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**An Evaluation of BioCOPE to Reduce Hydrogen  
Sulfide in Sediments, and Accelerate the Breakdown of  
Petroleum Hydrocarbons in Soil**

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## TABLE OF CONTENTS

|  |          |
|--|----------|
| <b>Abstract.....</b>                       | <b>1</b> |
| <b>Introduction.....</b>                   | <b>1</b> |
| <b>Objectives.....</b>                     | <b>1</b> |
| <b>Experimental Method and Design.....</b> | <b>1</b> |
| Test A-Hydrogen Sulfide in Sediments ..... | 1        |
| Test B-Petroleum Hydrocarbons in Soil..... | 2        |
| <b>Test Results and Discussion .....</b>   | <b>2</b> |
| Test A-Hydrogen Sulfide in Sediments ..... | 2        |
| Test B-Petroleum Hydrocarbons in Soil..... | 3        |
| <b>Summary .....</b>                       | <b>5</b> |
| <b>Disclaimer .....</b>                    | <b>5</b> |

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## **Abstract**

BioCOPE, Inc. manufactures a multi-enzyme product designed for use in waste water treatment systems. BioCOPE and BioCOPE B are multi-enzyme based wastewater treatment products. Essentially both products breaks down the “food” and makes it available for naturally occurring bacteria present in the water column. The bacteria can metabolize contaminants at an accelerated rate thereby decreasing the time necessary to treat waste waters.

This bench test is the first time BioCOPE products have been applied to contaminated soils. Two products, BioCOPE and BioCOPE B were applied to petroleum hydrocarbon and hydrogen sulfide contaminated soils. The purpose of the experiments was to evaluate how well each product performs under ambient conditions. This was accomplished by comparing degradation rates of contaminated soil not treated against contaminated soils treated with BioCOPE and BioCOPE B.

## **Introduction**

BioCOPE, Inc. and the Rocky Mountain Oilfield Testing Center (RMOTC) entered into a Cooperative Research and Development Agreement (CRADA) to conduct two bench tests. Test A involved an experiment for reducing hydrogen sulfide (H<sub>2</sub>S) in sediments. Test B was an evaluation of the degradation rate for petroleum hydrocarbons in sediments. These tests were conducted under ambient conditions at Naval Petroleum Reserve No. 3.

The data presented in this report was collected from July 11, 2000 through October 17, 2000. This report will detail the experimental design, materials and methods used to conduct the test, test procedures, laboratory results, and a summary of those results.

## **Objectives**

1. To evaluate how effective BioCOPE and BioCOPE B are in reducing H<sub>2</sub>S levels in sediments contaminated by oil field production processes.
2. To evaluate the degradation rate of petroleum hydrocarbons in sediments contaminated by oil field production processes when BioCOPE and BioCOPE B were applied.

## **Experimental Method and Design**

RMOTC and BioCOPE designed two separate bench experiments. The experiments were conducted simultaneously by RMOTC at Naval Petroleum Reserve No. 3 (NPR-3) under ambient conditions from July through September 2000.

### **Test A-Hydrogen Sulfide in Sediments**

The test for reduction of hydrogen sulfide in sediments spanned slightly more than 60 days. Four 30-gallon rubber containers were set-up outside. The containers were filled with sludge-like sediments removed from an oil-skimming pit. This pit receives approximately 35,000 barrels of produced water per day. As the solids drop out of the water, they collect in the pit.

Each container received approximately 20 gallons or 0.14 m<sup>3</sup> of sediment. Before beginning the experiment, composite samples were collected from each container. The samples were analyzed for hydrogen sulfide content in sediments using EPA Method 376.1 to establish baseline levels of sulfides in the sediments. Field technicians analyzed the pH levels of the sediments to ensure they remained neutral or within 6.5-7.5 standard units.

The containers were labeled “Control, I, II, III, and IV.” The control container only received the hydrogen sulfide contaminated sediment. Container II received an addition of 70 ml of BioCOPE and the sediments were stirred. Container III received 70 ml of BioCOPE B and the sediments were stirred. In Container IV BioCOPE was sprayed onto the sediments. The sediments in this container were not stirred.

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BioCOPE and BioCOPE B were added weekly to each container with the exception of the Control container. Each container received 14 ml of BioCOPE or BioCOPE B as prescribed. The sediments in Container II and III were stirred while Container IV was not stirred.

Initially samples were scheduled for collection biweekly or every 14 days. Due to a significant price increase from the commercial laboratory used to analyze the samples, sampling frequency was reduced to monthly.

### **Test B-Petroleum Hydrocarbons in Soil**

The test for accelerating the breakdown of petroleum hydrocarbons in soil by applying BioCOPE and BioCOPE B lasted slightly more than 60 days. Six 30-gallon rubber containers were set-up outside. The containers were filled with petroleum hydrocarbon contaminated soil collected from a landfarm onsite. Each container received approximately 20 gallons or 0.14 m<sup>3</sup> of sediment. Before beginning the experiment, composite samples were collected from each container. The samples were analyzed for total petroleum hydrocarbon (TPH) content using EPA Method 8015. Throughout the duration of the pH levels of the soil was monitored to ensure the soil remained within 6.5-7.5 standard units.

The containers were labeled “Control IA, Control IIA, II, III, IV, and V.” Control container IA received only petroleum hydrocarbon contaminated soil and 200 ml of deionized water. The soil in this container was stirred. Control container IIA received the same volume of contaminated soil, 200 ml of deionized water but the soil in this container was not stirred during the duration of the test. The purpose of setting up two control containers was to demonstrate the effect of oxygenating the soil through stirring.

The remaining four containers were filled with the same volume of soil. To Container II, 200 ml of BioCOPE was added. This container was not stirred during the test. Container III and V received 200 ml of BioCOPE and the soil was stirred during the test. Container V also received a baseline and a weekly addition of 5 grams of nitrogen and phosphorous fertilizer. Finally, BioCOPE B was added to Container IV. The soil in Container IV was oxygenated as well.

BioCOPE and BioCOPE B were added weekly to each container with the exception of the control containers. These containers received weekly amendments of deionized water. The remaining containers received 20 ml of BioCOPE or BioCOPE B weekly as prescribed. The soil in Containers IA, III, IV, and V were stirred while Containers IIA and II remained dormant. Potable water was added to each container as needed to maintain soil moisture content of 30-40%. The moisture levels were determined by visual inspection of the soil.

Composite samples were collected on or around the 30<sup>th</sup> and 60<sup>th</sup> days of the test.

## ***Test Results and Discussion***

### **Test A-Hydrogen Sulfide in Sediments**

The purpose of this experiment was to determine if BioCOPE and BioCOPE B were effective in reducing the levels of H<sub>2</sub>S in sediments. Four 30-gallon containers were filled with approximately 20-gallons (0.143 m<sup>3</sup>) of hydrogen sulfide contaminated sediments. The containers were placed outside to simulate “landfarm” conditions.

The table below suggests that the total sulfide in the soil quickly dissipate after the application of BioCOPE and BioCOPE B to the sediments.

| <b>H<sub>2</sub>S in Sediments (mg/kg) EPA Method 376.1</b>               |                                 |   |   |  |
|---|---------------------------------|---|---|--|
| <b>Sample Dates</b>   | <b>Control<br/>No additions</b> | <b>Container II<br/>Add<br/>BioCOPE, stir</b> | <b>Container III<br/>Add<br/>BioCOPE, B,<br/>stir</b> | <b>Container IV<br/>Spray<br/>BioCOPE, do<br/>not stir</b> |
| 7/11/00 (Baseline sample)   | 310                             | 312   | 192   | 276  |
| 7/28/00   | 296                             | 106   | >50*  | 76   |
| 8/23/00   | 62                              | >50*  | >50*  | >50*   |
| *50 mg/kg is the limit of detection or reporting limit for this procedure |                                 |   |   |  |

| <b>Percent Decrease of Hydrogen Sulfide<br/>(EPA Method 376.1)</b>        |                                 |   |   |  |
|---|---------------------------------|---|---|--|
| <b>Sample Dates</b>   | <b>Control<br/>No additions</b> | <b>Container II<br/>Add BioCOPE,<br/>stir</b> | <b>Container III<br/>Add BioCOPE,<br/>B, stir</b> | <b>Container IV<br/>Spray<br/>BioCOPE, do<br/>not stir</b> |
| % decrease from 7/11/00*<br>to 7/28/00                                    | 4.51%                           | 66.03%  | 74.50%  | 72.50%   |
| % decrease from 7/28/00<br>to 8/23/00                                     | 79.05%                          | 53.80%  | 0.00%   | 35.53%   |
| Total % decrease  | 80.00%                          | 84.30%  | 74.50%  | 82.25%   |
| *50 mg/kg is the limit of detection or reporting limit for this procedure |                                 |   |   |  |

Nothing was added to the sediments in the Control container and the sediments were not stirred during the test. Sulfide concentrations in this container fell 4.5% from July 11 to July 28. The concentrations fell an additional 79% from July 28 to August 23. Total percent decrease for this container was 80 percent.

Container II received an initial slug of 70 ml of BioCOPE and subsequent weekly treatments of 14 ml. The sediments in this container were stirred weekly. After initial treatment, sulfide levels were reduced by 66%. After the second sampling event, those levels were reduced to below the limit of detection. Hydrogen sulfide levels fell by more than 84% over the duration of the test.

Container III received an initial slug of 70 ml of BioCOPE B and subsequent weekly treatments of 14 ml. The sediments were stirred weekly. Sulfide levels were reduced by almost 75% to below the limit of detection after initial treatment.

Container IV received an initial treatment of 70 ml of BioCOPE. This time the product was sprayed onto the sediments instead of poured onto the sediments. Fourteen ml of BioCOPE was sprayed onto the sediments weekly. The sediments in this container were not stirred. Sulfide levels were reduced by nearly 73% after initial treatment. Results from the second sampling event showed an additional reduction of 35%. Total percent decrease for this container was 82 percent.

#### **Test B-Petroleum Hydrocarbons in Soil**

The purpose of this experiment was to determine if BioCOPE and BioCOPE B were effective in accelerating the breakdown of petroleum hydrocarbons in soil. Six 30-gallon containers were filled with approximately 20-gallons (0.143 m<sup>3</sup>) of petroleum hydrocarbon contaminated soil. The containers were placed outside to simulate "landfarm" conditions.

The table below suggests that the total petroleum hydrocarbons in the soil quickly dissipate after the application of BioCOPE and BioCOPE B to the sediments. It should be noted that the reduced concentrations of petroleum hydrocarbons might also be attributed to the volatilization of the petroleum compounds under ambient conditions.

| <b>Total Petroleum Hydrocarbons-Diesel Range Organics<br/>(EPA Method 8015B Modified)</b>        |                                       |   |  |  |   |  |
|--|---------------------------------------|---|--|--|---|--|
| <b>Sample Dates</b>  | <b>Control IA<br/>Add water, stir</b> | <b>Control IIA<br/>Add water, do not stir</b> | <b>Container II<br/>Add BioCOPE, do not stir</b> | <b>Container III<br/>Add BioCOPE, stir</b> | <b>Container IV<br/>Add BioCOPE B, stir</b> | <b>Container V<br/>Add BioCOPE, fertilizer, stir</b> |
| 7/11/00*   | 8940                                  | 10300   | 11700  | 8270                                       | 8210  | 9460   |
| 8/23/00  | 3820                                  | 2560  | 2980   | 3410                                       | 3900  | 4380   |
| 9/27/00  | 1240                                  | 3930  | 3170   | 2220                                       | 4680  | 2970   |
| 10/16/00<br>(resample)   |                                       |   |  |  | 3800  |  |
| *Baseline sample<br>(1000 mg/kg is the limit of detection or reporting limit for this procedure) |                                       |   |  |  |   |  |

| <b>Percent Decrease of Total Petroleum Hydrocarbons-Diesel Range Organics<br/>(EPA Method 8015B Modified)</b> |                                       |   |  |  |   |  |
|---|---------------------------------------|---|--|--|---|--|
| <b>Sample Dates</b>   | <b>Control IA<br/>Add water, stir</b> | <b>Control IIA<br/>Add water, do not stir</b> | <b>Container II<br/>Add BioCOPE, do not stir</b> | <b>Container III<br/>Add BioCOPE, stir</b> | <b>Container IV<br/>Add BioCOPE B, stir</b> | <b>Container V<br/>Add BioCOPE, fertilizer, stir</b> |
| % decrease from 7/11/00* to 8/23/00   | 57.27%                                | 75.15%  | 74.53%   | 58.77%                                     | 52.50%                                      | 53.70%   |
| % decrease from 8/23/00 to 9/27/00  | 67.54%                                | -53.52%                                       | -6.38%   | 34.90%                                     | -20.00%                                     | 32.19%   |
| Total % decrease  | 86.13%                                | 61.84%  | 72.91%   | 73.16%                                     | 43.00%                                      | 68.60%   |
| *Baseline sample<br>(1000 mg/kg is the limit of detection or reporting limit for this procedure)              |                                       |   |  |  |   |  |

Two control containers were set up, Control IA and Control IIA, respectively. Control IA received only water and the soil was stirred. Control IIA also received a prescribed amount of water but the soil was not stirred. Petroleum concentrations were reduced by 57% in container IA and 75% in IIA. From August 23 to September 27, the concentrations fell an additional 68% in container IA. Petroleum concentrations in container IIA increased by 54% over the baseline results during this same period. Total percent decrease for container IA was 86% and an overall 62% reduction in container IIA.

Container II received an initial slug of 200 ml of BioCOPE and subsequent weekly treatments of 20 ml. The sediments in this container were not stirred. After initial treatment, petroleum hydrocarbon levels were reduced by 75%. Hydrocarbon concentrations increased slightly by 6% indicating that significant

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degradation of the petroleum compounds did not occur after initial treatment. TPH levels were reduced by 73% over the duration of the test.

Container III received an initial slug of 200 ml of BioCOPE and subsequent weekly treatments of 20 ml. The sediments were stirred weekly. Petroleum hydrocarbon levels were reduced by 59% after initial treatment. Further treatment yielded an additional 35% reduction over baseline values. Total levels of petroleum compounds were reduced by 73%.

Container IV received an initial treatment of 200 ml of BioCOPE B. 20 ml of BioCOPE B was added onto the sediments weekly. The sediments in this container were stirred. TPH levels were reduced by nearly 53% after initial treatment. Results from the second sampling event showed an increase of 20%. This bin was re-sampled. TPH levels were in line this time. Results showed a minor reduction of 2.5%. Total percent decrease for this container was 54 percent.

Container V received an initial treatment of 200 ml of BioCOPE and weekly additions of 20 ml of BioCOPE and 5g of fertilizer. The sediments in this container were stirred weekly. TPH levels were reduced by 54% over baseline levels. These concentrations were reduced by an additional 32% from August 23 to September 27. Total percent decrease for this container was 69%.

## **Summary**

- ✍ The sulfide concentrations in the control container were reduced by a total of 80% without treatment.
- ✍ H<sub>2</sub>S concentrations in Container II generated the greatest total overall decrease (84.30%). Monthly decreases were consistent over 60 day test period.
- ✍ Additional reductions in sulfide concentrations for Container III could not be quantified after July 28 testing due to detection limits for this procedure.
- ✍ Overall percent decrease for Container IV was 82.25%. The greatest reduction in sulfide concentrations was seen between July 11 and July 28. BioCOPE B was added to this container.
- ✍ In conclusion for the sulfide concentrations containers II, III, and IV showed an average reduction in sulfide sediments average 71.7% from July 11 through July 28 while the control containers average reduction was 4.51%.
- ✍ When comparing TPH Control container IA and containers III, IV, and V it appears stirring enhances hydrocarbon degradation.
- ✍ The greatest total reduction of TPH levels in Container II was seen from July 11 to August 23. TPH concentrations tested slightly higher for the period August 23 through September 27. The sediments in this container were not stirred.
- ✍ TPH degradation rates were consistent in container III. Total percent decrease in TPH concentrations was 73.16%.
- ✍ BioCOPE B was added to container IV. TPH concentrations were reduced by 43% from baseline concentrations. This container yielded the poorest results for the TPH experiment.
- ✍ The TPH levels in Container V were consistent and comparable to Container III. The addition of fertilizer to the soil does not appear to enhance remediation in this case.

## **Disclaimer**

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