was more extensive and included more time points. Samples were taken at 48,72, and 96 hours for the sample and control. The sample consisted of 600 ml seawater, 6 ml Megaborg oil, and 6 ml of the oil-eater provided. The control consisted of 600 ml seawater, and 6 ml Megaborg oil. The sample and control were stirred constantly at a very low speed. Sampling procedure: Vigorously stir the solution and remove 100 ml. Extract for TRPH analysis. After 90 hours the client requested addition of more seawater to improve the efficiency of the oil-eater, this was performed. A final analysis for TRPH was performed at 216 hours and was a complete sample extraction. In order to better compare the control and oil-eater results, results are shown in % Recoverable Oil, assuming that 1 gram of oil is equal to 1 ml of oil (since oil density is unknown). The percent recoverable oil is calculated as follows:

equation	TRPH g/ml	100 ml			
not clear		1000 g/ml	100	=	%
	theoretical amount of oil		1000 mg/g		
	extracted in each aliquot = 1 g				

TRPH and % Recoverable Oil for each time are shown for the sample and control in tables 1 and 2, respectively. Megaborg oil itself was found to have a TRPH of 1,070,000 mg/l.

Sincerely,

Mary Riddle

Mary Riddle
Research Scientist

Approved:

Donald E. Johnson

Donald E. Johnson, Ph.D.
Director

SAN ANTONIO TEXAS

Table 1

01-3108-092
Sample With Oil-Eater II

Time Elapsed	TRPH (mg/10)	% Recoverable Oil
48 hours	7520	75.2
72 hours	6910	69.1
96 hours	5990	59.9
*216 hours	529	5.3

95% Reduction of TPH in 216 hours. Chemical reduction of TPH.

94.7% residual weight reduction in 216 hours. Physical reduction of oil.

* Total sample analyzed

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SUMMARY OF BETX TEST

The objective was to have a third party testing laboratory show how OSE II (OIL SPILL EATER II Concentrate) worked well even on Benzene, Ethyl Benezene, Toulene and Xylene. The final composition – after all dilutions were performed, was 2,000 parts water to one (1) part OSE II Concentrate.

Even at this low level, the total BETX was reduced 32%. The correlation of strengths should prove that at 1,000 to one (1) reduction would have been 64%, a 500 to one (1) reduction would have been 80%; a 100 to one (1) reduction would have been 98%, almost completely Biodegraded.

At 2,000 to one (1) OSE II is a cost-effective product for Ballast Water Treatment.

The reduction correlation's with the increasing ratios also show that OSE II is an effective product for gasoline and diesel spills. OSE II would reduce gasoline or diesel spills on the surface and around leaking Underground Storage Tanks. OSE II would also be a good product to clean up any oil sheen on water surfaces and concrete surfaces.

Steven R. Pedigo
Chairman

CHEMICAL ANALYSIS, INC.

Chemical ❄ Polymer ❄ Design

Research and Development
Consultation
Legal and Expert Witness

Failure Analysis
Formula Analysis
Engineering Design

March 14, 1990

Mr. Steve Pedigo

Subject: BETX Analysis
CAI Lab. No. 3229

Dear Mr. Pedigo:

Chemical Analysis, Inc. being a third party independent laboratory was employed to evaluate a product identified as Oil Spill Eater and its affect on BETX solution. The procedural method was provided to our laboratory which outlined the preparation of several solutions.

Solution I: BETX

COMPONENTS	% BY VOLUME
Benzene	5.0
Ethylbenzene	5.0
Toluene	5.0
Xylene	5.0
Florida Sea Water	80.0
TOTAL	100.0%

Solution II: OSE-Florida Sea Water

COMPONENTS	% BY VOLUME
Oil Spill Eater	0.20
Florida Sea Water	99.80
TOTAL	100.0%

The percentage ratio of these two components represents a 1 to 500 mix ratio respectively.

3001 Skyway Circle North, Suite 100. Las Colinas Irving, Texas 75038 (214) 255-4100

Solution III: BETX/OSE-Florida Sea Water

COMPONENTS	% BY VOLUME
Solution I	50.00
Solution II	50.00
TOTAL	100.0%

Solution IV: BETX/OSE-Florida Sea Water Solution

COMPONENTS	% BY VOLUME
Solution III	50.00
Florida Sea Water	50.00
TOTAL	100.0%

Final Solution Composition:

COMPONENTS	% BY VOLUME
Aromatics	5.00
OSE Additive	0.05 (1:2000 weight ratio)
Florida Sea Water	94.95
TOTAL	100.0%

The final solution identifies the composition of the final mixture when the various solutions are prepared and mixed together based on the procedural instructions. The resultant final solution was allowed to stir for a period of (96) hours and the volume of BETX aromatic content was evaluated. The initial percent volume of aromatic discontinuous phase in the final solution represented five percent after the test. As a result of the evaluation, it was observed that 1.6% of the BETX solution had decreased from the discontinuous aromatic phase; this represented a 32% volume reduction in the aromatic content. Turbidity was observed to have increased in the water phase which indicated that incompatable components were incorporated into the water phase.

The 1:2000 weight ratio concentration of OSE in the final solution is based on the assumption that the OSE additive is 100% active; if the OSE is less than 100% active then one needs to proportionate the concentration accordingly.

If there are any questions or if we can be of further assistance, please advise.

Sincerely yours,
CHEMICAL ANALYSIS, INC.

Galen W. Hartman
Laboratory Director

GWH/cmc