

# 11<sup>th</sup> Quarterly Report (No-Cost Extension)

## Paraffin Control in Oil Wells Using Anaerobic Microorganisms

**Period Covered by the Report:** April 16, 2008 to July 15, 2008

**Date of Report:** August 21, 2008

**EPA Grant Number:** X83-2428-01

**Title:** Paraffin Control in Oil Wells Using Anaerobic Microorganisms

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**Institution:** The University of Oklahoma

**EPA Project Officer:** Bala Krishnan

**Project Period:** 10-16-07 to 10-15-08 (Year 3)

**Project Amount:** No-cost extension

**Research Category:** Petroleum Environmental Technology, Wellbore Cleanout

### Objective(s) of the Research Project:

Paraffins that form waxy deposits upon removal from reservoirs have been implicated in numerous oil field problems leading to reductions in oil recovery. In oil reservoirs, anaerobic conditions usually predominate. Thus the addition of anaerobic microbial populations that can definitively biodegrade paraffins under such conditions may be of great use to treat wax accumulations. For this project, our aim was to evaluate the feasibility of using anaerobic microbial consortia to biodegrade waxy hydrocarbons in order to treat paraffin accumulations in oil reservoirs.

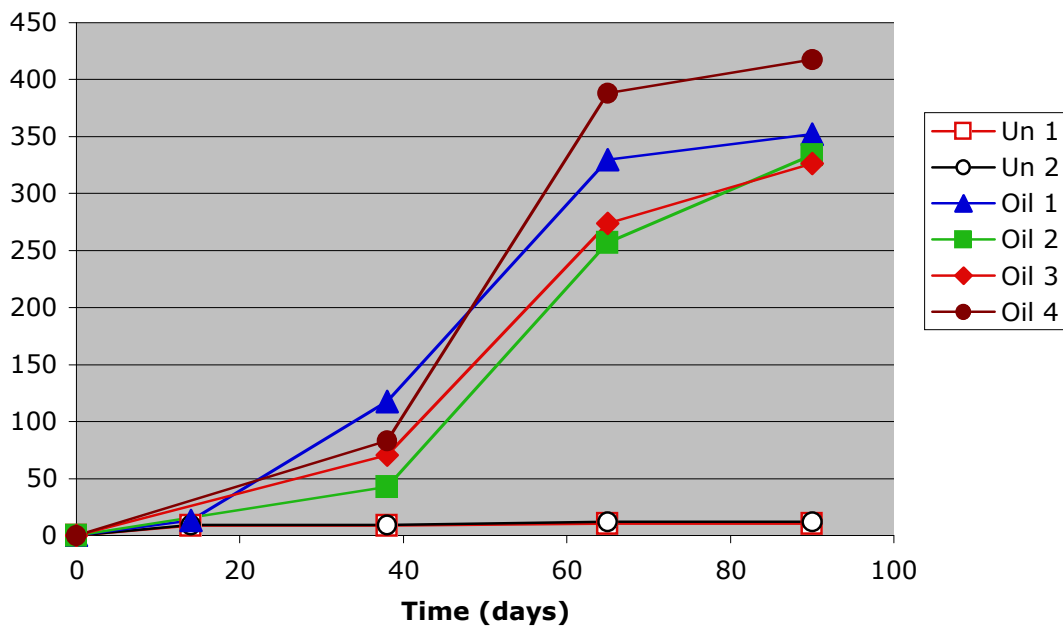
### Progress Summary/ Accomplishments:

In the previous report, we showed that anaerobic populations enriched from gas condensate-contaminated aquifer sediments on long-chain paraffins produced enhanced levels of methane and consumed enhanced levels of sulfate relative to controls. During this reporting period, we have established a high temperature GC method to analyze the remaining paraffin content in those cultures showing enhanced levels of methane and sulfate reduction. This analysis is currently underway and the data will be presented in the subsequent report.

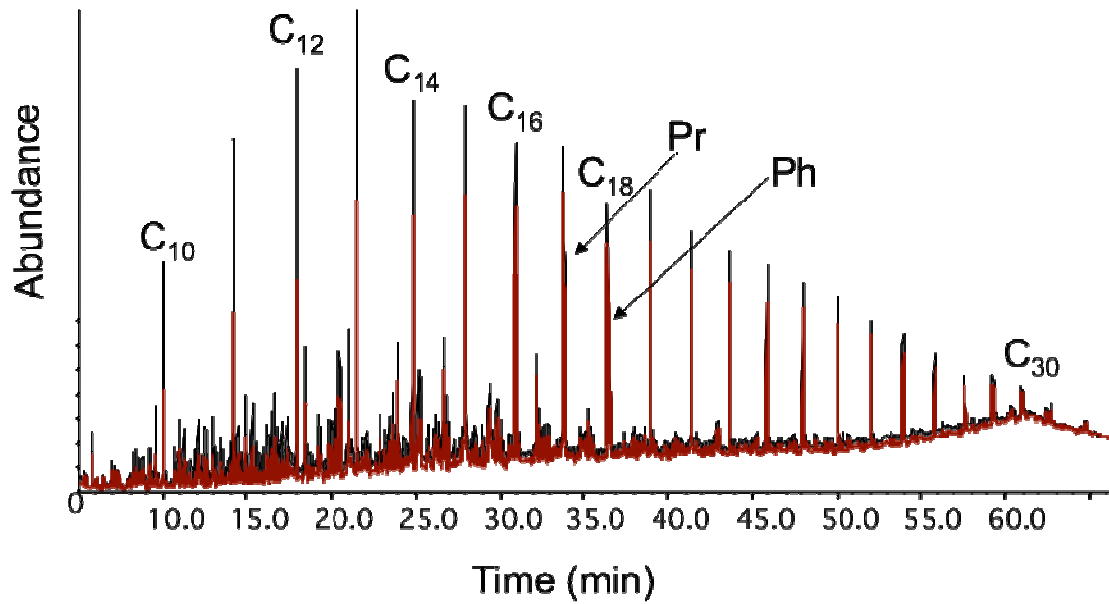
During the previous reporting period, we had transferred some of the promising cultures into fresh medium under conditions of methanogenesis (no added electron acceptor) and sulfate reduction (sodium sulfate supplied at ~ 10 mM). Transfers were amended with another aliquot of field paraffins and the experiment also included paraffin-free controls. After about 130 days of incubation, only a few of the cultures are showing enhanced levels of methane production relative to controls. Sulfate analysis in these cultures is underway. More incubation time is needed to further assess the activity of the transferred cultures.

We also previously described the establishment of sulfate-reducing and methanogenic enrichment cultures from two Alaska North Slope oilfield production water samples on a variety of different paraffinic substrates at 55°C. Methanogenesis

appeared to be the preferred electron-accepting process in the initial incubations for both of the production water samples. Upon transferring these cultures into fresh medium containing paraffinic crude oil, enhanced methanogenesis ensued in enrichments derived from one of the production water samples relative to substrate-unamended controls, shown in Figure 1. The oil fraction was examined from these methanogenic cultures for evidence of biodegradation by organic extraction and GC-MS analysis. Quantification of each individual straight-chained alkane was made by comparison of its peak area response relative to the branched-chained alkanes pristane and phytane. For this method of quantification, a decrease in the resulting peak area ratio is suggestive of biodegradation. In examining the gas chromatograms of the paraffinic oil in sterile controls versus the non-sterile incubations, it was evident that some of the hydrocarbon constituents were depleted (Figure 2). Table 1 shows that alkanes of the range C<sub>10</sub> to C<sub>16</sub> were indeed partly consumed in the live incubations, wherein the peak area ratios of these compounds relative to pristane and phytane decreased relative to those in the sterile controls. These results showed that compounds in paraffinic oil were biodegraded under methanogenic and thermophilic conditions. These promising cultures were further transferred and monitoring for continued activity is ongoing.



**Figure 1.** Methane production by a methanogenic consortium incubated with a paraffinic oil under thermophilic conditions. Closed symbols indicate methane production in oil-amended replicates and open symbols indicate that from oil-free controls.



**Figure 2.** Comparison of the total ion chromatograms of crude oil layers obtained from transferred Field B production water samples producing enhanced levels of methane relative to unamended incubations (shown in red) versus sterile oil-amended incubations (black). Pr = pristane, Ph= phytane.

**Table 1.** Alkane-to-pristane and alkane-to-phytane peak area ratios for oils incubated in the presence (inoculated) or absence (sterile) of a methanogenic population enriched from a hot oilfield production well on Alaska's North Slope. A decrease in these ratios in the sterile versus inoculated incubations is suggestive of biodegradation (as seen for alkanes ranging from C<sub>10</sub> to C<sub>16</sub>).

Alkane	Alkane/Pristane		Alkane/Phytane	
	Sterile	Inoculated	Sterile	Inoculated
<b>C<sub>10</sub></b>	<b>0.874</b>	<b>0.490</b>	<b>1.034</b>	<b>0.558</b>
<b>C<sub>11</sub></b>	<b>1.621</b>	<b>0.820</b>	<b>1.621</b>	<b>0.976</b>
<b>C<sub>12</sub></b>	<b>1.647</b>	<b>1.083</b>	<b>1.949</b>	<b>1.287</b>
<b>C<sub>13</sub></b>	<b>2.064</b>	<b>1.480</b>	<b>2.443</b>	<b>1.740</b>
<b>C<sub>14</sub></b>	<b>1.884</b>	<b>1.501</b>	<b>2.229</b>	<b>1.835</b>
<b>C<sub>15</sub></b>	<b>1.629</b>	<b>1.402</b>	<b>1.928</b>	<b>1.561</b>
<b>C<sub>16</sub></b>	<b>1.511</b>	<b>1.366</b>	<b>1.789</b>	<b>1.547</b>
C <sub>17</sub>	1.792	1.652	2.121	2.112
C <sub>18</sub>	1.226	1.180	1.451	1.380
C <sub>19</sub>	1.128	1.072	1.335	1.207
C <sub>20</sub>	1.058	1.057	1.252	1.197

**Publications/ Presentations:**

Gieg, L. M., Duncan, K. E., Suflita, J.M. **2006**. Anaerobic Paraffin Biodegradation. *In*: Abstracts of the 11<sup>th</sup> International Symposium on Microbial Ecology, Vienna, Austria, August 20 - 25 (poster presentation).

Gieg, L.M., Davidova, I.A., Duncan, K.E., Suflita, J.M. **2007**. Paraffin Control in Oil Wells Using Anaerobic Microorganisms. Oral presentation at the 14th IPEC Meeting, Houston, TX, November 6 – 9.

Gieg, L.M.; I.A. Davidova; J.M. Suflita. **2008**. Thermophilic methanogenesis and sulfate reduction by oilfield populations incubated with selected hydrocarbon substrates. 12<sup>th</sup> International Symposium on Microbial Ecology, Aug. 17-22, Cairns, Australia.

**Future activities:**

This project is continuing as a no-cost extension. Maintenance and monitoring of all enrichment cultures capable of utilizing paraffinic substrates are ongoing as is method development to assess alterations of paraffin composition and metabolites analysis.

**Supplemental Keywords:** paraffin treatment, anaerobe, biodegradation, oilfield reservoir

**Relevant Web Sites:** Not applicable at this time.