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Microbial EOR -- Critical Aspects Learned From The Lab

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Abstract

DuPont and BP have been working together to develop Microbial EOR targeted at viscous oil in the Schrader Bluff formation on the North Slope of Alaska. The goal of this program was a 5% increase in the recovery factor. Mechanisms to be assessed in the original agreement included

1. Viscosity reduction of the oil by transformation or degradation of heavy components in the oil – thus improving the oil - water mobility ratio.
2. Drastic reduction (to $\sim <0.01$ dynes/cm) in the interfacial tension between water and the oil

After extensive fundamental research we have learned many critical aspects of microbial EOR that made the application of these two mechanisms to the Schrader Bluff formation impractical. Instead, we have demonstrated two site appropriate mechanisms that achieved, in the lab, the targeted increase in the recovery factor.

1. Improved flow conformance and increased sweep efficiency by preferential plugging of high permeable zones thereby forcing water to produce oil from previously unswept parts of the reservoir.
2. Reduced oil / rock surface tension and a subsequent reduction in the oil “wetting” the rock. This results in changes in the relative permeability of the oil and the water and ultimately lower residual oil saturation.

This paper describes the key laboratory tests used to evaluate these four mechanisms. The cornerstones of our work have been the detailed characterization

of the waters, the oil, the formation matrix and the microbial community. In addition we describe our search for useful microbes isolated from a variety of environmental samples collected from the Milne Point Unit (MPU) of the Alaskan North Slope. These samples were taken over several years and included injection, production and power fluid waters. These samples were used to understand the temporal changes in the microbial populations and to provide inoculum for our enrichment cultures.

Our ongoing research has provided many insights into the appropriate application of microbial EOR. The unique aspects of each production area, the nature of the oil, the water, the formation matrix, and the background microbial population and their complex interactions must all be assessed when considering the potential application of microbial EOR. The amount of work described below for assessing potential MEOR mechanisms is extensive. However, this process has been streamlined and we have been able to assess new target reservoirs for potential MEOR treatments in about 6 months.

Introduction

Much work has been done to understand the application of MEOR in an oil reservoir setting (Bryant, 2000). Treatments include both both huff n puff and interwell water flooding applications (Nagase, 2001). Our focus has been the application of MEOR as an interwell treatment. In this application, the reservoir is inoculated with a selected strain and fed with an optimized set of nutrients in a manner that accomplishes the desired effect deep in the reservoir. A potential target reservoir must meet a set of criteria. The criteria we have developed are listed in Table 1. The fields must be under water flood. Water flooding is the means to transport the microbes and nutrients into the reservoir. To support microbial growth, an efficient electron acceptor is necessary. In the anaerobic environment of a reservoir, oxygen is not considered a viable option due to corrosion issues and limited carrying capacity in the injected water. Addition of sulfate could encourage oil well souring by sulfate reducing

organisms. Therefore, we have restricted ourselves to a limited set of electron acceptors, concentrating mostly on nitrate. The pore throat diameter of the rock (Table 1) must be high enough to allow passage of microbes (Nelson, 2009). The next three criteria in Table 1, temperature, salinity and pH, are key to determining the type and diversity of life seen in the reservoir. In most cases it is the combination of the temperature and salinity that are the determining factors since pH is often buffered to a neutral value due to natural minerals in the clays or cements of the rock. Figure 1 illustrates that the combination of a higher salt concentration and temperature than listed in Table 1 can make a challenging environment for microbial growth.

Very high oil viscosity, (Table 1), makes it difficult to evaluate fluids from a reservoir in sandpack or microbiological experiments.

Down hole pressure is also a consideration. At extreme pressures only pressure tolerate microbes (piezophiles) will operate efficiently.

Table 1 – Criteria for MEOR applications.

Production mode	Secondary – fields under waterflood
Metabolic Pathway	Anaerobic with restricted electron acceptor
Permeability	>50 to 100 milliDarcy
Reservoir Temperature	< 60 – 70°C (<140 – 160°F)
Salinity (injected and produced waters)	~<6% Total dissolved solids (TDS)
pH	5 – 9 (6 – 8 ideal)
Oil viscosity	<500 cp (preferred)
Down hole pressure	<3000 psi (preferred)

It is important to have a holistic understanding of the entire system that may impact the efficacy of the treatment. As illustrated in the following sections, our preferred approach is to use microbes or a consortium of microbes either isolated from the candidate well or from comparable well environments.

Viscosity reduction mechanism

Reducing the crude oil viscosity in situ for heavy oils can have a great impact on both the quality and the recoverability of heavy crudes – not only due to lower oil – water mobility ratio but also due to less viscous fingering (Kumar, 2005). This can result in watered out channels in the reservoir. High molecular weight paraffinic oils have been shown to exhibit a significant viscosity drop (Strappa, 2004 and Maure, 2005) due to MEOR treatments. In Maure et al (Maure, 2005), data indicates that biodegradation of high molecular weight paraffins leads to this viscosity

reduction. In contrast, the candidate wells we have examined in the Milne Point Field of the Alaskan North Slope (Thrasher, 2010) are not paraffinic. Our analyses including 2D NMR analysis (Figure 2) (Bax, 1986; Garbow, 1982; Martin, 1979; Mueller, 1979) and SARA analysis (ASTM D4124-01) supports this conclusion. Consequently, our viscosity reduction research focused on the selective modification of polyaromatic hydrocarbons in this candidate crude oil and not on selective degradation of long chain paraffins – since there were essentially none in this oil.

Our protocol for characterizing and selecting microbes is illustrated in Figure 3. Our preferred approach is to use microbes or consortia of microbes either isolated from the candidate well or from our MEOR culture collection. To start this process authentic injected water and produced fluid samples are collected from the candidate wells. DNA is harvested and the microbial population typed using standard molecular biology methods (Pham, 2008 and Pham in preparation). This provides a snap shot of the population of organisms in the well at that point in time. The inferred syntrophic microbial physiologies present in these well systems have been reviewed (Pham, 2008 and Pham in preparation). Using the known water chemistry and the microbe population characterization, we can infer the dominant metabolic activities occurring in the reservoir.

Produced fluids and injected water samples are maintained on wet ice after recovery in the field. A series of enrichments are started upon return to the lab. Anaerobic serum vials are used in the simplest type of enrichment (Figure 4). A variety of different nutrients are used in these enrichments in order to select for specific microbes. Many of these enrichments need to be started in order to test a wide variety of nutrient combinations with different environmental samples. Enrichment methods can be very powerful revealing rare strains that are not evident in DNA-based population studies. Typically, thousands of colonies are obtained in this manner (Figure 5). 16S rDNA Phylogenetic typing is used to discriminate between identical strains. Hundreds of unique strains are identified in this manner. These unique strains are preserved at -80°C and become part of our MEOR library. These unique strains then go through a series of “challenges” or selection for activity and function. These selection tests include growth studies, simple functional screens and complicated functional screens. For viscosity reduction of the oil it was necessary to measure the degradation of the oil and the change of the oil viscosity in the enrichments using the candidate organisms and a range of nutrient packages. Simple bottle enrichments were first used to screen for

organisms that could degrade aromatic molecules that represented the crude oil. A special ‘in-culture’ crude oil viscosity measurement device was developed (Groski, patents applied for) to measure the change in oil viscosity due to microbial action. A schematic and picture of this apparatus is shown in Figures 6 and 7 respectively. It consisted of multiple wells each containing the nutrient laden water and the crude oil. Agitation was done to the oil layer in such a way that the apparent viscosity could be determined independently for each well. The viscosity was calibrated using standard fluids. The entire apparatus was housed in an inert chamber that assured anaerobic operation (no oxygen present in the wells). This apparatus eliminated the need to sub sample oil out of the cultures for viscosity measurements. This reduced the volume and number of cultures in the screen. A selection of microbes and nutrient packages (based on nitrate reduction) was operated for over two months. In these cultures, we observed either no change in viscosity or an increase in viscosity due to the loss of light components.

In parallel with this (unsuccessful) effort to find a microbe that could decrease viscosity by selective degradation of high molecular weight components, we pursued the possibility of reducing viscosity by reducing the aromaticity of the compounds (Figure 8). We were successful in microbially reducing model aromatic compounds using hydrogen or carbon monoxide in enrichment cultures (Fallon, 2009). We further demonstrated that hydrogenation of crude oil leads to a viscosity reduction. We believe that the reduction or addition of hydrogen to the aromatic structures caused the rings to “pucker” and disrupted π/π stacking of the aromatic rings. This type of molecular stacking is often seen to be responsible for high viscosity in aromatic compounds.

Any viscosity reduction mechanism is subject to rate limitations. A significant amount of oil has to be degraded to get a measurable amount of viscosity reduction. Although no universal viscosity correlation for crude oil exists, our estimates based on recombining SARA fractions of various distillation cuts indicate that 5 to 10% of the oil must be degraded or transformed. This is a substantial amount and presents a challenge for achieving this transformation on an economical time scale. Fortunately there are estimates for rates for hydrocarbon transformation or degradation that can be gleaned from the literature (Head, 2003). Our estimates of the required economic rates are $\sim 10^2$ - 10^5 g/m²/yr. This compares favorably with rates of degradation using air, for example, aerobic fermentation of octane by *Pseudomonas oleovorans* ($\sim 3 \times 10^7$ g/m²/yr) [this requires that there be no mass transfer limitations for delivery of oxygen to the

microbes]. But does not compare favorably for in situ rate constants measured in oil reservoirs or estimates of fluxes at the base of oil columns ($\sim 10^{-6}$ to 10^{-1} g/m²/yr). Considering the amount of oil to be transformed and this disparity between the required rates and known rates, we believe mass transfer limitations make viscosity reduction not feasible to economically implement for MEOR.

Reduction of interfacial tension between oil and water

In conjunction with our attempts at developing a viscosity reduction mechanism, we also saw evidence of microbial effects on the interfacial tension between oil and water. Some of our enrichments using oil showed evidence of emulsification. Spontaneous emulsification of oil into water can lead to improved oil recovery especially (Banat, 1995 and Maudgalya, 2004) if that oil is heavy. We subsequently developed simple functional screens for a drop in interfacial tension between oil and water. With optimized nutrients, a selection of microbes from our screens altered IFT between crude oil and water (Figure 9). Microbe A (Figure 9) was known not to generate any biosurfactant and was consequently used as a negative control. The best organisms, E and F, dropped the IFT to about 15 dynes/cm. Although this is encouraging, it is not low enough to cause spontaneous emulsification. However, this may be good enough to help mobilize oil and increase oil recovery in some cases. Work in parallel with this (Fallon, 2009 and Fallon, 2009) did show promise for improving hydrocarbon – water emulsification – (bottom of Figure 9). Although IFT reduction did show some promise, we elected to develop other mechanisms for possible field tests. This is described in the next two sections.

Change in oil wetting of the matrix.

Through our investigation into modifying the interfacial tension between oil and water, we also investigated the possible changes of the surface tension between the oil and matrix sand. In order to pursue this effect, the experimental flow down (Figure 3) was adapted by using different functional screens to find organisms and nutrients that could lead to changes in the surface tension between oil and the matrix sand. Several different small scale screens were developed (described by Keeler 2009 and Keeler 2009) and oil recovery tests were done on sand packs as described below.

We discovered microbial strains that could drastically increase the contact angle of oil wetting an authentic sand grain (Figures 10 and 11) without having any measurable effect on the oil / water IFT (Keeler, 2009, and other application not yet

published). When suspended in brine matching the major salts found in the injection water, aged, oil-wetted sand shows complete coverage of the grains and a low oil-sand contact angle (Figure 10). Following exposure to our selected microbes, another sand grain (from the same source of sand and oil) shows a drastically increased oil-sand contact angle (Figure 11). More importantly, the sand grain appears to be virtually cleaned of the oil.

This microbial induced change in oil wetting of sand was tested for its effect on oil recovery in sand packs (Figures 12 and 13). Constrained sand packs were constructed using cleaned and dried produced sand collected at the well site. The permeability of the packs were about 4 Darcys. These sand packs were flooded with synthetic injection brine solution made to replicate the water chemistry of the actual injection brines used in the field. Stock tank oil was flooded onto the pack until the residual water saturation was reached. The oil was aged for 2 weeks. Synthetic injection brine was used to de-oil the pack (first control flood) while the water saturation and pressure drop were measured in and across the pack. The pack was re-oiled and de-oiled a second time while water saturation and pressure drop were again measured. These two control de-oiling curves were virtually identical (Figure 12). The sand pack was re-oiled for a third time. The sand pack was inoculated with the same microbes used in Figure 11 and shut in. After the shut in, flooding with the synthetic injection brine was resumed and again the water saturation of the pack and the pressure drop were measured. This de-oiling curve following the microbial process is shifted above the control floods giving more than 5% extra oil as a result of the treatment. The relative permeability curves were estimated by history matching the water saturation data and the pressure drop data (Figure 13). The relative permeability curves appear to show a decrease in the residual oil saturation consistent with the increased contact angle of the oil we observed on the sand. We believe the field application of this mechanism may be well suited to a huff n puff treatments as well as interwell treatments.

Permeability modification

Improved flow conformance by permeability modification is the fourth mechanism we investigated. As with the other mechanisms, the experimental flow down (Figure 3) was followed, but with different functional tests or screens used to test for microbial plugging as the means to achieve improved flow conformance (Figures 14 and 15).

The pressure drop data for hydraulically constrained slim tubes show a significant drop in permeability following MEOR treatments (Figure 14). These slim

tubes were packed with cleaned produced sand obtained from the well site. Permeabilities were about 4 to 6 Darcys. The slim tubes were flooded with synthetic brine, then flooded with stock tank oil and allowed to age with oil in contact with the sand. After 3 weeks of aging, they were flooded with synthetic brine, and then inoculated with a type of microbe that showed a great ability for plugging (Keeler, 2009). Two treatments were used with this microbe. In treatment 1, the microbe quickly formed plugs, but the plug was not durable. This is especially evident after day 40 (Figure 14). For treatment 2, a more durable plug formed (Figure 14). In both cases, the apparent permeability of this slim tube decreased by nearly an order of magnitude. As will be discussed below, the amount of nutrients required to see this effect is within an economic viable range (depending on the permeability variation of the formation).

A composite (hydraulically constrained) slim tube test shows the effectiveness of the MEOR process for permeability alteration that results in enhanced oil recovery (Figure 15). Two slim tubes were connected in parallel. One had a permeability about 10X higher than the other. Before they were connected in parallel, the slim tubes were prepared in the same manner as discussed above. Consequently both slim tubes were at residual water saturation at the start of the test. The oil used in this test was more viscous than the water resulting in a oil/water mobility ratio greater than 1. We measured pressure drop across the composite slim tube and the amount of produced oil at the exit of both slim tubes (Figure 15). The slim tubes were connected in parallel once they were at residual water saturation. Synthetic injection brine was then pumped into the parallel connected slim tubes. As expected, virtually all the brine flowed through the more permeable slim tube. This slim tube reached residual oil saturation quickly and became watered out. This was the condition of the slim tubes at day 0 in Figure 15. The pressure drop across the composite slim tube was about 1 psi while a little over 35% of the total oil in both slim tubes had been recovered. Virtually all of the produced oil was from the higher permeable slim tube. Inoculation and nutrient feed was started before day 5. The inoculation and nutrients were not directed specifically at either slim tube but were allowed to go to with the natural flow. By day 15, plugging had started as evidenced by the climb of the pressure drop across the composite slim tubes. Commensurate with this increased pressure drop, there was a significant increase in the amount of oil recovered. The pressure drop was erratic (in part because of the high oil water mobility ratio – as the lower permeability slim tube produced oil, its resistance to flow dropped). Despite the erratic pressure drop, additional oil continued to be

produced from the less permeable slim tube (Figure 15).

We believe that improved sweep efficiency by flow conformance is best implemented using interwell treatments, although with the proper protocols, huff n puff treatments could benefit as well.

Considerations for field implementaions

There are a host of ancillary issues with implementing MEOR in the field (Thrasher, 2010). Oil well souring, microbially induced corrosion and the safety and environmental impact of the micro-organism and the nutrient treatments are all important. Other considerations are the ability of the selected microbe to compete against native strains for the nutrients used. Table 2 illustrates the importance of using a nutrient package to sustain an inoculated organism against the native organisms. Enrichments were done using live waters from the well site. For each enrichment a desirable microbe (microbe #1) was inoculated and then fed with a set of nutrients. In two cases, (#1 and #4) the preferred microbe does not compete well – the population is still dominated by the native strains. The native strains are nearly as good at using the nutrients as the preferred microbe. For treatments #3 and especially #2, the preferred strain dramatically out competes the native strain for the nutrients.

Table 2: Effects of optimizing nutrients on the competitiveness of a desired strain compared to the background population. Four different nutrient systems are shown. Each nutrient system is a row in the table. Counts for the preferred microbe and the back ground microbes are show in the two middle columns. On the right side of this table is the ratio of these counts. This ratio needs to be much greater than 1 for the preferred microbe to effectively compete against the native strains.

Nutrient package	Colony counts Strain #1 preferred	Colony counts back-ground	Ratio of counts
#1	2.2 MM	2.3 MM	~1
#2	12.6MM	0.2 MM	63
#3	6.8 MM	0.5 MM	~14
#4	0.68 MM	0.88 MM	~0.8

Efficacy or the economics of the treatment is an obvious important consideration. The results of a simple economic model for demonstrating the effectiveness of improved flow conformance by bio plugging is shown in Figure 16. This model is a variable resistor flow model based on the Stiles type of model (Gray, 2008). The model takes into account only vertical sweep efficiencies (no arial efficiencies

are considered in the analysis). A key parameter needed in the model is the amount of permeability change for a given amount of nutrient consumed. This data was obtained from experiments like those described in Figures 14 and 15. The permeability variation, V , is plotted on the X axis. The predicted amount of oil produced for the amount of nutrient used is plotted on the Y axis. An estimate for the minimum economic limit for the treatment is shown as the shaded bar across this plot. Although we do not believe that this plot is accurate in an absolute sense, we believe the trends are correct. The model was run for a variety of reservoirs – each reservoir having a different permeability variation. For each reservoir with a given perm variation, a series of simulations were performed (Figure 16). Each simulation had a different amount of nutrients fed into the reservoir to create the plugging and the flow conformance. All simulations used an oil / water mobility ratio comparable to that used in the experiments shown in Figures 14 and 15 ($>>1$). The amount of additional oil recovered was calculated for each value of the perm variation and for the amount of nutrients used. All these simulation are shown as performance curves in Figure 16. All performance curves show that at low permeability variation there is no economic gain to doing the plugging treatment. In order for the treatment to be effective, high permeable streaks must be present in the formation that need to be plugged. The lowest curve used the least amount of nutrients (labeled 1X). At this low level of nutrients, there is not enough bio plugging occurring for effective flow conformance until a high perm variation of about 0.7 is exceeded. However, at 3X and 5X this amount, there appears to be an economic incentive for using this treatment at perm variations above about 0.3. This simulation does not take into account viscous fingering that can cause poor sweep efficiency at high oil/water mobility ratios (Kumar, 2005) in homogenous formations. We believe that this same treatment can reduce viscous fingering. Experiments are in the planning stages to test this.

A significant cost for performing MEOR is the preparation of the inoculum. It is important to be able to prepare substantial quantities of microbes at high rate in batch fermentors. The quantity of microbes produced in this fashion and used for the well inoculation will depend on the depth of the pay zone and the well configuration. We are fortunate to have extensive test fermentation capability needed to generate basic data required for fermentation scale up. Figure 17 illustrates batch fermentation rate data we collect to produce a microbe to be used for a future well test. It is important to produce a high concentration of microbes (optical density greater than about 8) in about day as was achieved in this example. Growth rate variations shown in Figure 17 are due to optimization of nutrients and differences in

the size of the test fermentations.

Conclusions

1. Viscosity reduction is not a viable MEOR mechanism. Rates and availability of key nutrients (electron acceptors) through out the reservoir make this impractical.
2. Drastic drop in the interfacial tension between water and oil – low enough to cause spontaneous emulsification does not appear to be practical. The amount of bio-produced surfactant necessary to produce spontaneous emulsification is too great. However, some drop of interfacial tension is possible and this may improve oil recovery.
3. Microbially driven changes in oil wettability of rock have been observed. This effect has been demonstrated in sand packs to result in a significant drop in residual oil saturations for oil wetted or mixed-wetted rock.
4. Enhanced flow conformance by permeability modification using microbes and specially designed nutrient packages is a viable mechanism.
5. Optimal use of selected microbes and nutrients and their proper application in the reservoir is the key to effective MEOR. However, the reservoir conditions must be amenable to microbial growth (temperature and salinity being the most important criteria) before a reservoir is considered for MEOR treatments.

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References

ASTM standard method D4124-01, *Standard Test Methods for Separation of Asphalt into Four Fractions*.

I. M. Banat, Biosurfactants Production and Possible Use in Microbial Enhanced Oil Recovery and Oil Pollution Remediation: A Review, *Bioresource Technology*, Vol. 51, (1995) pp 1-12.

A. Bax, and S Subramanian. "Sensitivity-enhanced two-dimensional heteronuclear shift correlation NMR spectroscopy." *Journal of Magnetic Resonance*. 67. (1986): 565-569.

S. L. Bryant, and T. P. Lockhart: Reservoir Engineering Analysis for Microbial Enhanced Oil Recovery. *SPE 79719, 2000 SPE Annual Technical Conference and Exhibition, Dalla, TX, 1-4 Oct., 2000*.

R. D. Fallon, S. C. Jackson, *Ring Reduction via Natural Anaerobic Microbiological Activity for Transformation of Crude Oil – Associated Aromatic Chemicals*. Published patent application US2009/0050315A

R. D. Fallon, *Method for Improving Hydrocarbon – Water Compatibility in a Subsurface Hydrocarbon Contaminated Site*. Published patent application US2009/0050317.

R. D. Fallon, *Method for Improved Hydrocarbon and Water Compatibility* Published patent application US2009/0050316.

J. Garbow, D Weitekamp, and A Pines. "Bilinear rotation decoupling of homonuclear scalar interactions." *Chemical Physics Letters*. 93. (1982): 504-509

M. R. Gray, et. al. Potential Microbial Enhanced Oil Recovery Processes: A Critical Analysis, SPE 114676, *2008 SPE Annual Technical Conference and Exhibition*, Denver Co. USA, 21-24 Sept. 2008.

D. Groski, S. C. Jackson, Patents applied for.

I. M. Head, D. M. Jones & S. R. Larter. 2003. *NATURE*. 426:344

S. J Keeler, E. R. Hendrickson, L. L. Hnatow, S. C. Jackson, *Identification, Characterization and Application of Shewanella putrefaciens (LH4:18), Useful in Microbially Enhance Oil Release*. Published patent application US2009/0260803..

S. J Keeler, E. R. Hendrickson, L. L. Hnatow, M. Perry, *Identification, Characterization and Application of Pseudomonas stutzeri (LH4:15), Useful in Microbially Enhance Oil Release*. Published patent application US2009/0263887.

M. Kumar, et. al., High Mobility Ratio Water Flood Performance Prediction: Challenges and New Insights, SPE 97671, *SPE International Improved Oil Recovery Conference in Asia Pacific*, Kuala Lumpur, Malaysia, 5-6 Decemeber 2005.

G. Martin, and A Zektzer. *Two-Dimensional NMR Methods for Establishing Molecular Connectivity*. Weinheim, Germany: VCH, 1988. 213-221.

S. Maudgalya, et. al. SPE 89473, Development of Bio-Surfactant Based Microbial Enhanced Oil

Recovery Procedure, 2004 SPE/DOE Fourteenth Symposium on Improved Oil Recovery, Tulsa OK, USA, 17-21 April, 2004.

Maure, et. al.: Biotechnology Application to EOR in Talara Off-shore Oil fields, Northwest Peru, SPE94934 *SPE Latin American and Caribbean Petroleum Engineering Conference*, Rio de Janeiro, Brazil, 20-23 June 2005

L. Mueller, "Sensitivity enhanced detection of weak nuclei using heteronuclear multiple quantum coherence." *Journal of the American Chemical Society*. 101. (1979): 4481-4484.

K. Nagase, et. al: Improvement of Sweep Efficiency by Microbial EOR Process in Fuyu Oilfield, China. SPE 68720, *SPE Asia Pacific Oil and Gas Conference and Exhibition, Jakarta, Indonesia 17-19 April 2001*.

P. H. Nelson, Pore-throat sizes in sandstones, tight sandstones, and shales. *AAPG Bulletin*, V. 93, no. 3 (March 2009), pp. 329-340.

V. D. Pham, L.L. Hnatow, S. Zhang, R.D. Fallon, S.C. Jackson, J.-F. Tomb, E.F. DeLong, and S.J. Keeler: Characterizing Microbial Diversity in Production Water from an Alaskan Mesothermic Petroleum Reservoir with Two Independent Molecular Methods. *Environmental Microbiology*, 2008.

V. D. Pham, L. L. Hnatow, S Zhang, R. D. Fallon, E. F. DeLong and S J. Keeler, Microbial diversity profiles of fluids from low-temperature petroleum reservoirs with and without exogenous water perturbation, paper in preparation.

L. A. Strappa, et. al.: A Novel and Successful MEOR Pilot Project in a Strong Water-Drive Reservoir Vizcacheras Field, Argentina, SPE 89456, *SPE/DOE Fourteenth Symposium on Improved Oil Recovery*, Tulsa, OK, USA, 17-21 April 2004.

D. Thrasher, et. al. SPE 129701, Microbial EOR-From Lab to Field, 2010 *SPE Improved Oil Recovery Symposium*, Tulsa Oklahoma, USA, 24-28 April 2010.

Figures

Figure 1 – Combinations of temperature and salt content can make for more challenging environments for MEOR. Fields evaluated using the protocol described in the text are shown as diamonds. RMOTC is the Rocky Mountain Oil Test Center in Wyoming.

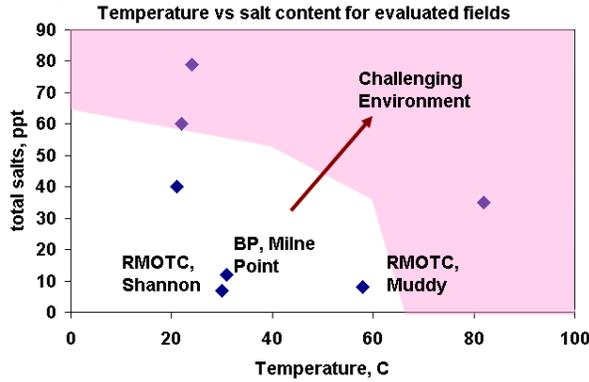


Figure 2 – 2-dimensional Nuclear Magnetic Resonance [2D NMR] (Bax, 1986; Garbow, 1982; Martin, 1979; Mueller, 1979) of the oil targeted for viscosity reduction. The proton NMR signal is plotted on the “X” axis. The carbon NMR signal is plotted on the “Y” axis. The colored islands within the plot give the intensity of proton and carbon signal for various structural units of the crude oil components. A poly aromatic molecule with saturated side chains is drawn in the plot with the NMR chemical shifts for comparison. This data shows the strong aromatic nature of this candidate oil.

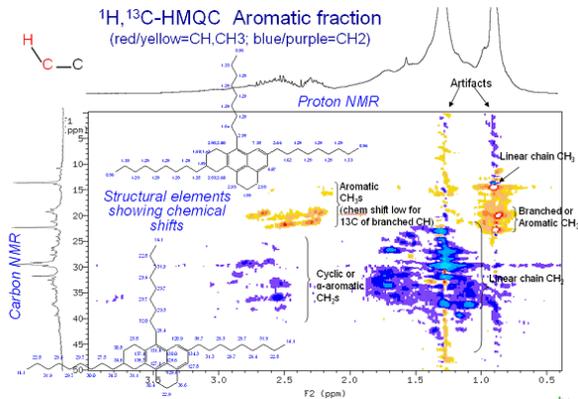


Figure 3 – Experimental flow down for finding useful microbes from a candidate well system.

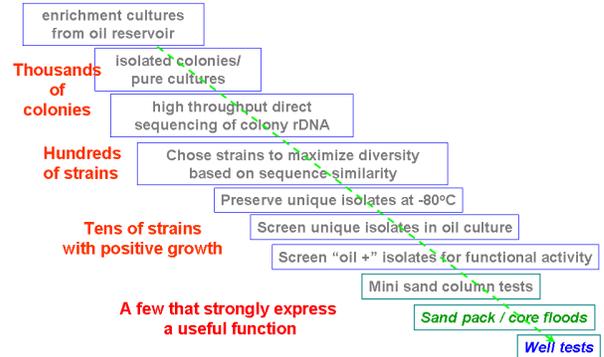


Figure 4: Picture of bottle enrichments.



Figure 5: Picture of colonies (small circular structures) of pure strains or consortia that have grown up after being "streaked" out from one of the enrichments.



Figure 6: Schematic diagram of the in culture oil viscosity measurement apparatus.

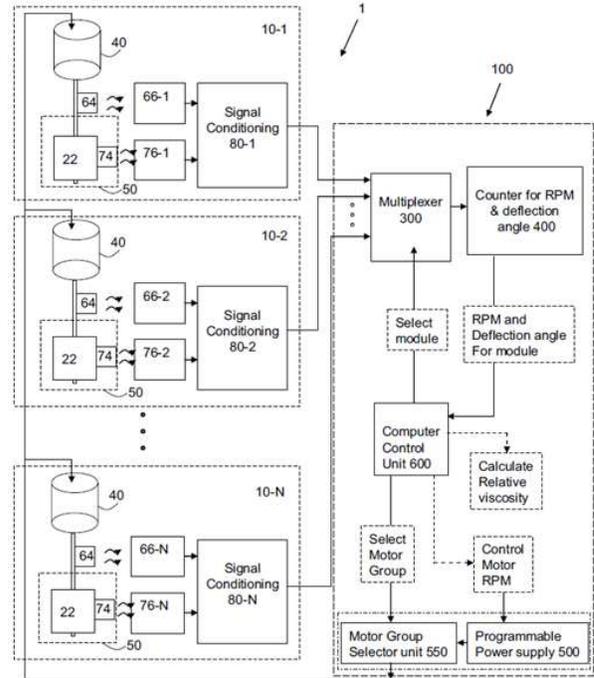


Figure 7: Photograph of in culture oil viscosity measurement apparatus.

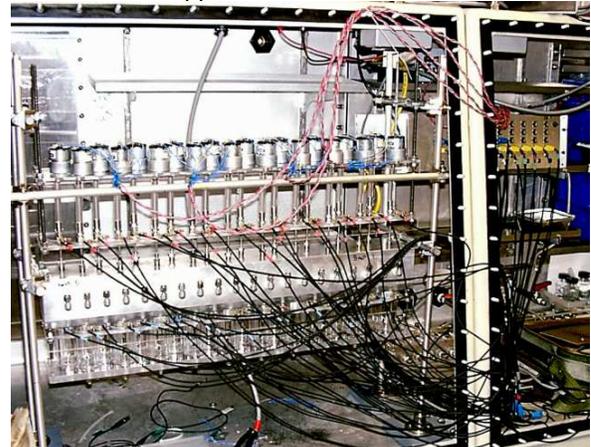


Figure 8: Preferential reduction of PAHs leading to viscosity reduction (from ref. 16).

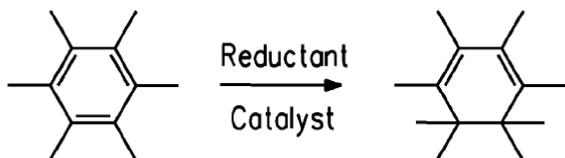


Figure 9: Top: Measurements of modest change in oil-water interfacial tension as a result of microbial activity.

Bottom: Emulsification due to microbial activity.

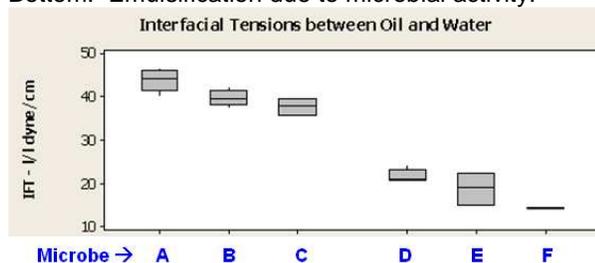


Figure 10: A sand grain (from produced sand collected at the well site) with the crude oil covering most of the sand. The sand was from Milne Point (Thrasher, 2010). Note the low contact angle of the oil on the sand. Water used here is the same as Figure 11 – no microbes present. The water composition was developed to represent the actual injection water chemistry used at the well site.

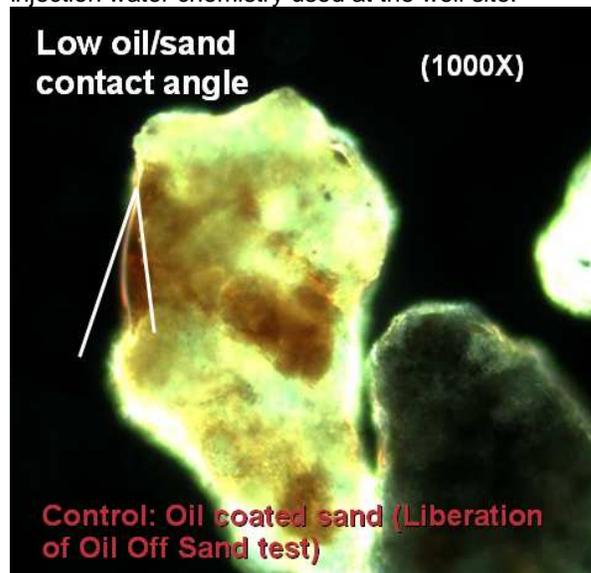


Figure 11: Modification of oil wetted sand using selected microbes. Note the high contact angle of the oil left on the sand. Sand, oil and brine are identical to that used in Figure 10.

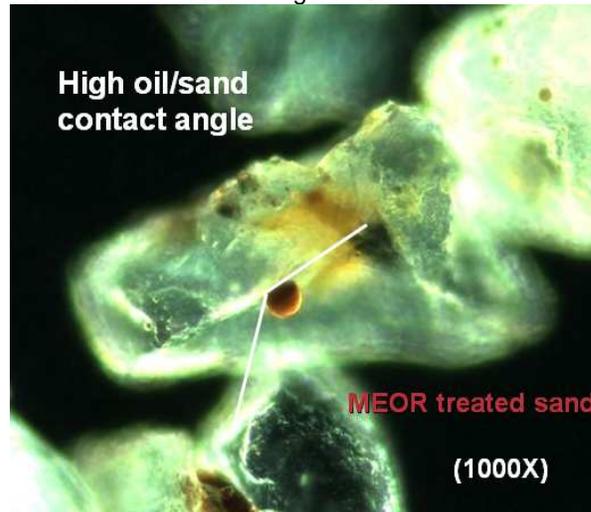


Figure 12: De-oiling curve (top) and pressure drop curves (bottom) for ambient condition sand pack.

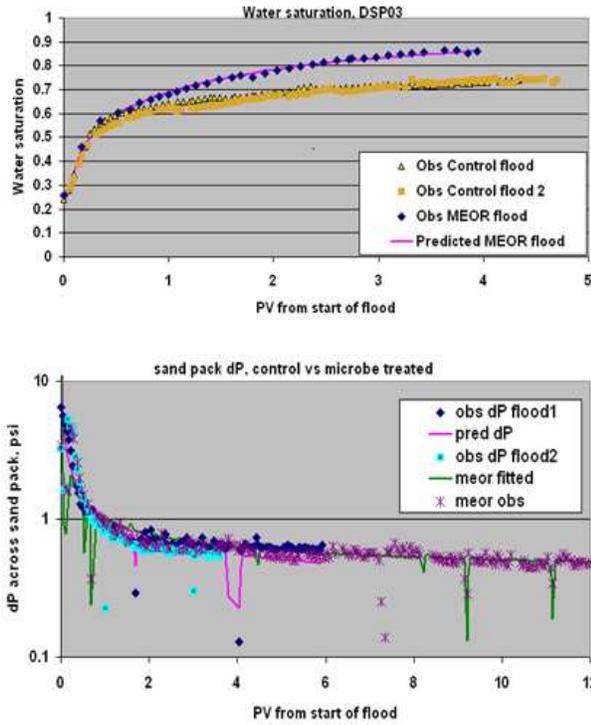


Figure 13: Relative permeability curves determined by history matching the data in Figure 12.

Changes in relative permeability (Corey function)

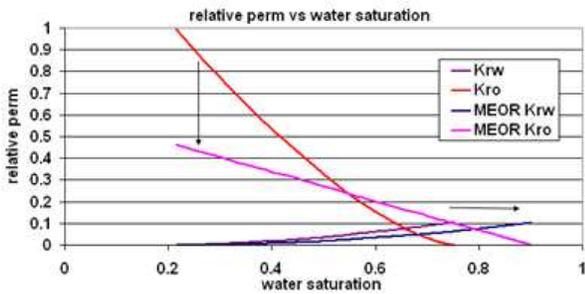


Figure 14: Measured pressure drop in slim tubes using two types of treatments. The X axis is the time the experiment was on stream (days). The Y axis shows the pressure drop across the slim tube. The black dots are the pressure drop data across the length of the slim tube. The dotted line at bottom is the pressure drop of an identical but untreated slim tube. Both treatments used the same microbe but differed in how the microbe formed the plug in the slim tube.

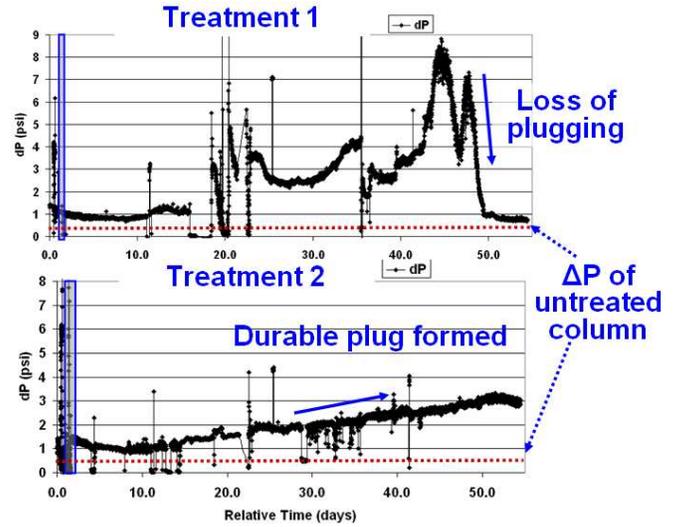


Figure 15: Composit slim tube results. Pressure drop is shown as black dots (scale to the left). Percent of oil recovered is shown as red squares (scale to right).

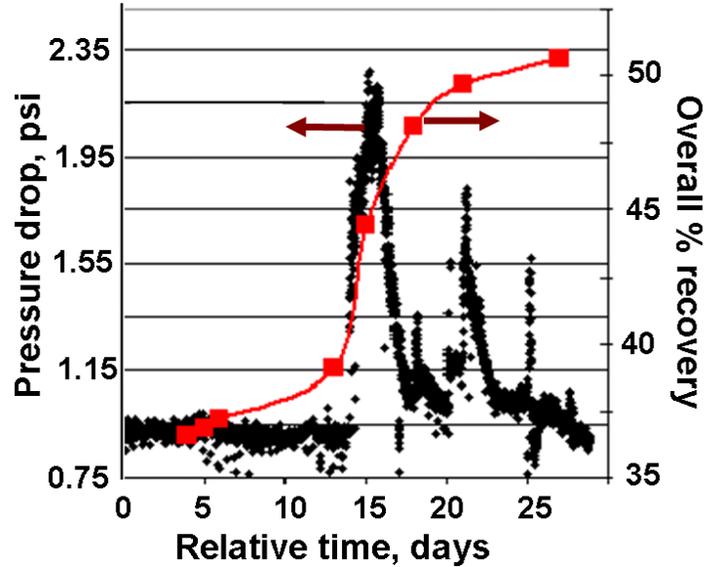


Figure 16: Simple economic model for flow conformance.

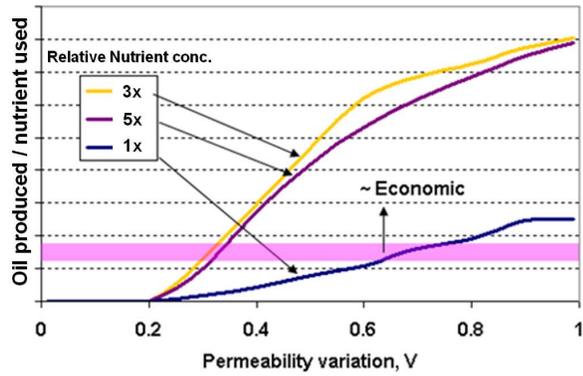


Figure 17: Fermentation growth curves for a MEOR organism. Optical density (Y axis) is a measure of the concentration of microbes grown in the batch fermentor.

