Annual Summary

Paraffin Control in Oil Wells Using Anaerobic Microorganisms

Period Covered by the Report: October 15, 2005 to October 14, 2006
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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: 10-15-06 to 10-14-07 (Year 2)
Project Amount: $149, 298
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. Our aim is to evaluate the feasibility of using anaerobic microbial consortia to biodegrade waxy hydrocarbons in order to ameliorate paraffin accumulations in oil reservoirs.

Progress Summary/ Accomplishments:

For this project, we have been cultivating microbial populations from a variety of sources for the potential to degrade and treat waxy paraffins under anaerobic conditions. Enrichment cultures derived from hydrocarbon-contaminated marine sediments in San Diego Bay have shown enhanced levels of sulfate reduction when C_{28}, C_{40}, or C_{50} is provided as the paraffinic substrate relative to substrate-free controls (Figure 1). These cultures continue to be transferred and cultivated for potential paraffin-treating activity. The paraffin-utilizing enrichments are also able to utilize alkanes as low as C_6 (hexane) based on sulfate reduction measurements. Molecular analyses were used to help identify the organisms in these enrichments responsible for anaerobic paraffin decay. Denaturing gradient gel electrophoresis (DGGE) analysis, cloning, and sequencing of the 16S rDNA (~1500 bp) amplified from the enrichments were used to assess the diversity and identity of the requisite bacteria. A comparison of the 16S rDNA clone library from the C_{28}, C_{40}, and C_{50}-degrading enrichment cultures revealed close affinity to several known hydrocarbon-degrading sulfate-reducing members of the delta proteobacteria (Figure 2).

Enrichments set up from other marine sediments under methanogenic conditions (e.g., no added electron acceptor) have also shown enhanced levels of methane production relative to controls. This effect was most dramatically observed when C_{28} was supplied as the sole paraffin source, but some enhanced methane levels were also

measured when C_{40}, C_{50}, or a commerically-available high molecular weight waxy mixture, Polywax (~ C_{30} to C_{100}, Polywax 655, Supelco) was supplied as the paraffin substrate. These enrichments also continue to be cultivated as potential paraffin-treatment cultures. Experiments are in the planning phases to incubate the cultures with fully deuterated C_{28} in order to help with the identification of metabolites that may be produced during anaerobic paraffin degradation.

We have also established incubations using anaerobic sediments from a freshwater, hydrocarbon-impacted aquifer to determine the ability of the microbial population to utilize Polywax under sulfate-reducing and methanogenic conditions. These sediments are already known to degrade alkanes in oils up to C_{34} in length under anaerobic conditions (*Townsend et al., 2003, Environ. Sci. Technol. 37: 5213-5218*). Further, we established parallel incubations using a sediment-free, oil-degrading methanogenic population enriched from the same site as an inoculum. Incubations were amended with ANS oil (from Alaska's North Slope), Polywax, or a mixture of ANS oil and Polywax. Over the course of approximately 7 months, we observed enhanced levels sulfate reduction over substrate-free controls when the enrichments were established in the presence of all three substrates. This was observed for incubations containing sediments as well as for those containing the sediment-free oil-degrading inoculum. When the cultures were re-amended with sulfate, sulfate reduction continued relative to the substrate-free controls, attesting to the ability of the enrichments to use a wide range of paraffins under sulfate-reducing conditions. In contrast, no enhanced levels of methane production from the added Polywax were observed in the methanogenic incubations.

Publications/ Presentations:

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

Future activities:

Enrichment and monitoring of the above-described cultures for the ability to degrade waxy paraffins under anaerobic conditions will continue. Experiments will be conducted to determine the nutritional requirements of some of the enrichment cultures to improve growth and deduce salinity tolerance. In the next phase of the project, enrichments will be challenged with field paraffins and paraffinic oils. Some cultures will also be established under more thermophilic conditions using field samples such as oil production waters. The concentration of paraffins will be assessed in addition to measures of electron-accepting processes.

Supplemental Keywords: paraffin treatment, anaerobe, biodegradation, oil field reservoir

Relevant Web Sites: Not applicable at this time.

Figure 1

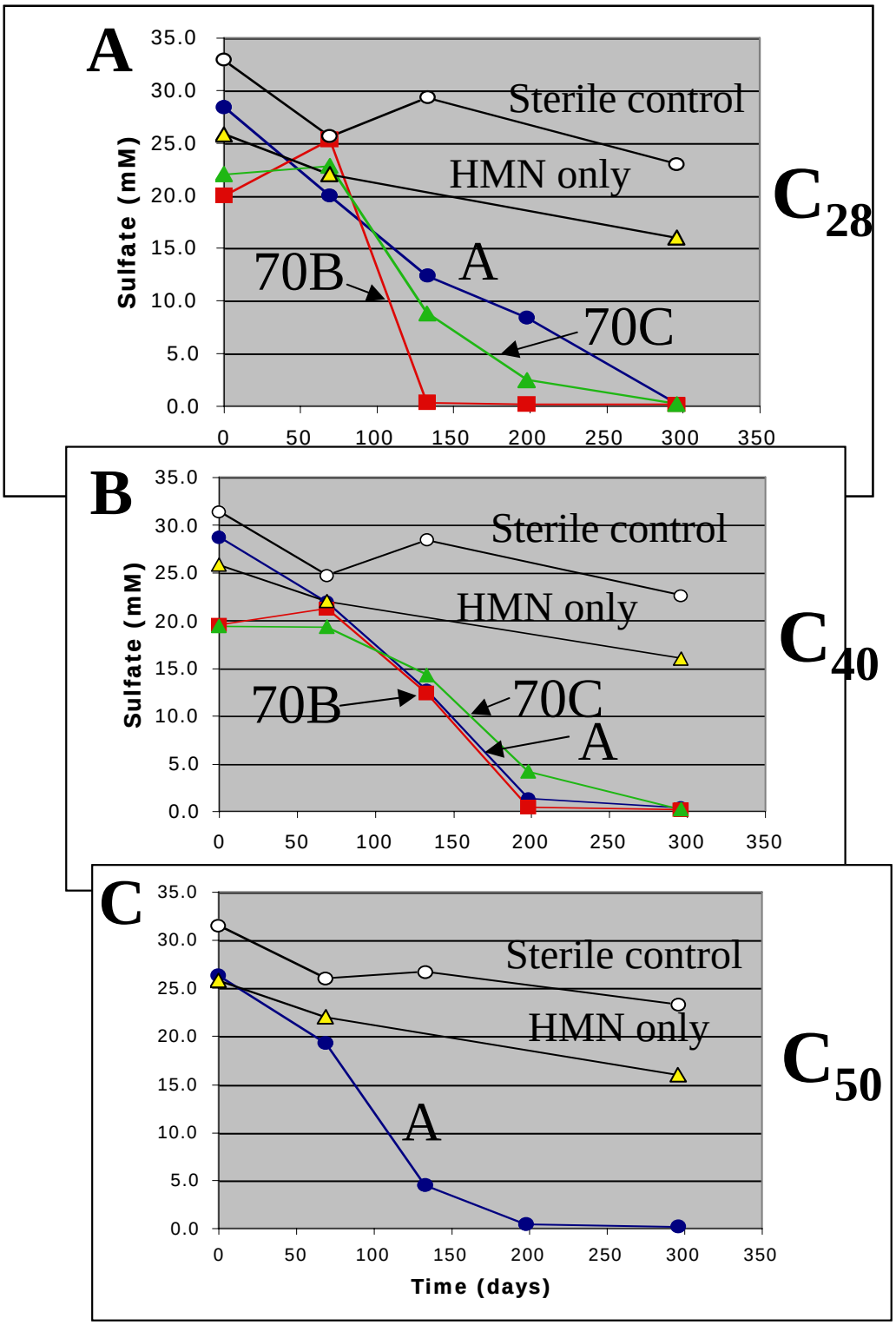
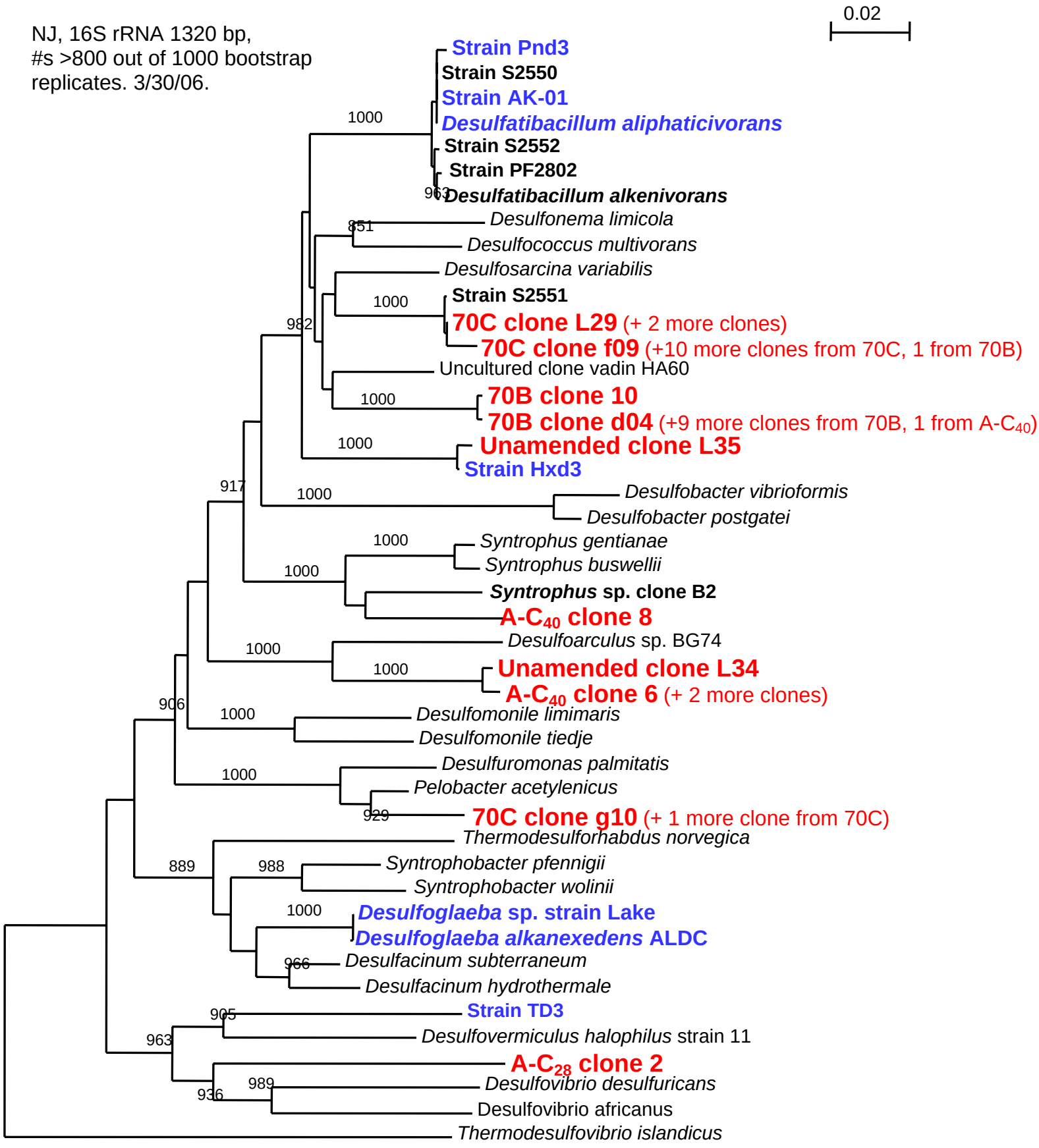

A
B
C
Sterile control
HMN only
70B
A
70C
C_{28}
C_{40}
C_{50}
Sulfate (mM)
Time (days)
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0
50
100
150
200
250
300
350

Figure 2


NJ, 16S rRNA 1320 bp,
#s >800 out of 1000 bootstrap
replicates. 3/30/06.
0.02
Strain Pnd3
Strain S2550
Strain AK-01
Desulfatibacillum aliphaticivorans
Strain S2552
Strain PF2802
Desulfatibacillum alkenivorans
Desulfonema limicola
Desulfococcus multivorans
Desulfosarcina variabilis
Strain S2551
70C clone L29 (+ 2 more clones)
70C clone f09 (+10 more clones from 70C, 1 from 70B)
Uncultured clone vadin HA60
70B clone 10
70B clone d04 (+9 more clones from 70B, 1 from A-C40)
Unamended clone L35
Strain Hxd3
Desulfobacter vibrioformis
Desulfobacter postgatei
Syntrophus gentianae
Syntrophus buswellii
Syntrophus sp. clone B2
A-C40 clone 8
Desulfoarculus sp. BG74
Unamended clone L34
A-C40 clone 6 (+ 2 more clones)
Desulfomonile limimaris
Desulfomonile tiedje
Desulfuromonas palmitatis
Pelobacter acetylenicus
70C clone g10 (+ 1 more clone from 70C)
Thermodesulforhabdus norvegica
Syntrophobacter pfennigii
Syntrophobacter wolinii
Desulfoglaeba sp. strain Lake
Desulfoglaeba alkanexedens ALDC
Desulfacinum subterraneum
Desulfacinum hydrothermale
Strain TD3
Desulfovermiculus halophilus strain 11
A-C28 clone 2
Desulfovibrio desulfuricans
Desulfovibrio africanus
Thermodesulfovibrio islandicus
1000
963
851
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