

# Development of an In Situ Biosurfactant Production Technology for Enhanced Oil Recovery

DE-FC26-04NT15522

## Goal

The ultimate goal of the project is to move biosurfactant-effected oil recovery from laboratory investigations to field applications. The project will determine 1) the prevalence of biosurfactant producers in oil reservoirs, 2) the efficacy of using nutritional supplements to stimulate biosurfactant production in situ, 3) the relationship between biosurfactant production and biofilm formation, and 4) the efficacy of the nutrient and flow regimes in mobilizing entrapped oil. Researchers will work closely with several independent oil producers to demonstrate this technology in the field.

## Performers

University of Oklahoma  
Norman, OK

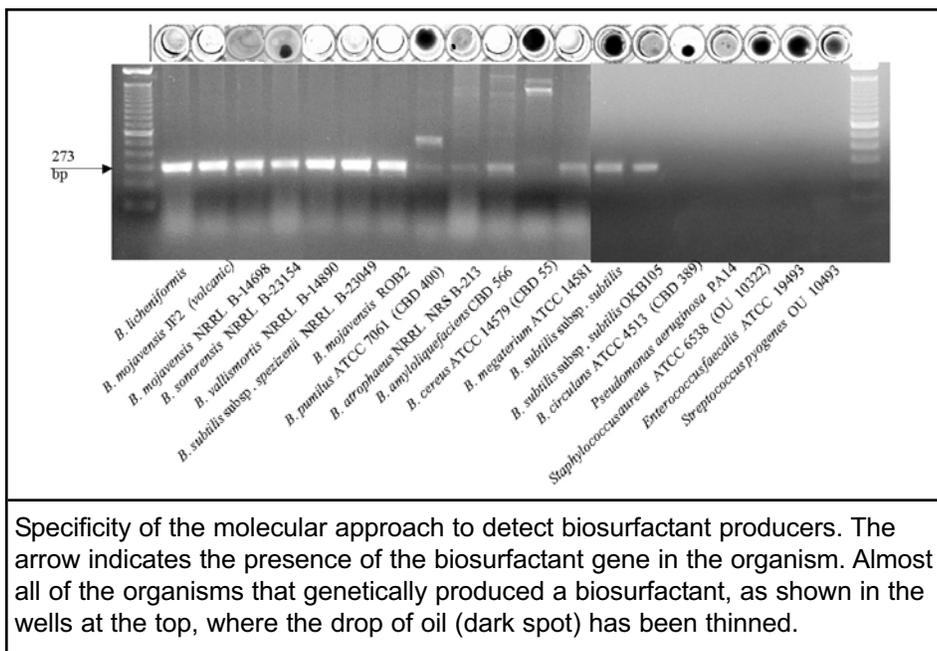
Arrow Holding, Inc.  
Norman, OK

## Results

The ultimate goal of the project is to move biosurfactant-mediated oil recovery from laboratory investigations to actual field applications. The project has developed cultivation-dependent and cultivation-independent approaches to detect and enumerate microorganisms that produce biosurfactants in oil field brines. Seven different oil formations were surveyed, ranging in salinities from about 2 percent to 15 percent. Indigenous biosurfactant producers were in very low numbers in samples from these reservoirs. The low numbers of biosurfactant producers in oilfield brines suggest that inoculation of wells will be needed. A very effective biosurfactant producer was obtained for use as an inoculum, and a nutrient package was developed that triggers its growth and results in biosurfactant production in brine samples with salinities up to 11 percent. This microorganism and the nutrient package were tested in the field and shown to produce large amounts of the biosurfactant. A second field test showed an increase in oil production.

## Benefits

The long-term economic potential for



Specificity of the molecular approach to detect biosurfactant producers. The arrow indicates the presence of the biosurfactant gene in the organism. Almost all of the organisms that genetically produced a biosurfactant, as shown in the wells at the top, where the drop of oil (dark spot) has been thinned.

enhanced oil recovery (EOR) is large, with more than 300 billion barrels of oil remaining in domestic reservoirs after conventional technologies reach their economic limit. Actual EOR production in the United States has never been very large—less than 10 percent of total domestic production—even though economic incentives have been used to stimulate the development and application of EOR processes. The U.S. DOE Reservoir Database contains more than 600 reservoirs with over 12 billion barrels of currently unrecoverable oil that are potential targets for microbially enhanced oil recovery (MEOR). If MEOR could be applied successfully to reduce the residual oil saturation by 10 percent in a quarter of these reservoirs, more than 300 million barrels of oil could be added to U.S. oil reserves. This would stimulate oil production from domestic reservoirs and reduce our Nation's dependence on foreign imports.

## Background

Additional information is needed to move biosurfactant-effected oil recovery from laboratory-based studies to actual field applications. First, it is necessary to know if oilfields contain biosurfactant-producing microorganisms or whether these cells must be injected. Second, it is important to know which nutrients to inject and how to inject them (continuous versus intermittent; high or low concentration) to stimulate and maintain in-situ biosurfactant production.

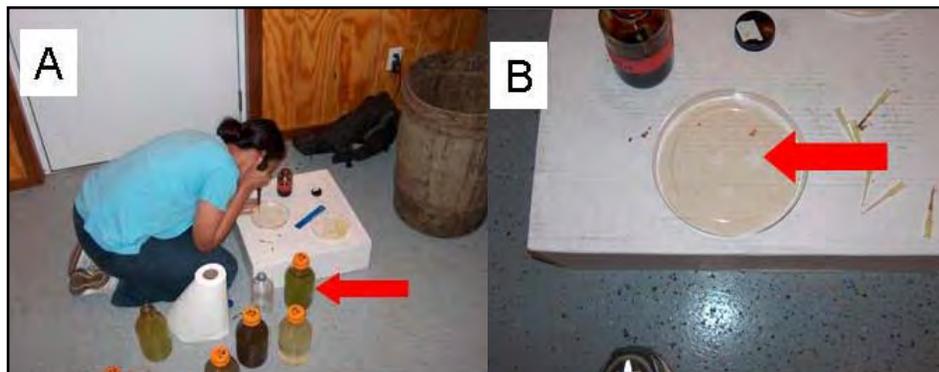
## Summary

Tools were developed to detect and quantify biosurfactant-producing microorganisms in produced fluids from oil reservoirs. First, the most probable number (MPN) enumeration approach was modified by using a growth medium designed to support biofilm formation and by adding a small drop of oil to the culture once it has grown to detect biosurfactant production. Second, a cultivation-independent approach was developed to detect biosurfactant-producing microorganisms by detecting the presence of the genes involved in the synthesis of lipopeptide biosurfactants. Other cultivation-independent approaches were used to detect microorganisms that are members of the genus *Bacillus* because this genus contains most of the microorganisms known to produce lipopeptide biosurfactants, in particular the detection of the *gyrA* gene and the 16S rRNA gene of *Bacillus* species. The cultivation-dependent (MPN) method did not detect biosurfactant producers in any of seven oil formations that were sampled. However, low numbers of biosurfactant producers were detected by enrichment cultivation approaches in all formations. The genes for biosurfactant production were detected in five of the seven reservoirs sampled.

The project systematically tested a number of nutrient components to determine the formulation that best stimulated biosurfactant production in brine samples from different oil reservoirs. The approach was to add the nutrients directly to the brine to

mimic actual field conditions as closely as possible because the brine contains the indigenous microbial populations that may compete for these nutrients. Since the brine did not contain any indigenous biosurfactant-producing microorganisms, the efficacy of bioaugmentation was tested where non-indigenous biosurfactant-producing microorganisms were added along with the nutrients. The inoculum was a *Bacillus licheniformis* strain. The nutrient formulation that best stimulated biosurfactant activity consisted of nitrate and either glucose or molasses. This nutrient formation supported growth of the inoculum strain even in the presence of indigenous oilfield microorganisms in volumes sufficient for use as inocula for oil reservoirs (~75,000 liters).

The inoculum preparation technology that was developed requires microbiological skill but does not rely on expensive or specialized equipment and provides a low-cost solution to supply the large volumes of microbial cultures needed for the inoculation of oil reservoirs. One of the major technical questions concerning the efficacy of MEOR is whether the injected bacterium will produce sufficient amounts of the biosurfactant. It was shown in a controlled field trial that large amounts of biosurfactants (about 90 mg per liter) were made in the reservoir. Recently, a second field trial showed that additional oil was recovered and that the microbial process is economic. The results are significant because they show that inoculation of oil reservoirs with



Noha Youssef conducting an oil-spreading assay for biosurfactant activity. Arrow highlights the green color of the tracer used to show when the treatment package containing cells and nutrients was recovered from the formation. B: Closer view of the oil-spreading assay. The arrow shows clear zones in a film of oil. This is due to the presence of the biosurfactant. Samples from control wells were negative or showed much smaller zones of clearing. Biosurfactant production was verified by high-pressure liquid chromatography.

bacteria is technically and economically feasible.

#### Current Status (January 2007)

The project is in its third year. A number of techniques were developed, both cultivation-dependent and cultivation-independent, to detect biosurfactant producers, sampled a number of different oil formations, and successfully stimulated the growth of biosurfactant producers in oilfield brines. The major task of the project, which was to determine the prevalence of biosurfactant

producers in oil formations within the first two years of the project, was completed.

#### Funding

The goal of the Oil Exploration and Production Program Solicitation DE-PS26-04NT15450-0 is to expand the knowledge base to bring efficient, economically competitive, and environmentally acceptable new fossil resources and technology options into the marketplace and improve U.S. national security by reducing dependence on imported oil.

### **Publications**

Maudgalya, R. Knapp, M., and McInerney, M.J. "Microbial enhanced oil recovery: A review of past, present, and future," 2007 SPE Production and Operations Symposium, Oklahoma City, OK, March 31-April 3, 2007, SPE 106978.

N. Youssef, Simpson, D.R., Duncan, K.E., McInerney, M.J., Folmsbee, M., Fincher, T., and Knapp, R.M., "In-situ biosurfactant production by injected *Bacillus* strains in a limestone petroleum reservoir," Applied Environmental Microbiology, March issue.

McInerney, M.J., Jenneman, G.E, Voordouw, G., and Sublette, K.L., Oil Field Microbiology, in Hurst, C.J., Crawford, R.L., Garland, J.I., Mills, A.L., Schmidt, S.K., and Stezenbach, L.D. (ed.), Manual of Environmental Microbiology, American Society for Microbiology, Washington, D.C., in press, 2006.

Folmsbee, M., Duncan, K.E., Han, S-O., Nagle, D.P., Jennings, E., and McInerney, M.J., "Re-identification of *Bacillus licheniformis* strain JF-2 as *Bacillus mojavensis* strain JF-2," Systematic and Applied Microbiology, in press, 2006.

Youssef, N.H., Duncan, K.E., and McInerney, M.J., "Importance of the 3-hydroxy fatty acid composition of lipopeptides for biosurfactant activity," Applied and Environmental Microbiology, Vol. 71, pp. 7690-7695, 2005.

McInerney, M.J., Nagle, D.P., and Knapp, R.M., "Microbially enhanced oil recovery: past, present, and future, pp. 215-237, in Magot, M., and Ollivier, B., Petroleum Microbiology, American Society for Microbiology Press, Washington, D.C., 2005.

Maudgalya, S., McInerney, M.J., Knapp, R.M., Nagle, D.P., and Folmsbee, M.M., "Tertiary oil recovery with microbial biosurfactant treatment of low-permeability Berea sandstone cores," SPE 94213, 2005 SPE Production and Operations Symposium, Oklahoma City, OK, April 17-19, 2005.

Folmsbee, M., McInerney, M.J., and Nagle, D.P., "Anaerobic growth of *Bacillus mojavensis* JF-2 and three other *Bacillus* strains requires deoxyribonucleotides or DNA," Applied and Environmental Microbiology, Vol. 70, pp. 5252-5257, 2004.

Maudgalya, S., McInerney, M.J., Knapp, R.M., Nagle, D.P., and Folmsbee, M.M., "Development of bio-surfactant based microbial enhanced oil recovery procedure," SPE 89473, SPE/DOE Fourteenth Symposium on Improved Oil Recovery, Tulsa, OK, April, 17-21, 2004.

**Project Start:** October 1, 2004

**Project End:** September 30, 2007

**Anticipated DOE Contribution:** \$675,588

**Performer Contribution:** \$180,237 (21 percent of total)

### **Contact Information**

NETL – Virginia Weyland (virginia.weyland@netl.doe.gov or 918-699-2041)

U. of Oklahoma – Michael J. McInerney (mcinerney@ou.edu or 405-325-6050)