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*March 18, 2005*

### SUMMARY

#### OIL SPILL EATER II BIOREMEDIATION TEST

##### *Performed by the University of Mexico*

The University of Mexico was asked by our Distributor in Mexico to perform Bioremediation (reduction) Tests, utilizing OSE II (ARA Bioderas) on a heavy crude oil (Maya) and a light crude oil (Olmec).

The goal of this test was to determine the rate of extent of Bioremediation (reduction) of light and heavy crude oil from Mexico over a 30 day period.

There were three test matrices, OSE II - Bioremediation Olmec, OSE II Bioderas on Maya and crude oil without OSE II Biodera. There was a day Olmec tests ran on each matrices to determine the initial crude oil. Then OSE II - Biodera was applied to the Olmec initial crude oil level. Then OSE II -Biodera was applied to the Olmec and Maya crude oil. Subsequent tests on all three matrices were performed on day 7, day 15 and day 30.

The control test (with no OSE II Bioderas) showed virtually no bioremediation (reduction) of crude oil. The lighter crude oil showed a quick reduction in the first seven days then reduction showed, however, the total reduction was 84.78%. The heavier crude oil showed a slow start and then a dramatic reduction in the last 15 days. This also, was a significant reduction. It can be extrapolated that there would have been 100% reduction in less than 45 days.

These tests tracked with what has occurred over and over since 1989. The lighter crude oil (Olmec) showing a great initial reduction, was because it is easier to break down shorter chained hydrocarbons. Once the shorter chain hydrocarbons

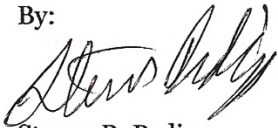
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were reduced, the reduction on longer chained hydrocarbons started; which is a slower process. The 82.82% reduction would make using OSE II – Bioderas - in a water spill, a far superior cleanup compared to any other method in the world. All other methods or mechanical processes are not capable of getting rid of 30% of a spill.

The heavy-end oil, Maya, showed a slow reduction at first, because longer chain hydrocarbons are more difficult to break down; however, once the process starts, the reduction is very rapid.

When OSE II – Biodera – is used, and the toxicity rapidly reduced, the adhesion properties are reduced, the oil is made to float, limiting the impact of the spill and the flammability is reduced. Therefore, once OSE II is applied, the spills environmental impact is eliminated, while OSE II reduces the oil to Carbon Dioxide and water.

By:

A handwritten signature in black ink, appearing to read "Steven R. Pedigo". The signature is written in a cursive, flowing style.

Steven R. Pedigo  
Chairman/OSEI Corp.



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March 19, 2005

**Interpretation of University of Mexico's  
Bioremediation Tests of OSE II**

**REPORT OF RESULTS**

TEST: Evaluation of an application product for bioremediation of petroleum spill.

LABORATORY WHERE THE TESTS WERE PERFORMED: Ecologia microbial

PERSON RESPONSIBLE: M.en C. Gabriel Pineda Flores

PERSON RESPONSIBLE FOR EXTERNAL SERVICES: Q. B. P. Norma Pescador Elizondo.

Number of products analyzed: one

Name: Bioderra

Company: Flusell de Hidalgo S.A. de C.V.

**CHARACTERISTICS OF THE TEST**

To determine the efficiency of the product, a test to quantify the diminishment of the total content of the crude oil of Maya and Olmec by the metabolic action of microorganisms in a test of fresh water. The tests were done in two ways: one with Bioderra OSE II and the second without the addition of the product.

**DEVELOPMENT**

Three series of matrices on four different dates were performed. And, Erlenmeyer Flask of 250ml, which contained 100ml of fresh water, 10ml of crude oil plus 10ml of the freshwater Bioderra (OSI II) mixture (proportion: 12.8 ounces of fresh water plus 0.3 oz of Bioderra). One series contained Maya heavy

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crude, the second was Olmec light crude, and the third was the control, to which was only added Maya oil and fresh water.

The three series were maintained under constant agitation and ambient temperature for 30 days. The ASTM 5520 method was used to evaluate the diminishment of the crude oil to quantify the total hydrocarbons by a gravimetric separation (division) the following formula was used:

$$\text{mgHC/L} = \frac{(A-B) \times 1000}{M1 \text{ of muestra (tested?)}}$$

Where:

Mg HC/L: milligrams per liter of total hydrocarbons

A: Weight of beaker (vaso) with crude oil

B: Weight of the beaker

The volume of each sample tested was 10ml (a little unsure here but I think this is pretty close).

The determination of the diminishment of the oil was carried out at 0, 7, 15, and 30 days from the initiation of the test.

Effect of the product "Bioderra" (OSE II) on the elimination of total hydrocarbons on different types of crude oil – (See Attached University of Mexico Tests).

In figure 1 it was observed that the most notable diminishment of oil was in the system (test) that contained Bioderra (OSE II) and Olmec oil reaching its maximum at 30 days of testing. In the case of Maya oil the diminishment was notable after 30 days of testing, the control did not show any diminishment in the oil content.

## DISCUSSION

In accordance with the obtained results it is possible to determine that the product "Bioderra" (OSE II) stimulates the process of the elimination of crude oil by microbial action, also that in the control test the reduction of oil is minimal.

The stimulation of the elimination of the oil is most notable when you refer to the light crude oil (Olmec) with an 84.78% of reduction and in the case of the heavy crude oil (Maya) reduction is less, with a maximum 54.46% value.

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## CONCLUSIONS

The product named Bioderra (OSE II) of the Flusell Company . . . is considered a product **THAT STIMULATES THE ELIMINATION OF CRUDE OIL in tested conditions.** The elimination is greater in the light crude oil (Olmecca) and is less in the heavy oil (Maya)



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### REPORTE DE RESULTADOS

**PRUEBA:** Evaluación de un producto de aplicación para biorremediación de derrames de petróleo

**LABORATORIO DONDE SE REALIZÓ LA PRUEBA:** Ecología microbiana

**PERSONAL PARTICIPANTE:** M. en C. Gabriel Pineda Flores

**RESPONSABLE DEL SERVICIO EXTERNO:** Q.B.P. Norma Pescador Elizondo

Número de productos analizados : uno (1)

1. Nombre: Bioderra (sin número de lote)

Compañía: flusell de hidalgo s.a. de c.v.

### CARACTERÍSTICAS DE LA PRUEBA

Para determinar la eficiencia del producto, se procedió a cuantificar la disminución del contenido total de petróleo crudo tipo Maya y Olmeca, por la actividad metabólica de microorganismos de una muestra de agua natural dulce. Se probaron dos condiciones: una con Bioderra y la segunda sin la adición del producto.



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### DESARROLLO

Se utilizaron tres series de cuatro matraces Erlenmeyer de 250 ml, los cuales contenían 100 ml de agua natural dulce, 10 ml de petróleo crudo más 10 ml de la mezcla agua dulce-Bioderra (proporción: 12.8 oz agua natural dulce más 0.3 oz de Bioderra). Una serie contenía petróleo tipo Maya (crudo pesado), la segunda tipo Olmeca (crudo ligero) y la tercera fue el sistema testigo, al cual solo se le adicionó petróleo Maya y el agua natural dulce.

Las tres series de mantuvieron en agitación constante y a temperatura ambiente durante 30 días. Para evaluar la disminución del petróleo crudo se aplicó el método ASTM 5520F (1) para cuantificar hidrocarburos totales por un método gravimétrico de partición, en el cual utiliza la siguiente fórmula:

$$\text{mg HC/L} = \frac{(A-B) \times 1000}{\text{ml de muestra}}$$

En donde:

mg HC/L: miligramos por litro de hidrocarburos totales

A: Peso del vaso con petróleo crudo

B: Peso del vaso a peso constante

El volumen de muestra analizada por cada muestreo y matraz fue de 10 ml.

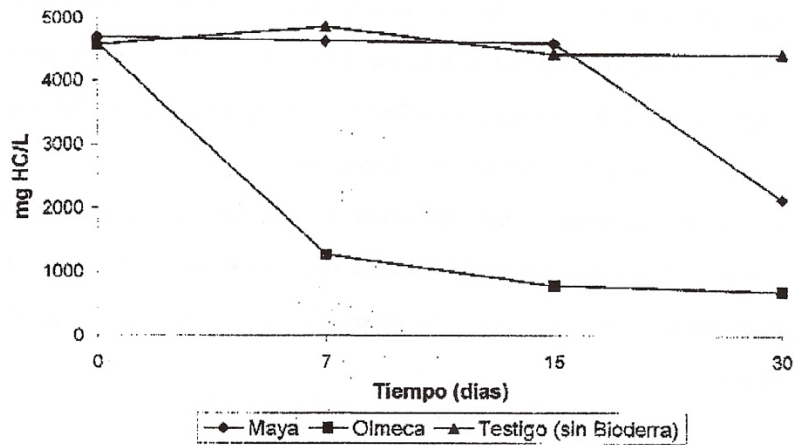
Las determinaciones de la disminución del petróleo se realizaron a los 0, 7, 15 y 30 días de iniciada la prueba.



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### RESULTADOS



**Efecto del producto "Bioderra" sobre la eliminación de hidrocarburos totales de diferentes tipos de petróleo crudo**

En la figura 1 se observa que la disminución de petróleo más notable se presentó en el sistema que contenía Bioderra con petróleo Olmeca, siendo ésta máxima a los 30 días de desarrollo de la prueba. En el caso del petróleo Maya su disminución fue notable hasta los 30 días de prueba, el sistema testigo no presentó una disminución en el contenido de petróleo.





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Tabla I. Efecto del producto "Bioderra" sobre el porcentaje de remoción de hidrocarburos totales de diferentes tipos de petróleo crudo

Tiempo (días)	% de remoción		
	Maya	Olmeca	Testigo (sin Bioderra)
0	0	0	0
7	1.48	72.39	0
15	2.12	82.82	3.28
30	54.46	84.78	3.06

En la tabla I se observa que el mayor porcentaje de remoción se presentó en el sistema que contenía petróleo Olmeca y después en el sistema compuesto por crudo tipo Maya. La remoción de petróleo en el sistema testigo se considera insignificante.

#### DISCUSIÓN

De acuerdo con los resultados obtenidos, es posible determinar que el producto "Bioderra" estimula el proceso de eliminación de petróleo crudo por actividad microbiana, ya que en el sistema testigo la remoción de petróleo es mínima y este contenía microorganismos, petróleo pero carecía del producto Bioderra.

La estimulación en la eliminación de petróleo es más marcada cuando se trata de crudo ligero (tipo Olmeca) con un 84.78% de remoción y en el caso de petróleo pesado (tipo Maya) su remoción es menor, con un 54.46% como valor máximo.



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**CONCLUSIONES**

El producto denominado Bioderra (sin número de lote) de la compañía flusell de hidalgo s.a. de c.v. se considera como un producto que ESTIMULA LA ELIMINACIÓN DE PETRÓLEO CRUDO en las condiciones de la prueba aplicadas. La eliminación es más abundante en petróleo de tipo ligero (Olmeca) y es menor para crudo de tipo pesado (Maya).

**BIBLIOGRAFÍA**

1. APHA, AWWA, WEF (1995). Standard Methods for the examination of water and wastewater, 19<sup>th</sup> ed., APHA Washington DC, 5-30 to 5-35 p.

M. en C. Gabriel Pineda Flores

Realizó los análisis

Q.B.P. Norma Pescador Elizondo

Jefe del laboratorio de Ecología Microbiana