

SYBRON CHEMICALS INC.



**MICROBIAL
CHARACTERIZATION
STUDY
for
AMOCO PRODUCTION**

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October 1993

Presented by:

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PURPOSE

It is the understanding of Sybron Chemicals Inc.'s Environmental Services Division (Sybron) that Amoco Production (Amoco) is evaluating bioremediation as a remedial alternative for the treatment of petroleum condensate contaminated soils and groundwater located at the South Sprint Facility. The objective of this preliminary biological analysis was to characterize the microbial quality of the impacted soil and groundwater. The following parameters were determined:

- Total aerobic heterotrophic bacterial count (TPC);
- Selective plate count (SPC - "petroleum condensate" degrading microorganisms);
- pH;
- N-NH₃;
- O-PO₄; and
- Soil percent moisture.

CONCLUSIONS

- (1) The results of the preliminary microbial characterization study indicate that petroleum hydrocarbon degrading microorganisms are present in the samples analyzed; however, the counts are about one to two orders of magnitude below optimum. Stimulation and/or augmentation of the microbial community present in the soil should enhance selective microbial counts; therefore, increasing condensate degradation rates.
- (2) Total aerobic heterotrophic microorganism counts are present at acceptable levels in the soil and low by two or greater orders of magnitude in the water samples analyzed.
- (3) The aquifer environment may be growth limiting due to low pH concentrations. The pH concentration detected in the water samples (especially sample number 1-W) is not optimal for biodegradation of hydrocarbon contaminants. The pH observed in three of the groundwater samples was approximately 6.2 and the pH observed in sample 1-W was 2.0. A pH of 7.0 to 7.5 is desirable. The low pH detected in groundwater sample 1-W should not be a problem because the soil and groundwater located in this area of the site are not contaminated according to the concentration map supplied by Amoco.
- (4) The pH observed in the soil samples was slightly high, but within an acceptable range for hydrocarbon degradation.
- (5) The macronutrient ortho-phosphate and ammonia nitrogen concentrations in the soil and groundwater are low in most site areas.

RECOMMENDATIONS

A treatability study is recommended to establish the optimum conditions for in-situ bioremediation by stimulation and/or bioaugmentation, if needed. Respirometry will be used to determine nutrient requirements, optimum pH, select cultures for augmentation, if required, and estimate target compound degradation rates.

MATERIALS AND METHODS

Standard U.S. Environmental Protection agency (EPA) methods or Standard Methods for the Examination of Water and Wastewater (SM) were used to analyze the following parameters:

- Orthophosphate phosphorus ($O-PO_4$) - SM method 4500-P.
- Rapidly accessible nitrogen as ammonia ($N-NH_3$) - EPA method 350.2.
- pH - EPA method 9040 or 9045 when applicable.
- Moisture content - EPA method 160.3.

Total aerobic heterotrophic microbial counts were determined using standard microbiological spread plate methods. Hydrocarbon degrading petroleum microorganisms were enumerated using condensate as the sole carbon source.

RESULTS AND DISCUSSION

The results of the preliminary microbial characterization study indicate that petroleum condensate degrading microorganisms are present and that the aquifer environment is likely to be growth limiting. The data are summarized in Table 1.

As stated in the conclusions, the pH of the groundwater, especially the sample collected at location 1-W, is not at an optimum for hydrocarbon biodegradation. A pH of 7.0-7.5 is desirable for the microbial degradation of hydrocarbons. According to site information provided by Amoco, the soils and groundwater in the area from which sample 1-W was collected are not impacted by condensate contaminants; therefore, the low pH detected in sample 1-W should not have a negative impact on the degradation of the contaminated areas. In general, the macronutrient ortho-phosphate and ammonia nitrogen concentrations are low.

The total number of aerobic microorganisms as well as condensate degrading microorganisms present in the groundwater are two to three orders of magnitude less than the numbers achievable under ideal conditions. It is interesting to note that the total and condensate degrading microorganisms counts are nearly equal in most of the groundwater

samples. This indicates that most of the indigenous microorganisms present in the groundwater are capable of degrading the hydrocarbon contaminants. The total number of aerobic heterotrophic microorganisms detected in the soils are present at acceptable levels; however, the condensate degrading microorganisms counts are one to two orders of magnitude less than the numbers achievable under ideal conditions.

A treatability study is recommended to establish those conditions that will enhance subsurface microbial degradation.

SCOPE OF WORK

Oxygen utilization rates (respirometry) will be used to determine those conditions of pH, nutrient loading and microbial augmentation, if needed, that will accelerate degradation of the target compounds, i.e., aromatic petroleum hydrocarbons. Contaminant degradation rates will be determined.

Task 1: Review Data

Task 2: Treatability Study Part I (3 weeks - Respirometry)

Subtask 2.1: Sampling

Subtask 2.2: Respirometry

Task 3: Report of Findings (2 to 3 weeks from end of Task 2)

Task 1: Review of Data

The historical data and report documents describing the soil and groundwater quality will be reviewed.

Task 2: Treatability Study Part 1 (Respirometry)

Subtask 2.1: Sampling

Soil and groundwater sampling will be performed in accordance with a QA/QC plan that insures proper sample handling and documentation.

The soil for the treatability study should be obtained from the region of the site from which samples 3 and 4 (W&S) were collected. Samples of this description are the most ideal for the determination of biodegradation rates and abiotic mechanisms of loss such as volatilization.

Approximately 5 kg of soil and 20 liters of groundwater will be required for the treatability study.

Subtask 2.2: Respirometry

The respirometers will be 1 liter aerobic stirred batch reactors containing groundwater and about 100 grams of soil in suspension. Approximately eight reactors will be set up in duplicate at pH values representative of site conditions and 7.5. Nutrients will be added and the pH adjusted as required. Cumulative oxygen uptake and oxygen uptake rates will be measured and used to establish the optimum conditions for microbial growth and biodegradation of the hydrocarbon contaminants. Two of the microcosms will be augmented with Sybron cultures to determine whether bioaugmentation is a feasible alternative. Two of the microcosms will be abiotic controls for estimation of the percent of contaminant loss attributable to non-biological mechanisms. Contaminant biodegradation rates will be determined.

The parameters to be monitored are:

- (1) Cumulative oxygen uptake;
- (2) Oxygen uptake rate;
- (3) Contaminant concentration;
- (4) Microbial counts - total and hydrocarbon degrading;
- (5) pH; and
- (6) Macronutrient concentrations.

SCHEDULE

Sybron will commence work on the project as soon as notice to proceed is received. The entire study, i.e., Part I and Part II and the final report will take 5 to 6 weeks.

ESTIMATED COST

Task I: Review Data	No Charge
Task 2: Treatability Study Part I (3 weeks - Respirometry)	
Subtask 2.1: Sampling (to be performed by Amoco)	NA
Subtask 2.2: Respirometry	3,000.00
Task 3: Final Report (2 to 3 weeks from end of Task 2)	<u>500.00</u>
Total	\$3,500.00

Table 1
Soil and Groundwater Characterization

Sample Number	Matrix	pH	O-PO ₄ (ppm)	N-NH ₃ (ppm)	Moisture (% solid)	Bacterial Counts		BTEX
						Total (CFU/gm)	*Selective (CFU/gm)	
1-W	water	2.0	4.0	60	NA	-0-	-0-	clean
2-W	water	6.2	-0-	100	NA	4.0X10 ³	7.0X10 ²	268 ppb
3-W	water	6.2	-0-	-0-	NA	5.0x10 ³	1.0x10 ³	3424 ppb
4-W	water	6.3	-0-	2	NA	4.0x10 ³	3.6x10 ³	7600 ppb
1-S	soil	7.9	2.0	-0-	81	8.5x10 ⁶	9.9x10 ⁵	clean
2-S	soil	8.1	2.0	-0-	83	2.4x10 ⁶	9.0x10 ⁴	low
3-S	soil	8.1	2.0	-0-	85	1.6x10 ⁵	2.7x10 ⁴	medium
4-S	soil	8.2	2.0	-0-	84	4.3x10 ⁴	8.3x10 ⁴	high
Condensate	Condensate	5.9	NA	NA	NA	NA	NA	NA

* Selective plate counts performed using condensate

NA = not applicable

