Effects of Selected Thermophilic Microorganisms on Crude Oils
at Elevated Temperatures and Pressures

1991 Annual Report

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Abstract

During the past several years, a considerable amount of work has been carried out showing that microbially enhanced oil recovery (MEOR) is promising and the resulting biotechnology may be deliverable. In this laboratory systematic studies are being conducted which deal with the effects of thermophilic and thermoadapted bacteria on the chemical and physical properties of selected types of crude oils at elevated temperatures and pressures. Particular attention is being paid to heavy crude oils such as Boscan and Cerro Negro (Venezuela), Monterey (California) and those from Alabama and Arkansas. Current studies indicate that during the biotreatment several properties of crude oils are affected. The oils are (1) emulsified; (2) acidified; (3) there is a qualitative and quantitative change in light and heavy fractions of the crudes; (4) there are chemical changes in fractions containing sulfur compounds; (5) there is an apparent solubilization of trace metals; and (6) the qualitative and quantitative chemical and physical changes appear to be microbial species dependent. Effects on heavy crude oils are also compared to those on lighter oils such as oils from the Wyoming petroleum reserve. Microbial oil interactions are monitored routinely by a consortium of analytical techniques which are continuously upgraded and are capable of multiparameter analysis. The results generated in fiscal year 1991, describing (1) through (6), are presented and discussed in this report.

1. Objectives

The objective of this program is to determine the chemical and physical effects of thermophilic and temperature adapted organisms on crude oils at elevated temperatures and pressures, e.g., 85°C and higher and pressures up to 2500 psi. The information gained from these studies will be used to explore the effects of the same set of organisms on samples of oil containing sand cores under experimental conditions which match those found in reservoirs, i.e., different temperatures, pressures, salinities and permeabilities. A number of parameters known to define the best candidate for microbial enhanced oil recovery (MEOR) are known. As defined by laboratory studies generated in this program, the best MEOR candidates will be used for scale up and technical feasibility studies leading to field applications.

2. Background

Over the past several years active groups at Universities, Government Institutions and Industry in the U.S.A. and abroad have laid the groundwork for MEOR (King and Stevens, 1986; King, 1987; Bryant, 1991). The past record indicates that MEOR is a promising technology. However, additional work is needed because most field tests were inconclusive or failures. Further, the chemistry and biochemistry of microorganism-oil interactions is by and large still unknown and, most bacteria either as single strains or mixtures were not tested in the laboratory under reservoir conditions. Nevertheless, available data allow us to suggest several mechanisms for the action of microorganisms in MEOR biotechnology (Bryant and Douglas, 1987; Moses 1991). These include: (1) production of gases (CO_2 , H_2 , N_2 , CH_4) which can increase pressure in the reservoir and reduce oil viscosity; (2) microbial production of low molecular weight acids, which cause rock solubilization; (3) production of biosurfactants which decrease surface and interfacial tensions; (4) microbially mediated changes in wettability; and (5) production of polymers which facilitate mobility. It has been suggested (Grula, 1986) that some of the desirable properties of bacteria to be used in MEOR should include microorganisms capable of large production of "oil releasing" metabolites (e.g., low molecular weight alcohols, acids, surfactants and gases); they should not require expensive nutrients, they should be anaerobic and/or be capable of growing in the presence of low oxygen concentrations and be able to withstand relatively high pressures and salinities. Also, such microorganisms should be easily grown in facilities above ground, remain viable over extended time and be transportable. Once underground they should continue to be viable and resume their activity upon refeeding. At the 1986 First International MEOR Workshop, it was recommended (Donaldson, 1986) that a search be conducted for anaerobic and thermophilic bacteria capable of breaking down heavy oils under reservoir conditions. Studies at the Brookhaven National Laboratory (BNL) have generated results which clearly indicate the significance of this approach (Premuzic and Lin, 1991-a). At the 1986 workshop, it was also recognized that the use of microorganisms in well bore clean out was also promising, although additional research in the areas dealing with control of sulfate reducing bacteria, corrosion prevention, reduced production of H_2S and well plugging is still needed. Further, recent work indicates (Premuzic and Lin, 1991-b,c) that biochemical treatment of crude oils may have useful applications in down stream operations.

A number of microorganisms have been found to be present in formation waters and were viable under certain reservoir conditions (Donaldson, 1986). The usefulness of these bacteria appears to be limited because, for these organisms to grow, reservoirs have to be within a given depth, salinity, temperature and permeability range and also be resistant to increases in the concentration of products generated by their action. Thermophilic organisms live under harsh conditions, such as low pH (1-3) and high temperatures (up to 110°C) and some are known to grow under alkaline conditions (Brock, 1978 and 1985). These organisms can use inorganic (e.g., sulfides, elemental sulfur, ferrous ion) and organic energy sources. Some of these bacteria are also capable of switching from an aerobic to anaerobic metabolism. Further, the natural habitats of these organisms are geothermal brines which means that they are tolerant of high salt concentrations. Thus, the general properties

of thermophilic organisms, although not fully explored, already indicate that they possess a number of desirable properties for MEOR.

The MEOR program at BNL has generated additional data which indicates that there are several advantages in using thermophilic chemotropic organisms:

1. Active at high temperature and pressures
2. Use constituents indigenous to the substrate
3. Withstand pH extremes
4. Tolerate high salt concentrations
5. Biodegrade high molecular weight compounds

Further, in this program, experimental strategies are being developed which allow selected microorganisms to adapt to conditions in which practically the sole source of energy for their growth is the indigenous crude oil. Detailed studies are also being conducted of the chemical and biochemical interactions between thermophilic, temperature adapted, acidophilic, chemotropic organisms and crude oils whose chemical and physical properties are known and explore the feasibility of MEOR biotechnology based on the biochemical, chemical and physical properties of these interactions. In this report, the progress in studies dealing with the biotreatment of crude oils over a wide range of experimental conditions will be discussed. Several topics will be addressed, which will include emulsification, qualitative and quantitative changes in the composition of low and high molecular fractions of crude oils, as well as in the composition of fractions containing sulfur and organometallic compounds.

3. Experimental

Interactions between microorganisms and crude oils are complex and involve multiple processes. In order to understand better the chemistry and biochemistry of these processes extensive studies and analyses of several variable parameters are needed. To accomplish some of these requirements we have expanded and/or modified our instrumental capabilities, so that many variable parameters can be measured at any given time. Expansion and modification of instrumentation capabilities are carried out as necessary.

Instrumentation

Gas Chromatography-Mass Spectrometry (GC/MS). A Perkin-Elmer model 8700 microprocessor-controlled gas chromatograph with multiramp temperature programming, soft key entry of all temperature and time parameters on color VDU, dual-channel screen graphics, dual-channel integral data handling, and reintegration has been interfaced with a Perkin-Elmer Finnigan Ion Trap Detector (ITD). The Ion Trap Detector System has a mass range of 20-650 mass units and encompasses NBS/EPA Mass Spectral Library containing over 42,000 mass spectra.

The interfaced system has been used routinely and is dedicated to the MEOR program, and the associated operational details of the system have been reported (Premuzic and Lin, 1989-d-i).

Metal Complexes

Hewlett-Packard gas chromatograph model HP 5921A equipped with atomic emission detector was used for the determination of metal complexes. For multi-elemental analyses, Induced Coupled Plasma Mass Spectrometry (ICP-MS) was used.

Sulfur Analysis

Total sulfur was determined by combustion (Huffman Laboratories, Golden, CO). The organic sulfur compounds were monitored by GC from 40°C-300°C equipped with FPD. A J&W, DBI column was used throughout. Characterization of changes in groups of sulfur compounds present in oil, e.g., sulfides, thiophenes and sulfoxides has been carried out by means of XANES analysis (Premuzic and Lin, 1990).

Growth and Adaptation of Microorganisms

Bioreactors, culture media and growth conditions have already been described (Premuzic and Lin, 1988, 1989-d-i) and were used in the work reported here without any modifications. A methodology has also been developed (Premuzic and Lin, 1989) which makes it possible to adapt microorganisms to different pressures and temperatures. In this methodology, modified bioreactors allow for continuous microbial growth in a medium in which a temperature gradient is maintained over a temperature range from 4°C to >85°C.

4. Results and Discussion

A. Emulsification

The effects of different microorganisms on crude oils depend on microbial species and types of oils used (Premuzic and Lin, 1991-a-c). The changes brought about by biotreatment result in different emulsions, and removal of organic sulfur and trace metals. There are also qualitative and quantitative changes in the distribution of high and low molecular weight fractions, all with noticeable species dependence. In order to further explore and document the above observations, measurements of additional properties of biotreated oils have been introduced in the continuing studies of bioprocess(es) characteristics.

Several crude oils were incubated with two species of bacteria over a period of two months and the viscosity of the produced emulsions were then measured with an LVT viscometer 25°C. The results are shown in Table 1. Variations in viscosity are consistent with reported microbial species-oil type dependencies.

Table 1

Oil	Viscosity in Centipoises	
	BNL-4-26	BNL-4-25
Prudhoe Bay (Alaska)	3.9	5.2
Naval Petroleum Reserve		
PR3 (Wyoming)	5.3	4.0
Wilmington (California)	3.5	3.7

Naval Petroleum Reserve (PR3) crude oil was treated with four different types of bacteria under identical experimental conditions. The produced oil emulsions containing the oil were then extracted with methylene chloride and the solvent removed by evaporation. Results are shown in Table 2, both in terms of Klett units and grams of oil per liter of emulsions produced.

Table 2

Klett Units	Microbial Strain	Oil Content in Emulsion(g/l)
50	BNL-4-21	3.58
200	BNL-4-22	11.0
700	BNL-4-23	18.0
30	BNL-4-24	3.03

The results summarized in Table 2 show a marked bacterial species dependence on the amount and the extent of emulsification. Gas Chromatography-Mass Spectrometry comparison of BNL-4-23 treated oil in emulsion and control (aqueous phase + nutrients + oil only) shows that C-15 to C-27 hydrocarbons have been emulsified as shown in Figures 1 and 2. It is to be understood that there is some loss of <C-15 hydrocarbons due to evaporation step in the extraction procedure.

B. Changes in the Chemical Composition of Crudes

Studies dealing with the variations in the interaction between different types of microorganisms and crude oils have been continued. Earlier observations (Premuzic and Lin, 1991-a,b) have indicated that the composition of crude oils in terms of organic compounds present changes during the biotreatment in favor of lighter components. Concurrent with this biochemical alteration of crude oils, there is also a change in the composition of organic sulfur compounds. Most recent studies (Premuzic and Lin, 1991-c), have shown that biotreatment of Boscan heavy crude oil from Venezuela with different microorganisms from the Brookhaven collection leads to significant chemical changes in the composition of the crude. Thus the biotreatment of Boscan crude oil with BNL-4-22 microorganism causes the following changes in the chemical composition of crude oil as shown in Figures 3 and 4:

- (i) Decrease in the C20 to C30 alkanes.
- (ii) Increase in the <20 type alkanes.
- (iii) There is an overall formation of lighter hydrocarbons.

Changes in the chemical composition of Boscan crude brought about by the biotreatment of the crude are consistent with those observed for Cerro Negro (Premuzic and Lin, 1991-a). However, they differ in chemical detail, and further indicate the significance of microbial species/crude oil type variety of interactions. These observations also imply that the biochemical mechanisms may differ with microbial species and/or types of crude oils being biotreated.

Figure 1, GC Trace of Biotreated and Extracted Emulsified Oil.

Figure 2. GC Trace of Control (non-treated extracted oil).

Figure 3. Aromatic Fraction of Boscan Crude m/e 57 Scan: A Before treatment and B After the Treatment with BNL-4-22.

Figure 4. Aromatic Fraction of Boscan Crude m/e 91 Scan: A Before Treatment and B After the Treatment with BNL-4-22. Legend: 1 Toluene; 2 C2-Benzene; 3 C3-Benzene, and 4 Naphthalene.

C. Sulfur Constituents of Crudes

Biotreatment of Boscan crude oil from Venezuela containing an average of 5% sulfur with BNL-4-22, BNL-4-23 and BNL-4-24 for seven days resulted in an overall lowering of organic sulfur content by 10-25%. Although kinetic studies under optimum conditions have as yet to be carried out, BNL-4-22 removed 25% of original sulfur in the crude, and a more detailed GC-MS analysis has been carried out on an untreated and treated sample of the crude. The GC trace using a flame photometric detector is shown in Figure 5. The results show that while there is a decrease in the overall content of organic sulfur there are also significant changes, both qualitatively and quantitatively in the content of thianaphthalenes. By and large, there is an overall decrease in the content of various substituted thianaphthalenes with the exception of thionaphthalene (peak 2, Figure 5) itself. This result is currently being further explored in order to determine whether this particular trend is characteristic of BNL-4-22 interaction with Boscan crude oil and differs from other microbial interactions studied in this program. An analytical tool exceptionally useful to follow total changes in sulfur compounds present in crude oils is the XANES analyses described earlier (Premuzic and Lin, Annual Report, BNL 45368, 1990). Results of comparative analyses by XANES of Boscan and Cerro Negro crude oils are shown in Table 3. Preliminary results reported in Table 3 suggest that the biotreatment decreases the sulfide and thiophene contents of the crudes and increases the sulfoxide contents. Since volatile products from biotreatment containing sulfur have not been detected to date, and there is a detected decrease in sulfur, a possible implication of these results is that the products are soluble in the water phase which is always present in the culture medium. Detailed experimental protocol is now being developed to investigate the chemical nature of the "sulfoxides" and other products which may be present in the aqueous phase. A possible outcome of this could be that some of the products may be water soluble sulfones responsible for the concurrent emulsification of the oil.

Table 3

XANES analysis of sulfides, thiophenes and sulfoxide contents of untreated and treated crude oils

Crude Oil	Microorganisms	Treatment	Relative Content		
			Sulfide	Thiophene	Sulfoxide
Boscan	0	untreated	0.198	0.738	0.064
	BNL-4-22	treatment*	0.159	0.655	0.186
	BNL-4-23	treatment*	0.121	0.743	0.135
Cerro Negro	0	untreated	0.147	0.781	0.072
	BNL-4-22	treatment*	0.179	0.683	0.138
	BNL-4-23	treatment*	0.103	0.713	0.184

* Treatment see Premuzic and Lin, 1991 a.

**FPD Trace of Boscan Crude Oil: (A) Before and
(B) After the Treatment with BNL-4-22**

Figure 5. FPD Trace of Boscan Oil: A Before and B After the Treatment with BNL-4-22.

D. Organometallic Compounds

We have reported earlier that during the biotreatment of Cerro Negro and Boscan crude oils, trace metals are removed concurrently with the removal of organic sulfur (Premuzic and Lin, 1991-a-c). In our original studies, only nickel porphyrin complexes have been monitored. We have now initiated studies to determine how many metals can be removed by bioprocessing of crude oils. To accomplish this, samples of biotreated and untreated crude oils are digested by nitric acid vapors, followed by dissolution in 1% aqueous nitric acid. The acid solutions are then analyzed by Induced Coupled Plasma Mass Spectrometry (ICP-MS). Preliminary results for Cerro Negro treated with BNL-4-23 over a period of ten days are shown in Table 4. These results indicate that other trace metals are also being removed. However, because of the digestion method used, arsenic, selenium and mercury, may have been volatilized and therefore "lost" during the treatment. Modification and optimization of the procedure is now in progress. The results are very promising, particularly in terms of downstream processing of crude oils.

Table 4

Changes in the selected trace metals contents of Cerro Negro oil treated with BNL-4-23.

Metal	Metal Content $\mu\text{g/ml}$	
	Untreated	Treated
V	3330	2290
Ni	926	639
Mg	78	6.8
Sr	9.6	1.7
Mn	15.8	1.8

E. Enhancement

Trends suggesting that different strains of microorganisms acting on the same oil interact differently appear to be consistent and warrant additional exploratory work. Studies have been initiated in which the biochemical effects caused by microbial treatment of crude oils are compared to those due to microorganisms with and without process enhancement. Thus, treatment of Wilmington, California crude oil with several microorganisms, Table 5, has shown that BNL-4-24, although not the best emulsion producer, is highly efficient in terms of conversion of organic sulfur compounds as shown by examples given in Figures 6, 7, and 8. All gas chromatographic traces are on the same scale and run under identical conditions. It is interesting to note that the same organism used in this experiment, when acting on heavy fractions (200°C fraction) of Wilmington oil, produced a larger extent of emulsification than when acting on the whole crude oil (Premuzic and Lin, 1991-a).

Table 5
Microbial Treatment of Wilmington Crude

Microorganism	Days incubated	Viscosity at the end of biotreatment in cps	Emulsion in Klett units
BNL-4-21	20	3.6	180
BNL-4-22	20	3.0	400
BNL-4-23	23	3.0	180
BNL-4-24	30	3.6	55
Control	55	3.3	7.5

Futher, it has also been observed that in some instances thermophilic microorganisms react with crude oils in a manner which causes the crude to be more efficiently emulsified. This information implies that chemically different emulsifying agents may be produced and further, the yields of naturally produced emulsifying agents, may also vary as a function of microbial species and the chemical composition of oils. If this is so, then some microbial species may be more suitable as producers of surface active agents while others may be better biochemical processors of crudes. Consortium of such organisms may enhance the overall effect. While ongoing R&D will further clarify this concept, preliminary experiments have been conducted in which the "enhancement effect" was tested. Thus, a sample of the Wilmington crude oil was emulsified with Tergitol (a commercial emulsifying agent) and then treated with BNL-4-24. The results are shown in Table 6. A considerable enhancement is achievable in terms of extent of emulsification and in terms of removal of organic sulfur compounds as shown in Figures 9 and 10, all in a much shorter period of time (seven days, compare Table 5). Similar experiments have been carried out with Boscan and Cerro Negro oils. The results are consistent and are shown in Table 7.

Figure 6. Gas Chromatography-flame Photometric detector (GCFPD) Trace (sulfur emission intensity) of Untreated Wilmington, CA, Crude. Molecular Markers A: Thiophene; B: Benothiophene; C: Phenyl Sulfide; D: Dibenzothiophene; E: C-1 Dibenzothiophene.

Figure 7. Gas Chromatography-flame Photometric detector (GCFPD) Trace (sulfur emission intensity) of Wilmington, CA, Crude Biotreated with BNL-4-21. C, D, E as in Figure 6.

Figure 8. Gas Chromatography-flame Photometric detector (GCFPD) Trace (sulfur emission intensity) of Wilmington, CA, Crude Biotreated with BNL-4-24. C, D, E as in Figure 6.

Figure 9. Gas Chromatography-flame Photometric detector (GCFPD) Trace (sulfur emission intensity) of Wilmington, CA, Crude Pre-emulsified with 0.5% Tergitol and Biotreated with BNL-4-24.

Figure 10. Gas Chromatography-flame Photometric detector (GCFPD) Trace (sulfur emission intensity) of Wilmington, CA, Crude Pre-emulsified with 0.5% Tergitol and Biotreated with BNL-4-24.

Table 6
BNL-4-24 Treatment of Pre-emulsified Wilmington Crude Oil

	Days Incubated	Viscosity at the end of biotreatment in cps	Emulsion in Klett units
BNL-4-24 + 0.1% oil + 1% Tergitol	7	2.79	7400
BNL-4-24 + 0.1% oil + 0.5% Tergitol	7	3.83	8000

Table 7
Biotreatment of Pre-emulsified Crude Oils

Oil	Microorganism	Media*	Treatment**	Emulsion in Klett units
Boscan	BNL-4-22	N.B. (10%C)	0	210
	BNL-4-33	N.B. (10%C)	0	140
	0	control	0	5
Cerro Negro	BNL-4-22	N.B. (10%C)	0	120
	BNL-4-23	N.B. (10%C)	0	145
	0	control	0	24
Boscan	BNL-4-22	Mod (No.C)	Emulsified	6000
	BNL-4-23	Mod (No.C)	Emulsified	5500
	0	Control	Emulsified	2800
Cerro Negro	BNL-4-22,23	(Oil Only)	Emulsified	2600
	BNL-4-22,23	Control	Emulsified	2600
	BNL-4-22	(Bacteria)	Emulsified	3800
	BNL-4-23	Mod(No.C)	Emulsified	4800
	0	Mod(No.C)	Emulsified	2800
Cerro Negro	BNL-4-22,23	Control	Emulsified	2600
	BNL-4-22,23	(Oil Only)	Emulsified	2600
	BNL-4-22,23	(Bacteria Only)	Emulsified	2600

*Nutrient (10%C) added; ** "0" means that the organisms were grown under conditions in which the organism generated the emulsification process in the medium. "Emulsified" means that the oil was pre-emulsified and then biotreated. The resulting emulsion is the cumulative result of pre-emulsification and emulsification generated by the microorganism.

5. Expansion of Microbial Reference Library

The reference library of aerobic and anaerobic microorganisms has been further expanded by addition of thermoadapted microorganisms. The data base is currently being enlarged by gas-chromatographic and mass-spectrometric data. The twenty-seven additional organic sulfur standard compounds and their GC-MS information have been logged in. Also, GC-MS characterization of thermo-adapted microbial strain has been initiated.

6. Presentations

1. Premuzic, E.T. Biochemical mechanisms of microbial transformation of energy related materials. Presented to the New York State Power Institute, 1991.
2. Premuzic, E. T., and Lin, M. S. Prospects for thermophilic microorganisms in microbial enhanced oil recovery (MEOR)--Part II. Presented at the SPE International Symposium on Oil field Chemistry, Anaheim, CA, Feb. 1991.
3. Premuzic, E.T. 273rd Brookhaven Lecture "Biotechnology without genetic Engineering: The use of microbial processes," 1991.

7. Publications/Patents

1. Premuzic, E.T. and M. Lin. 1991. Prospects of thermophilic microorganisms in microbial enhanced oil recovery (MEOR). SPE International Symposium on Oil field Chemistry, SPE21015, pp. 143-148.
2. Premuzic, E.T. and M.S. Lin. Effects of selected microorganisms on crude oils at elevated temperatures and pressures, Enhanced Oil Recovery, 65, 133-134, DOE/BC-91/1 (DE91002231).
3. Premuzic, E.T. and M.S. Lin. Interactions between thermophilic microorganisms and crude oils: Recent developments. Proc. Bioprocessing of Fossil Fuels Workshop, P.E. Bayer, Editor, pp. 453-466, CONF-890884, 1990.
4. Premuzic, E.T. and M.S. Lin. Microbial enhanced recovery of oil at elevated temperatures and pressures, Patent Pending, 1991.
5. Premuzic, E.T. and M.S. Lin. Prospects for thermophilic microorganisms in microbial enhanced oil recovery (MEOR), in: Microbial Enhanced Oil Recovery, E. Donaldson, Editor, Elsevier Press, Ch. R-18, pp. 277-296, 1991.
6. Premuzic, E.T. and M.S. Lin. Interactions between thermophilic microorganisms and crude oils: Recent developments. Resources, Conservation, and Recycling, 5, 177-284, 1991.

8. Reports

1. Premuzic, E.T. and M.S. Lin. Effects of selected thermophilic micro-organisms on crude oils at elevated temperatures and pressures. Annual Report BNL 45368, 1990.
2. Premuzic, E.T. and M.S. Lin. Effects of selected thermophilic micro-organisms on crude oils at elevated temperatures and pressures. Summary Annual Report BNL 45005, 1990.
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6. ibid., Quarterly Report, 07/01/91-09/30/91. BNL 46872.

9. Highlights

1. Microbial species vs. oil type specificity was further documented and emphasized.
2. Biotreatment causes a decrease in the total content of organic sulfur compounds with varying effects on different compounds such as thio-naphthalenes.
3. Biotreatment lowers the content of C20 to C30 alkanes.
4. With some microbial species there is an increase in <C20 compounds with an overall formation of lighter hydrocarbons.
5. Further evidence has been gathered which implies that some microbial species biochemically prefer to convert higher molecular weight compounds to smaller, while others favor formation of better emulsions.
6. Additional experimental evidence has been generated which supports the earlier observation which indicated that trace metals are removed (most likely solubilized in aqueous phase) during biotreatment.
7. Pre-emulsification may lead to a significant enhancement in the overall biochemical effect.
8. Efficient emulsifying agents may be generated by some microorganisms and not others, with significant variable yields of desirable products.
9. Biochemical modification and/or removal of organic sulfur compounds with a concurrent removal of trace metals from Cerro Negro and Boscan crudes by microorganisms from the BNL collection is "a first," and indicates that biotreatment of high sulfur content crude oils may lead to a desulfurization process for these types of oils.

10. A prototype continuous temperature gradient incubator has been constructed, tested, and further modified. The most recent model can culture microbial strains in a continuous mode over a temperature range of 4°C to 80°C. This is a unique tool for the development of thermoadapted bacteria capable of interaction with crude oils under different temperature regimes, including low and high extremes.

10. Recommendations

1. Currently available data indicates a considerable complexity in the processes by which microorganisms interact with crude oils. However, certain trends are beginning to emerge as seen in molecular weight distribution, sulphur content, emulsification and changes in trace metal contents. Different effects are also observed when different oils are treated by the same microorganisms. For example, Boscan crude oil is heavy due to immaturity and Cerro Negro is heavy due to biodegradation. It is clear that the microbial action on the biodegraded Cerro Negro is different than that on the Boscan not only because of their chemical differences, but also because the "naturally" biodegraded oil must change during the biotreatment by the microbial organism used in this program in a manner different to that occurring during biodegradation in a reservoir over geological periods of time. The above comments, based on the currently available experimental evidence, warrant the following recommendations:

1. Expand the studies to other immature biodegraded and heavy crude oils. Include Monterey crudes from California and those from Jurassic Smackover Formation of Alabama and Arkansas, all representing large domestic oil residues for future MEOR studies.
2. Continue exploratory work with bioreactors under varying conditions, biomass to oil ratios, and different strains of microorganisms already on hand.
3. Expand the GC/MS data base and complement with other chemical information, and use it as a tool in optimization studies. Include GC-MS characterization of thermophilic and thermoadapted microbial species tested.
4. Determine trends in the nature and extent of emulsification vs. pre-emulsification.
5. Explore strain/oil interactions in terms of chemical specificities and the enhancement potential.
6. Continue systematic studies of biochemical effects on sulfur containing organic components of crudes. Particular attention should be given to the extent of biochemical alteration and the chemical nature of products. The latter may include useful sulfone type emulsifying agents.
7. Collaborative effort with the Chevron Oil Field Research Company has been established. This effort should be expanded to involve detailed studies of the chemical nature of crude oil fraction containing key molecular markers. These should include saturate fractions, aromatic fractions containing sulfur, and metal compounds and polar fractions.
8. Expand studies in the area of trace metal bioremoval from crude oils.
9. Expand XANES studies of changes of organic sulfur compounds due to biotreatment of crude oils.
10. Initiate the exploration of the chemical nature of biochemically produced emulsifying agents under the experimental conditions used in this program.

11. Explore the utility of 6 and 8 in the development of remediation and/or downstream oil enhancement biotechnology.

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