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**APPLICATION OF MICROBIAL PROCESSES TO VISCOSITY  
REDUCTION OF HEAVY CRUDE OIL — FINAL REPORT**

By  
**William R. Finnerty**

**May 1987**

**Performed Under Contract No. AS19-81BC10507**

**University of Georgia  
Athens, Georgia**



**National Energy Technology Laboratory  
National Petroleum Technology Office  
U.S. DEPARTMENT OF ENERGY  
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## INTRODUCTION

This final report summarizes the results obtained from research conducted at the University of Georgia during the period 1981-1986. The objectives of the research program, in context of basic research directed to the overall goals of the U.S. Department of Energy program in "Microbial Enhanced Oil Recovery", were to determine whether microorganisms and/or microbial processes are applicable to the modification of heavy oil.

Literature concerning the level of microbial activity within the reservoir is scant. Reports exist concerning the growth of sulfate-reducing bacteria, souring of "sweet" crudes, plugging and other deleterious activities. Recent studies have indicated the presence of microorganisms within the oil reservoir with the realization that oil reservoirs are not, necessarily, sterile, biologically refractory environments. The source of the indigenous microflora is further unknown with possible point source contamination arising from surface origins or, alternatively, of subterranean origin.

The application of surface-active chemicals to enhanced oil recovery (EOR) has long represented an active developmental area in oil recovery technology. The consideration and application of biologically produced, surface-active chemicals has been generally overlooked, primarily due to the lack of a definitive literature. Literature Reviews discuss the broad generalities of biologically produced surface-active products in context of theory, sources, types and applications<sup>2,3</sup>.

The production of surface-active products by microorganisms has been recognized for years and a systematic characterization of these microbial products is beginning to develop in the literature. Recent studies have documented the production of numerous surface-active products by microorganisms. The development and application of such microbial-produced products may represent valuable and potentially useful chemicals in the recovery and production of oil.

The rationale underlying our approach to screening for microorganisms and/or microbial activities effective in heavy oil viscosity reduction was based on two possible mechanisms: 1) reduction of the average molecular weight of oil; and, 2) the production of specific biological products that would alter the physical properties of heavy oil. The reduction of the average molecular weight of heavy oils would necessitate the microbial depolymerization and/or degradation of the high molecular weight constituents such as asphaltenes and resin acids to smaller molecular weight components, thus, effectively reducing the average molecular weight of the oil. Alternatively, the production of surface-active products by microorganisms growing at the expense of oil or nonhydrocarbon substrates would serve to generate macro- and/or micro-emulsions of oil-in-water having lower viscosities than the parent crude oil. The following discussion summarizes our studies on the viscosity reduction of heavy oils by microorganisms.

### ENRICHMENT AND SELECTION OF MICROORGANISMS

The enrichment of microorganisms was accomplished through conventional enrichment techniques. A variety of soil samples ranging from randomly selected soils to soils subject to chronic exposure with oil were supplemented with Venezuelan Monagas crude, Venezuelan Cerro Negro crude, West Texas crude, and asphaltenes derived from each of these crudes. The culture flasks were incubated aerobically at room temperature with shaking for varying periods of time. Serial transfer of these primary enrichments were made at selected intervals to fresh oil-containing media and maintained through a minimum of 5 serial

passages. The final serial transfer was streaked onto nutrient broth-yeast extract agar plates for pure culture isolation. Each of the 300 bacterial isolates obtained in this manner was tested for their ability to grow in liquid culture at the expense of crude oils, asphaltene fractions, and pure alkanes.

#### SCREENING PROCEDURES FOR DETECTION OF BIOLOGICALLY PRODUCED SURFACE-ACTIVE PRODUCTS

Screening procedures were devised to determine the ability of bacterial isolates to produce surface-active agents. Screening procedures developed were culture broth tensiometry, indicator plates containing red blood cells and indicator plates containing crude oil. Enrichment culture procedures yielded in excess of 300 individual bacterial isolates. Crude oil-containing agar plates were prepared by the addition of oil to complete basal salts medium containing 2% agar and sonicated while maintaining a 70°C temperature. This oil-in-water suspension was poured into petri plates (15 ml/plate) and allowed to solidify at room temperature. The oil plates were inoculated with the bacterial isolates, incubated at room temperature and inspected daily for clearing of the oil. The procedure allowed for the rapid presumptive screening of large numbers of bacterial isolates which produce extracellular products that were effective in the dispersion or release of oil. We identified approximately 200 isolates which caused the dispersion of oil. Out of 200 isolates, 77 bacterial isolates were studied with respect to the ability of their cell-free culture broths to form stable emulsions. There were 62 isolates (82%) which formed stable emulsions with hexadecane and 14 (18%) isolates which formed unstable emulsions.

#### TENSIOMETRIC CHARACTERISTICS OF SPENT GROWTH MEDIA

The surface tension and interfacial tension of spent culture broths derived from selected cultures grown on hydrocarbon (hexadecane) and non-hydrocarbon substrates were determined for evaluating the presence of surface-active products (Table 1).

TABLE 1  
TENSIOMETRIC PROPERTIES OF SELECTED SPENT CULTURE BROTHS

| Culture Broth | Surface tension |            | Interfacial tension |            |
|---------------|-----------------|------------|---------------------|------------|
|               | NBYE            | Hexadecane | NBYE                | Hexadecane |
|               | mN/m            |            |                     |            |
| E-6           | 63.0            | 39.4       | 22.8                | 34.5       |
| H-10          | 46.7            | 57.6       | 18.6                | 24.4       |
| H-11          | 48.2            | 59.3       | 19.4                | 26.3       |
| H-12          | 50.6            | 57.2       | 22.9                | 29.0       |
| H-13          | 49.2            | 30.4       | 20.5                | 11.8       |
| I-2           | 44.3            | 43.4       | 11.3                | 13.2       |
| J-14          | 48.1            | 52.0       | 16.0                | 21.4       |
| K-3           | 47.8            | 53.7       | 13.9                | 25.7       |
| K-4           | 44.8            | 52.8       | 13.9                | 21.6       |
| L-4           | 40.9            | 59.3       | 15.4                | 25.7       |
| L-9           | 36.0            | 64.6       | 8.2                 | 36.8       |
| L-10          | 49.8            | 62.8       | 24.6                | 30.2       |
| M-1           | 49.5            | 60.4       | 21.4                | 25.8       |
| M-8           | 41.8            | 45.0       | 7.1                 | 18.8       |
| M-9           | 41.7            | 49.6       | 10.2                | 20.5       |
| M-10          | 46.8            | 52.2       | 16.7                | 24.2       |

|                     |      |      |      |      |
|---------------------|------|------|------|------|
| N-5                 | 72.8 | 58.3 | 30.4 | 37.2 |
| P-1                 | 42.4 | 48.1 | 12.2 | 16.3 |
| P-2                 | 42.0 | 49.3 | 11.1 | 19.2 |
| P-4                 | 42.4 | 48.2 | 10.4 | 13.9 |
| P-5                 | 43.5 | 47.9 | 10.0 | 22.2 |
| P-6                 | 53.6 | 60.0 | 17.2 | 31.0 |
| P-7                 | 43.4 | 56.7 | 10.0 | 24.4 |
| P-10                | 40.0 | 48.0 | 9.7  | 15.8 |
| P-11                | 42.4 | 52.3 | 10.6 | 20.7 |
| R-2                 | 53.9 | 63.7 | 24.6 | 31.9 |
| R-5                 | 44.3 | 54.5 | 11.8 | 22.9 |
| R-6                 | 43.2 | 56.3 | 9.5  | 24.4 |
| NBYE Control        | 44.6 | -    | 18.0 | -    |
| Basal Salts Control | -    | 72.0 | -    | 49.0 |

The isolates group into 3 classes with respect to their ability to alter surface tension: 1) those organisms which decrease the surface tension of spent media; 2) those organisms which increase the surface tension of spent media; and 3) those organisms which do not change the surface tension of spent media.

Nutrient broth-yeast extract (NBYE) appears to contain surface-active component(s) which are metabolized by specific isolates with a corresponding increase in the spent culture broth surface activity. It is unknown whether spent culture broths derived from isolates that yielded lower tensions produced additional biosurfactant or altered constituents present in NBYE to surface-active components. If these isolates metabolized the surface-active component(s) present in NBYE as well as producing additional biosurfactant, then the effective net yield of surface active component was greater than indicated by the measured tensions. In contrast, all isolates grown at the expense of hexadecane yielded spent culture broths having surface and interfacial tensions that were decreased from control values.

#### DETECTION OF BIOSURFACTANT-PRODUCING MICROORGANISMS BY RED BLOOD CELL LYSIS

A red blood cell (RBC) agar plate assay was developed as a rapid screening method for detection of surface-active products produced by microorganisms. This method is based upon the ability of surface-active agents to lyse RBC. Red blood cell plates (Baltimore Biological Laboratories) were inoculated with presumptive or unknown biosurfactant-producing isolates. If an isolate produces biosurfactant following growth on the RBC agar medium, RBC lysis occurs resulting in clear zones of hemolysis surrounding the colony. If an isolate does not produce biosurfactant, then no RBC hemolysis occurs. Table 2 illustrates the application of this technique to biosurfactant production by a specific isolate and its correlation to interfacial tension measurements of the corresponding spent culture broth and solvent-extracted biosurfactant.

TABLE 2  
CORRELATION OF BIOSURFACTANT PRODUCTION WITH RED BLOOD CELL HEMOLYSIS  
AND SURFACE ACTIVITY

| Biosurfactant-Producing<br>Isolates | Interfacial Tension |               | RBC Hemolysis |
|-------------------------------------|---------------------|---------------|---------------|
|                                     | Spent Culture       | Extracted     |               |
|                                     | Broth               | Biosurfactant |               |
|                                     | mN/m                |               |               |
| MS-23                               | 15.7                | 1.6           | +             |
| MS-24                               | 18.1                | 2.5           | +             |

|   |      |      |   |
|---|------|------|---|
| MS-26                                       | 14.3 | <1.0 | + |
| MS-27                                       | 16.7 | <1.0 | + |
| MS-28                                       | 12.9 | <1.0 | + |
| <u>Non-Biosurfactant-Producing Isolates</u> |      |      |   |
| MS-1  | 33.4 | 15.2 | - |
| MS-11                                       | 37.1 | 19.0 | - |
| MS-15                                       | 40.5 | 21.0 | - |
| MS-29                                       | 41.1 | 23.8 | - |
| MS-30                                       | 44.5 | 25.2 | - |
| Control (basal salts medium)                | 49.0 | 47.0 | - |

To demonstrate that a surface-active agent rather than a lytic enzyme caused RBC lysis, the surface-active compound was extracted from the spent growth medium with organic solvents. The biosurfactant was reconstituted 10-fold more concentrated solution in an aqueous medium, after removal of the solvent, and placed into wells cut into the RBC agar medium. In each case, the product extracted from the spent growth medium of biosurfactant-positive isolates exhibited RBC lysis. Several different biosurfactant concentrations were tested for RBC lysis with biosurfactant concentration correlating positively with zone of hemolysis diameter. Factors which influence the zone diameter are water-solubility and diffusional coefficient of the biosurfactant. In general, highly water-soluble, low molecular weight biosurfactants exhibit larger zones of RBC hemolysis. This detection method is limited to microorganisms which produce water-soluble, diffusible, low molecular weight biosurfactants. Microorganisms which produce biosurfactants only during growth on alkanes are rarely, if ever, detected by this method.

#### CONFIRMATORY TESTS FOR BIOSURFACTANT PRODUCTION

Extracellular biosurfactant production by microorganisms is readily confirmed by culture broth tensiometry, i.e., measurement of surface or interfacial tension of spent growth media. A significant reduction (75% or greater over control values) of surface or interfacial tension relative to the control value indicates significant biosurfactant production. The biosurfactant is then extracted from the spent growth medium into an organic solvent and reconstituted in an aqueous medium at a 10-fold higher concentration. This concentration factor results in a further decrease in surface or interfacial tension if the biosurfactant was present at concentrations below the critical micelle concentration in the culture broth.

#### VISCOSITY REDUCTION OF BACTERIAL-TREATED HEAVY OIL

Selected isolates were grown in 3-liter Fernbach flasks containing 1-liter of complete basal medium plus 50 gms of Monagas crude oil. All isolates were pregrown on 0.5% hexadecane for a minimum of 2 days and an inoculum volume of 100 ml added to each flask. This culture was incubated aerobically on a rotary shaker for 6-7 days at 28°C. The oil was separated from the aqueous medium by allowing phase separation to occur in a 2-L separatory funnel.

A number of visual characteristics were associated with these cultures following growth on heavy oil. First, uniform dispersion of the crude oil occurred throughout the aqueous medium. This dispersion was stable for days (24-48 hrs) with swirling of the flask resulting in the uniform and immediate dispersion of the oil. Second, following phase separation, the volume of oil recovered was 2-3 times greater than the volume of the original oil. Third,

the crude oil recovered was significantly less adherent to glass surfaces, tending to separate cleanly from such surfaces. Fourth, the recovered oil exhibited significantly improved fluid properties, with flow characteristics considerably different from the original crude oil.

Visual characteristics are of considerably less value than the more quantifiable properties such as viscosity. Table 3 summarizes our results relating to viscosity changes occurring with Monagas heavy crude following treatment with selected bacterial isolates.

The increased volume of oil was determined to result from the formation of a stable oil-in-water emulsion containing 30% water as the continuous phase. The oil phase exhibited a conductivity of  $2.43 \times 10^{-3}$  mho/cm, a conductivity comparable to that of 0.02 M KCl, indicating an oil-in-water emulsion. The treatment of Monagas crude with cell-free culture broth containing 295 mg/liter of extracellular biosurfactant resulted in a 50% reduction of crude oil viscosity. The H-13 isolate grows well on Monagas crude oil. Analyses of Monagas crude by gas chromatography before and after bacterial growth indicates that the bacteria grow at the expense of the paraffin content, with bacterial growth removing all paraffins. This fact precludes the direct application of such isolates for MEOR by virtue of their metabolic capabilities to further down grade oil quality. Accordingly, attention was directed to extracellular biosurfactant as a chemical enhanced oil recovery agent.

TABLE 3  
VISCOSITY CHANGES IN BACTERIAL-TREATED HEAVY OIL

| Sample                           | Temperature | Viscosity(cp) | Percent | Decrease |
|----------------------------------|-------------|---------------|---------|----------|
| Monagas crude (dry) <sup>1</sup> | 40°C        | >25,000       |         |          |
|                                  | 60°C        | 4,690         |         |          |
| Monagas crude (wet) <sup>2</sup> | 40°C        | 6,510         |         |          |
|                                  | 60°C        | 1,070         |         |          |
| F-3 treated crude (dry)          | 40°C        | 14,000        | 44      |          |
|                                  | 60°C        | 2,040         | 56      |          |
| F-3 treated crude (wet)          | 40°C        | 151           | 98      |          |
|                                  | 60°C        | 64            | 94      |          |
| H-10 treated crude (dry)         | 40°C        | 5,912         | 76      |          |
|                                  | 60°C        | 1,003         | 79      |          |
| H-10 treated crude (wet)         | 40°C        | 5,500         | 15      |          |
|                                  | 60°C        | 978           | 9       |          |
| H-13 treated crude (dry)         | 40°C        | 10,160        | 60      |          |
|                                  | 60°C        | 1,670         | 64      |          |
| H-13 treated crude (wet)         | 40°C        | 452           | 93      |          |
|                                  | 60°C        | 163           | 85      |          |
| H-13 treated crude (dry)         | 40°C        | 9,462         | 62      |          |
|                                  | 60°C        | 2,785         | 41      |          |
| H-13A treated crude (wet)        | 40°C        | 145           | 98      |          |
|                                  | 60°C        | 76            | 93      |          |

- 1) Samples designated dry represent the equilibration of Monagas crude with minimum basal salts medium for 5 days, recovery of the oil, and pumping of the oil under vacuum with a water aspirator for 24 hrs.
- 2) Samples designated wet represent the recovered oil as a stable emulsion

These results demonstrate viscosity reduction of bacterial-treated Monagas heavy crude ranging from greater than 50% for dry oil to 98% for wet oil. Bacterial growth occurred for all isolates, at the expense of Monagas heavy oil with colony forming units estimated at  $10^{10}$ - $10^{11}$  cells/ml. One specific isolate (H-13) decreased significantly the tensiometric properties of spent culture broths as well as reducing heavy oil viscosity by greater than 90%. H-13 was cloned on complex medium and isolated colonies selected. A pure culture was selected and designated H-13A for further study and development.

#### TAXONOMY AND PHYSIOLOGY OF H-13A

H-13A is a gram-positive rod tentatively classified in either the coryneform or nocardioform group of bacteria. It grows at the expense of a number of hydrocarbon and non-hydrocarbon carbon substrates as sole sources of carbon and energy. Hydrocarbons which support growth are alkanes ( $C_8$  to  $C_{30}$ ) and paraffinic-containing crude oils. H-13A produces extracellular biosurfactant only when grown at the expense of alkanes. Extracellular biosurfactant by H-13A from a homologous series of alkanes as the sole source of carbon and energy yields (in mg dry weight biosurfactant/liter): octane, 0; decane, 0; undecane, 20; dodecane, 550; tridecane, 1850; tetradecane, 1370; pentadecane, 925; hexadecane, 875; heptadecane, 230; octadecane, 510; nonadecane; 28; eicosane, 12.

The relationship between the growth of H-13A, extracellular biosurfactant production and interfacial tension of the spent growth medium is shown in Figure 1.

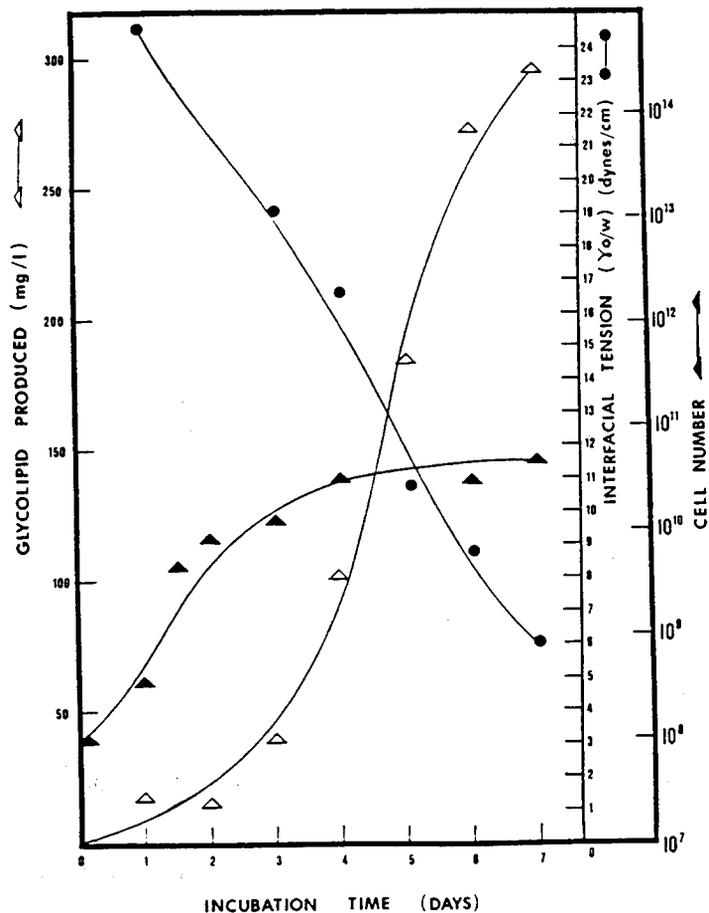


FIG. 1. Glycolipid production by H-13A grown on hexadecane.

Stationary growth phase occurs in 4-5 days, at which time the extracellular biosurfactant increases approximately 300%. Correspondingly, a 4-fold decrease in the interfacial tension of the growth medium occurs over 7 days. Biosurfactant appears to be synthesized over the total growth cycle of H-13A with maximum production occurring in the stationary growth phase. Extracellular biosurfactant synthesis appears to represent a physiological function associated only with growth of H-13A on alkanes. Experiments with high paraffin-containing crude oils, such as Pennsylvania light crude, indicate an extracellular biosurfactant yield in excess of 3 gms/liter of spent culture broth.

#### CHEMICAL PROPERTIES OF BIOSURFACTANT

The biosurfactant was extracted from the spent culture medium with ethyl-acetate:methanol (2:1;v/v) and fractionated by silicic acid column chromatography into neutral lipid, glycolipid and phospholipid fractions, with all surface-activity fractionating in the glycolipid fraction. Analysis of the glycolipid fraction by thin-layer chromatography (TLC) demonstrated the presence of one major component (~ 90%) and 5-6 minor components. Structural analyses performed on TLC purified major glycolipid (purified to 98% homogeneity by TLC) showed the presence of n-fatty acids, 2-hydroxy fatty acids, glycerol, trehalose and glucose. No phosphate or amino groups were detected. Prior studies indicated the presence of a peptide which appeared to be structurally related to the glycolipid. This peptide has been shown to result from the co-purification of a lipopeptide with glycolipid. The lipopeptide contains an unidentified fatty acid(s) and a peptide, perhaps a cyclic molecule. The lipopeptide can be separated from the glycolipid by diafiltration through UM-10 membranes. The lipopeptide partitions to the filtrate while the glycolipid remains in the retentate following extensive UM-10 diafiltration. Further structure details are presented elsewhere<sup>3,4,5,6</sup>.

#### PHYSICAL PROPERTIES OF BIOSURFACTANT

The CMC of the crude glycolipid was 1.5 mg/ml; the CMC for the purified major glycolipid was 1.0 mg/ml. The minimum interfacial tension (IFT), as measured against hexadecane at 25°C, was 0.25 mN/m for the crude glycolipid and 1.4 mN/m for the purified major glycolipid, indicating that both major and minor glycolipid components are required for maximum surface activity.

The interfacial tension of the crude glycolipid was measured as a function of alkane chain length to determine the equivalent alkane carbon number (EACN). The concept of EACN, as developed by Wade and colleagues<sup>7,8</sup>, exhibits for a surfactant a minimum IFT against an alkane of a specific carbon number or against a mixture of alkanes having an average EACN. This system has been used to evaluate the effectiveness of surfactants against crude oils having specific EACN values. The minimum IFT exhibited by the crude glycolipid was  $2 \times 10^{-2}$  mN/m against decane (Table 4). Addition of  $5 \times 10^{-2}$  pentanol (0.5% vol./vol.), as a cosurfactant, yielded a minimum IFT of  $6 \times 10^{-5}$  mN/m against undecane. Addition of a cosurfactant resulted in a shift from 10 to 11 as the EACN as well as in a significant reduction of IFT. Isopropanol and butanol were equally effective cosurfactants.

TABLE 4  
INTERFACIAL TENSION VERSUS ALKANE CARBON NUMBER  
Hexadecane-Derived Glycolipid<sup>a</sup>  
mN/m

| Alkane                    | mN/m                 |                            |
|---------------------------|----------------------|----------------------------|
|                           | Minus pentanol       | Plus pentanol <sup>b</sup> |
| Hexane                    | $5.7 \times 10^{-1}$ | $1.6 \times 10^{-1}$       |
| Octane                    | $3.0 \times 10^{-1}$ | $5.0 \times 10^{-2}$       |
| Nonane                    | -                    | $2.0 \times 10^{-2}$       |
| Decane                    | $2 \times 10^{-2}$   | $1.0 \times 10^{-3}$       |
| Undecane                  | $3 \times 10^{-2}$   | $6.0 \times 10^{-5}$       |
| Dodecane                  | $9 \times 10^{-2}$   | $1.4 \times 10^{-4}$       |
| Tridecane                 | -                    | $2.8 \times 10^{-4}$       |
| Tetradecane               | $1.6 \times 10^{-1}$ | $6 \times 10^{-2}$         |
| Hexadecane                | $2.5 \times 10^{-1}$ | $1.0 \times 10^{-1}$       |
| Octadecane                | $3.0 \times 10^{-1}$ | -                          |
| Hexadecane + Hexane (1:1) | -                    | $5.0 \times 10^{-5}$       |

<sup>a</sup>Glycolipid concentrations: 1.8 mg/ml; 1.7% salt; IFT measured at 40°C in spinning drop tensiometer

<sup>b</sup>Supplemented with 0.5% n-pentanol

Salinity: Supplementation of the glycolipid solutions with NaCl results in higher IFT values (Table 5). NaCl concentrations above 7.5% caused increased IFT values with precipitation of the glycolipid occurring at 10% NaCl.

TABLE 5  
EFFECT OF SALINITY ON SURFACE ACTIVITY OF GLYCOLIPID

| Sodium Chloride<br>(% wt.vol.) | Interfacial Tension <sup>a</sup> |
|--------------------------------|----------------------------------|
|                                | mN/m                             |
| 0%                             | $7.4 \times 10^{-3}$             |
| 1%                             | $2.1 \times 10^{-3}$             |
| 3%                             | $6.6 \times 10^{-3}$             |
| 5%                             | $1.0 \times 10^{-2}$             |
| 7.5%                           | $3.6 \times 10^{-2}$             |
| 10.0%                          | 11.2                             |

<sup>a</sup>IFT was dissolved in a 1.7% basal salts solution and measured at 50°C with a spinning drop tensiometer.

Divalent cations ( $\text{Ca}^{++}$ ,  $\text{Fe}^{++}$ ,  $\text{Mg}^{++}$ ) at 1 mM did not exert significant effects on the surface activity of glycolipid nor did temperature. Glycolipid preparations were stable to heating at 120°C under 20 psi for 15-30 minutes. The glycolipid exhibited maximum surface activity and highest solubility under basic conditions. At pH 2, glycolipid aggregated and exhibited some loss of surface activity. The acid-mediated aggregation was reversible with maximum surface activity restored by readjusting to pH 13.0.

Studies on glycolipid phase behavior indicate that the glycolipid-pentanol system forms a "middle" phase when mixed with undecane. The existence of a middle phase is associated with ultra-low IFT values. Maltese crosses were observed in the middle phase by plane-polarized light. This system has yet to

be optimized for microemulsion formation by varying parameters such as salinity, pH, coagent concentration or chain-length and temperature.

#### APPLICATIONS OF BIOSURFACTANT

The application of a biosurfactant requires consideration for the large scale preparation and down-stream processing of a product intended for use as a chemical enhanced oil recovery (CEOR) agent. The economics associated with such production technology will often preclude its commercial exploitation. We developed a test system for application's evaluation consisting of the spent culture growth medium of H-13A following growth on hexadecane as an economically viable and cost competitive source of biosurfactant. The cultures were monitored daily to determine maximum biosurfactant yield, harvested to remove the bacterial cell mass and the resulting spent culture broth reduced to one-half its original volume. This concentrated growth medium was dialyzed against 0.1 M phosphate buffer, pH 7.0, and was supplemented with 0.5% pentanol. This preparation was designated <sub>0</sub> standardized biosurfactant solution and was used for the following experiments .

#### OIL DISPLACEMENT FROM SAND PACKS

Preparation of Sand Packs. Oil-bearing sand was prepared with 40/50 mesh, nitric acid-washed, neutralized and heat-activated sand. The sand was coated with oil by suspending the sand in a solution of heavy oil dissolved in toluene, followed by vacuum evaporation of the solvent and air-drying overnight. The ratio of crude oil to sand was 0.5% and 1.0% on a weight basis.

The oil-sand was packed as a slurry into jacketed vertical glass columns forming a sand pack column. The sand pack was flooded prior to biosurfactant treatment with 0.1 M phosphate buffer, under specified conditions of pH, temperature and salinity.

Measurement of Oil Displacement. Total emulsified oil represents oil released from the sand pack in the form of an oil-in-water emulsion. The oil was measured by toluene extraction of the column eluate, followed by measurement of the absorbance of the toluene extract at 700 nm. A standard curve was prepared for each oil relating optical density to weight.

Total trapped oil represents the oil released from the sand but entrapped in the interstitial spaces. This oil was estimated by removing the sand from the column into distilled water. The trapped oil rose immediately to the surface and was extracted with toluene and its weight determined by optical density.

Total bound oil represents that oil which remained adsorbed to sand after biosurfactant treatment. This oil was estimated by extraction of the sand with toluene:methanol (1:1, vol/vol) and determining the optical density.

Total oil displacement represents the sum of total emulsified and total trapped oil released by biosurfactant treatment.

Surfactant-displaced oil represents that oil released by biosurfactant treatment alone.

#### RESULTS

The spent growth medium of hexadecane-grown H-13A was evaluated as a readily available source of extracellular biosurfactant. This option became necessary due to the lack of large volume process extraction equipment to recover adequate amounts of biosurfactant for displacement studies. The standardized biosurfactant solution was characterized with respect to physical and chemical properties (Table 6).

TABLE 6  
CHARACTERIZATION OF BIOSURFACTANT PREPARATION

| Property   | Concentrated Growth Medium |               |
|--|----------------------------|---------------|
|  | pH 7.0                     | pH 12.0       |
| $\gamma_{o/w}$ (25°C) <sup>a</sup>                                   | 10.0 dynes/cm              | 8.0 dynes/cm  |
| $\gamma_{o/w}$ supplemented with 0.5% n-pentanol (25°C) <sup>a</sup> | 8.0 dynes/cm               | 5.6 dynes/cm  |
| $\gamma_{o/w}$ supplemented with 0.5% n-pentanol (50°C) <sup>a</sup> | 1.5 dynes/cm               | 0.07 dynes/cm |
| Protein (mg/ml)  | 1.07                       | 1.07          |
| Glycolipid (mg/ml)   | 0.63                       | 0.63          |

<sup>a</sup>Measured against dodecane.

The standardized biosurfactant solution was observed to exhibit significantly lower interfacial tension values at pH 12.0. This pH value was adopted for all displacement studies.

#### Oil Displacement by Petroleum Sulfonates

Petroleum sulfonates were evaluated as synthetic surfactant controls for comparison to biosurfactant displacement studies. Pyronate and TRS 10-80 (Witco Chemical Co.) were optimized with respect to concentration and coagent for minimum interfacial tension values (Table 7).

TABLE 7  
OIL DISPLACEMENT BY PETROLEUM SULFONATES

|                        | Experiment A                    | Experiment B                                 |
|------------------------|---------------------------------|--|
| Bed Size               | 1" x 18"                        | 1" x 18"                                     |
| Amount of Oil          | 1000 mg                         | 1000 mg                                      |
| Temperature            | 122°F                           | 122°F  |
| Surfactant Flow Rate   | 0.5 ml/min                      | 0.5 ml/min                                   |
| Time                   | 24 hrs                          | 24 hrs                                       |
| Type of Oil            | Monagas crude                   | Monagas crude                                |
| Surfactant             | 5% pyronate +<br>1% isopropanol | 3% TRS 10-80 +<br>1% NaCl +<br>0.5% pentanol |
| $\gamma_{o/w}$         | $1 \times 10^{-1}$ dynes/cm     | $8.9 \times 10^{-3}$ dynes/cm                |
| Surfactant Volume      | 900 ml                          | 900 ml                                       |
| Total Oil Displacement | 0%                              | 62%  |

Pyronate was ineffective in the displacement of oil; whereas, TRS 10-80 displaced 62% oil in the effluent, indicating the effectiveness and efficiency of this surfactant in heavy oil displacement.

#### Continuous Flow Oil Displacement by Biosurfactant

The biosurfactant was evaluated under conditions of continuous flow at 50°C in the displacement of Monagas crude. The results shown in Table 8

illustrate the effectiveness of the biosurfactant under continuous flow for heavy oil displacement.

TABLE 8  
CONTINUOUS DISPLACEMENT OF HEAVY OIL BY BIOSURFACTANT AT 50°C

|                        | Experiment A  | Experiment B  |
|------------------------|---------------|---------------|
| Bed Size               | 1" x 18"      | 1" x 18"      |
| Amount of Oil          | 1%            | 0.5%          |
| Permeability           | 640 md        | 640 md        |
| Porosity               | 0.294         | 0.294         |
| Temperature            | 50°C          | 50°C          |
| Flow Rate              | 0.5 ml/min    | 0.5 ml/min    |
| pH                     | 12            | 12            |
| Type of Oil            | Monagas crude | Monagas crude |
| Time                   | 24 hrs        | 24 hrs        |
| Surfactant Volume      | 900 ml        | 900 ml        |
| Total Oil Displacement | 62%           | 80%           |

Discontinuous Oil Displacement by Biosurfactant at 30°C

Oil displacement under conditions of discontinuous flow of the biosurfactant solution was evaluated by alternating 8-12 hr soak cycles with collection of 1 bed volume of the biosurfactant solution. Results of these studies are shown in Table 9.

TABLE 9  
DISCONTINUOUS DISPLACEMENT OF HEAVY OIL BY BIOSURFACTANT AT 30°C

|                                     | EXPERIMENT    |                                |            |             |
|-------------------------------------|---------------|--------------------------------|------------|-------------|
|                                     | A             | B                              | C          | D           |
| Bed Size                            | 1" x 10"      | 1" x 10"                       | 1" x 10"   | 1" x 10"    |
| Amount of Oil                       | 0.5%          | 0.5%                           | 0.5%       | 0.5%        |
| Temperature                         | 30°C          | 30°C                           | 30°C       | 30°C        |
| Flow Rate                           | 2 ml/min      | 2 ml/min                       | 2 ml/min   | 2 ml/min    |
| pH                                  | 12            | 12                             | 12         | 12          |
| Oil Type                            | Monagas crude | Deasphaltened<br>Monagas crude | West Texas | Cerro Negro |
| Time                                | 56 hr         | 56 hr                          | 56 hr      | 56 hr       |
| Surfactant Volume/Flood             | 125 ml        | 125 ml                         | 125 ml     | 125 ml      |
| TOTAL EMULSIFIED OIL <sup>a</sup>   | 12%(4%)       | 18%(10%)                       | 0%(0%)     | 0%(0%)      |
| TOTAL TRAPPED OIL <sup>a</sup>      | 24%(32%)      | 15%(20%)                       | 23%(22%)   | 7%(5%)      |
| TOTAL BOUND OIL <sup>a</sup>        | 64%(58%)      | 67%(70%)                       | 77%(78%)   | 92%(95%)    |
| TOTAL OIL DISPLACEMENT <sup>a</sup> | 36%(36%)      | 33%(30%)                       | 23%(22%)   | 7%(5%)      |
| SURFACTANT DISPLACED OIL            | 0%            | 3%                             | 1%         | 2%          |

<sup>a</sup> Percentages listed are experimental values obtained followed by control values in parentheses. Controls were run with pH 12.0 buffer.

The biosurfactant was ineffective in the displacement of heavy crude oil by discontinuous flooding procedures. Interfacial tension values for the biosurfactant averaged 5 dynes/cm at 30°C, explaining, in part, the low efficiency in displacement of the surfactant at this temperature.

#### Oil Displacement by Biosurfactant at 50°C

The biosurfactant was evaluated at 50°C with discontinuous flow since studies with the isolated surfactant showed a decrease in interfacial tension values with increasing temperature. Table 10 shows the results of displacement experiments conducted at 50°C.

TABLE 10  
DISCONTINUOUS DISPLACEMENT OF HEAVY OIL BY BIOSURFACTANT AT 50°C

|                                     | EXPERIMENT    |             |                              |
|-------------------------------------|---------------|-------------|------------------------------|
|                                     | A             | B           | C                            |
| Bed Size                            | 1" x 10"      | 1" x 10"    | 1" x 10"                     |
| Amount of Oil                       | 0.1%          | 0.5%        | 0.5%                         |
| Temperature                         | 50°C          | 50°C        | 50°C                         |
| Flow Rate                           | 2 ml/min      | 2 ml/min    | 2 ml/min                     |
| pH                                  | 12            | 12          | 12                           |
| Salinity                            | 0%            | 0%          | 1%                           |
| Oil Type                            | Monagas crude | Cerro Negro | Deasphalted<br>Monagas crude |
| Time                                | 133 hr        | 127 hr      | 127 hr                       |
| Surfactant Volume/Flood             | 125 ml        | 125 ml      | 125 ml                       |
| TOTAL EMULSIFIED OIL <sup>a</sup>   | 25%(13%)      | 60%(0%)     | 12%(0%)                      |
| TOTAL TRAPPED OIL <sup>a</sup>      | 18%(11%)      | 17%(20%)    | 49%(61%)                     |
| TOTAL BOUND OIL <sup>a</sup>        | 57%(76%)      | 23%(80%)    | 39%(37%)                     |
| TOTAL OIL DISPLACEMENT <sup>a</sup> | 43%(24%)      | 77%(20%)    | 61%(61%)                     |
| SURFACTANT DISPLACED OIL            | 19%           | 57%         | 0%                           |

<sup>a</sup> Percentages listed are experimental values obtained followed by control values in parentheses. Controls were run with only pH 12.0 buffer.

Approximately 20% to 50% of the oil was displaced by the biosurfactant at 50°C. The pH 12-0 buffer control was effective in oil displacement, contributing an additive effect to the biosurfactant solution. A salinity of 1% NaCl reduced oil displacement to zero. This effect of salinity on the isolated biosurfactant was observed to increase interfacial tension values, indicating a decreased effectiveness of this biosurfactant in brine.

#### Oil Displacement by Biosurfactant Solution at 70°C

Discontinuous flow displacement of oil at 70°C was evaluated since the biosurfactant exhibited minimum interfacial tension values at this temperature. Table 11 contains the results of these experiments. A significant release of oil by the biosurfactant solution was observed at 70°C except in the case of a West Texas crude. Total oil displacement ranged from 48% to 90% with biosurfactant treatment.

TABLE 11  
DISCONTINUOUS DISPLACEMENT OF HEAVY OIL BY BIOSURFACTANT AT 70°C

|                                     | EXPERIMENT                   |               |            |             |
|-------------------------------------|------------------------------|---------------|------------|-------------|
|                                     | A                            | B             | C          | D           |
| Bed Size                            | 1" x 10"                     | 1" x 10"      | 1" x 10"   | 1" x 10"    |
| Amount of Oil                       | 0.5%                         | 0.5%          | 0.5%       | 0.5%        |
| Temperature                         | 70°C                         | 70°C          | 70°C       | 70°C        |
| Flow Rate                           | 2 ml/min                     | 2 ml/min      | 2 ml/min   | 2 ml/min    |
| pH                                  | 12                           | 12            | 12         | 12          |
| Oil Type                            | Deasphalted<br>Monagas crude | Monagas crude | West Texas | Cerro Negro |
| Time                                | 56 hrs                       | 56 hrs        | 24 hrs     | 24 hrs      |
| Surfactant Volume/Flood             | 125 ml                       | 125 ml        | 125 ml     | 125 ml      |
| TOTAL EMULSIFIED OIL <sup>a</sup>   | 76%(24%)                     | 27%(3%)       | 33%(8%)    | 40%(7%)     |
| TOTAL TRAPPED OIL <sup>a</sup>      | 14%(30%)                     | 51%(39%)      | 27%(52%)   | 8%(27%)     |
| TOTAL BOUND OIL <sup>a</sup>        | 10%(46%)                     | 22%(58%)      | 40%(40%)   | 52%(66%)    |
| TOTAL OIL DISPLACEMENT <sup>a</sup> | 90%(54%)                     | 78%(42%)      | 60%(60%)   | 48%(34%)    |
| SURFACTANT DISPLACED OIL            | 36%                          | 36%           | 0%         | 14%         |

<sup>a</sup> Percentages listed are experimental values obtained followed by control values in parentheses.

Controls ranged from 34% to 60% total oil displacement with an effective oil displacement by biosurfactant ranging between 14% to 36%.

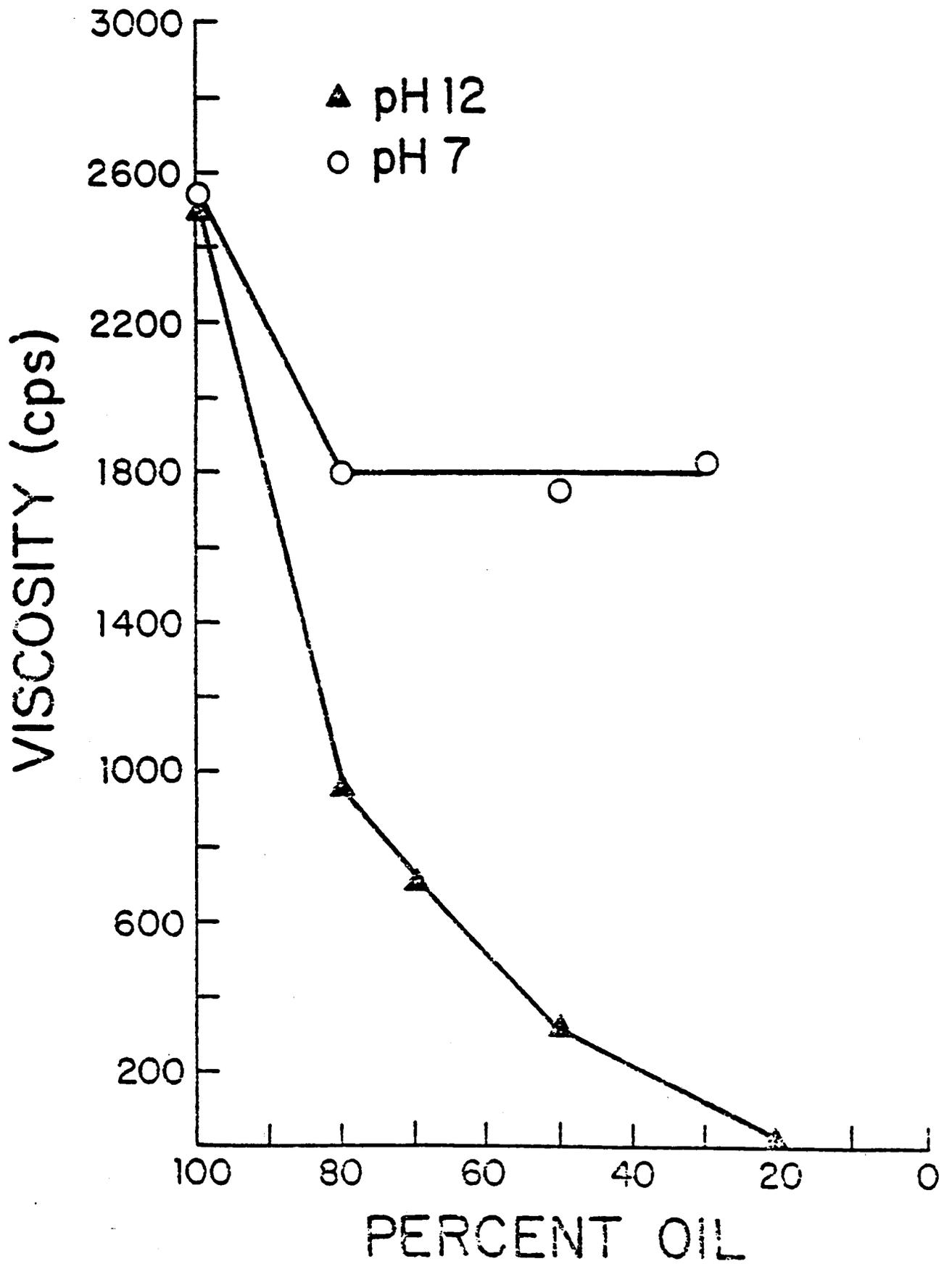
#### Viscosity Reduction of Heavy Oil by Biosurfactant

Viscosity reduction of Monagas crude by the biosurfactant at 70°C was tested as a function of the percent oil-in-water emulsion at pH 7.0 and pH 12.0. The 20% oil-in-water emulsion formed by biosurfactant treatment exhibited a viscosity reduction greater than 99% at pH 12.0; whereas, viscosity was reduced only 25% at pH 7.0 (Figure 2).

The spent growth medium of hexadecane-grown H-13A can be modified by volume reduction, coagent supplementation and pH adjustment, to offer a reasonable biosurfactant preparation having surface activity appropriate for testing in oil displacement. These studies indicate that this biosurfactant is effective in the displacement of heavy oil from sand packs by either continuous or discontinuous flow systems at 50°C or 70°C. The data further indicates that the biosurfactant is as effective as selected petroleum sulfonates in oil displacement phenomena. The inhibitory effect of salinity on the ability of this biosurfactant to displace oil represents a disadvantage in its application to high salinity reservoirs. Our preliminary data further indicates some degree of selectivity as to the type of oil which can be displaced by this biosurfactant. The biosurfactant is also quite effective in reducing the viscosity of heavy crude oils, a characteristic of importance to the transportation of such oils.

Other biosurfactants tested as oil recovery or displacement agents are few. Jenneman et al.<sup>10</sup> have reported on a biosurfactant-producing Bacillus licheniformis which mobilized crude oil with oil bank formation in sand packs. Future studies will reveal new surface-active molecules produced by microbial systems which have application to oil recovery technologies.

Figure 2



OIL DISPLACEMENT FROM CORES

Biosurfactant solution was evaluated for displacement of oil from a rock matrix. An Illinois naphthenic crude oil (API gravity 29.1) was used for these studies. The biosurfactant solution was supplemented with a synthetic brine solution consisting of NaCl, CaSO<sub>4</sub>, MgSO<sub>4</sub>, K<sub>2</sub>SO<sub>4</sub>, and Na<sub>2</sub>SO<sub>4</sub> with 365.3 ppm total dissolved solids. Core flood studies were conducted using a radial core configuration consisting of Berea sandstone 6" in diameter by 2" thick. The pore volume of the core was 175 ml with a porosity of 0.2 and an absolute permeability of 749 md. The core was conditioned by heating to 250°F for 2 hrs followed by heating at 850°F for 2 hrs and cooled. The procedure used for core flooding was core saturation by evacuation in produced water, determining the permeability to produced water by injection, saturating the core with Illinois crude oil by injection until pressure stabilized, determine the permeability to oil at residual water saturation (K<sub>ORW</sub>), water flood the core until pressure stabilized, determine permeability to water at residual oil saturation (K<sub>WRO</sub>), and inject biosurfactant solution at the rate of 1 ft/day.

Table 12 shows the effect of pH on interfacial tension of the biosurfactant solution at varying concentrations against Illinois crude oil.

TABLE 12

INTERFACIAL TENSION VERSUS pH FOR BIOSURFACTANT SOLUTION

| Biosurfactant Concentration, wt % | Interfacial Tension (dynes/cm) |        |
|-----------------------------------|--------------------------------|--------|
|                                   | pH 11.5                        | pH 6.2 |
| 0.5                               | 1.4 X 10 <sup>-2</sup>         | >2     |
| 1.0                               | 1.6 X 10 <sup>-2</sup>         | >2     |
| 2.0                               | 1.6 X 10 <sup>-2</sup>         | >2     |

Table 13 illustrates the changes in interfacial tension (IFT) by varying the concentration of biosurfactant.

TABLE 13

INTERFACIAL TENSION WITH ILLINOIS CRUDE OIL

| Biosurfactant Concentration, wt % | IFT (dynes/cm)         |
|-----------------------------------|------------------------|
| 1                                 | 1.8 X 10 <sup>-2</sup> |
| 2                                 | 1.5 X 10 <sup>-2</sup> |
| 4                                 | 1.2 X 10 <sup>-2</sup> |
| 6                                 | 1.7 X 10 <sup>-2</sup> |
| 8                                 | 1.1 X 10 <sup>-2</sup> |
| 10                                | 7.0 X 10 <sup>-4</sup> |
| 20                                | 7.0 X 10 <sup>-4</sup> |
| 40                                | 7.0 X 10 <sup>-4</sup> |
| 60                                | 1.0 X 10 <sup>-4</sup> |
| 80                                | 1.0 X 10 <sup>-4</sup> |
| 100                               | 1.0 X 10 <sup>-4</sup> |

Table 14 and Figure 4 summarizes core flood performance with H13-A biosurfactant. Figure 3 summarizes the control core flood performance lacking

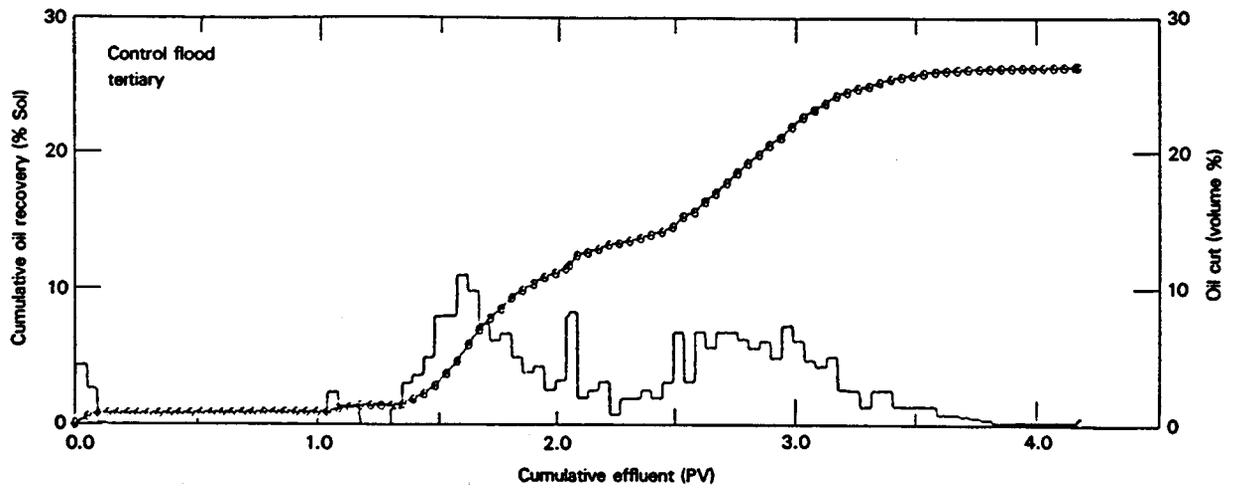


Fig. 3. Cumulative oil recovery and oil cut versus cumulative effluent

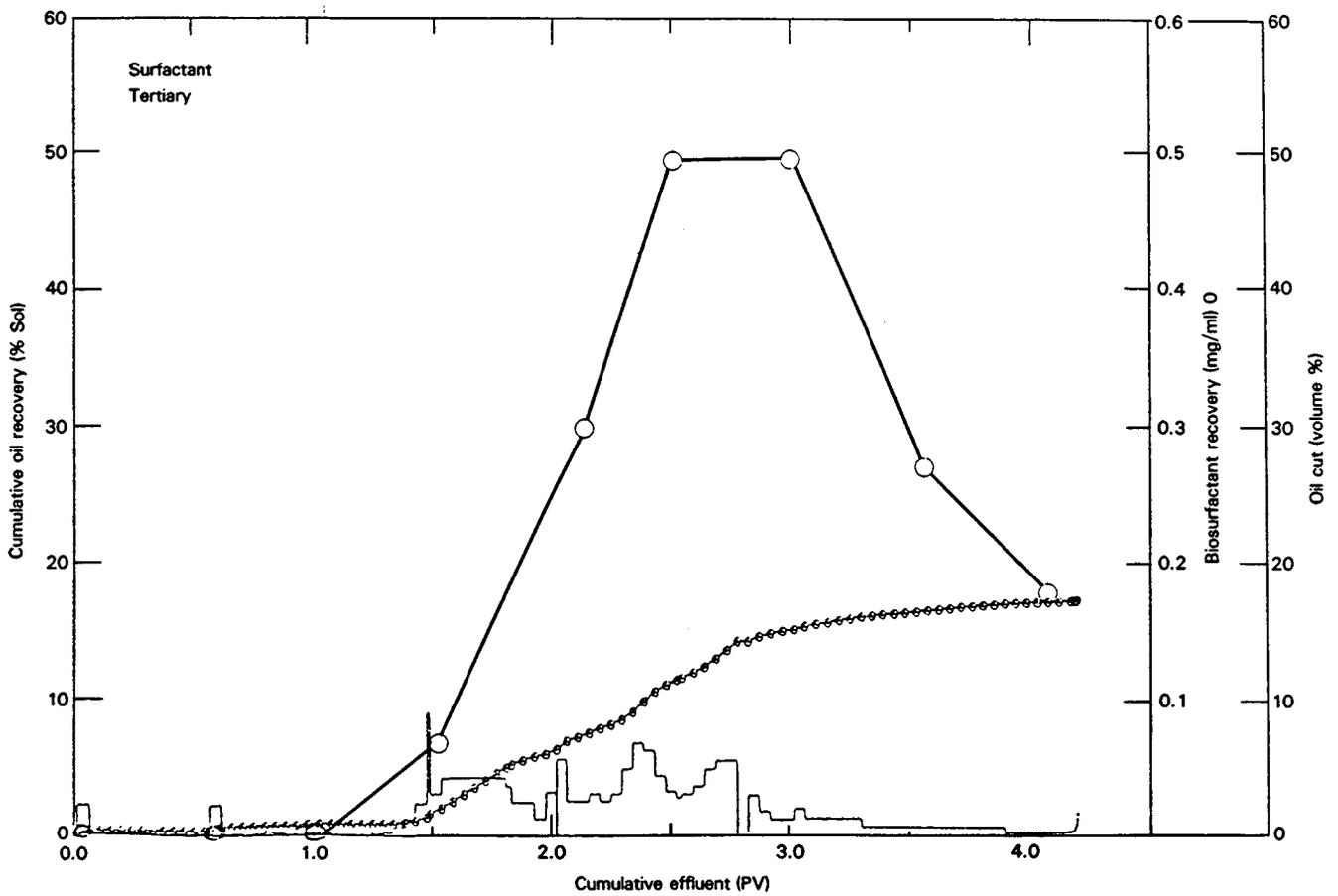


Fig. 4. Cumulative oil recovery and oil cut versus cumulative effluent

biosurfactant solution. As shown in Figure 4, approximately 95% of the biosurfactant slug was recovered in the effluent.

TABLE 14  
PERFORMANCE SUMMARY OF BIOSURFACTANT

| Run Parameters             | Units   | Control     | Biosurfactant |
|----------------------------|---------|-------------|---------------|
| Run Temperature            | 70°F    |             |               |
| <u>CORE PROPERTIES</u>     |         |             |               |
| Pore Volume                | ml      | 180         | 175           |
| Porosity                   | frac.   | .205        | .200          |
| Absolute perm              | md      | 535         | 749           |
| Eff Perm ( $K_{OWR}$ )     | md      | 18.0        | 17.4          |
| Eff Perm ( $K_{WRO}$ )     | md      | 2.6         | 10.5          |
| <u>Fluid Injection</u>     |         |             |               |
| Produced Water             | (PV) ml | (0.2) 36    | (0.2) 35      |
| Crude Oil                  | (PV) ml | (2.1) 378.1 | (2.1) 367.5   |
| Fresh Water                | (PV) ml | (2) 360     | (2.1) 367.5   |
| Biosurfactant Slug         | (PV) ml | (1) 180 (a) | (1) 175       |
| Fresh Water                | (PV) ml | (3) 580     | (3) 525       |
| <u>Oil Recovery</u>        |         |             |               |
| Initial Soil Sat (Soi)     | (PV) ml | (0.73) 131  | (0.73) 123    |
| Water Flood Recovery       | (PV) ml | (0.32) 58   | (0.30) 53     |
| Water Flood Residual (Sor) | (PV) ml | (0.41) 73   | (0.40) 70     |
| Tertiary Oil Recovery      | (PV) ml | (0.11) 19   | (0.07) 12     |
| Residual Sat (Sof)         | (PV) ml | (0.3) 54    | (0.33) 58     |
| Total Recovery             | (PV) ml | (0.43) 77   | (0.37) 65     |

(a) Injection fluid lacks biosurfactant.

The major findings from this set of experiments demonstrated that:

1. The biosurfactant solution reduced interfacial tension between crude oil and water at low concentrations, suggesting the potential for its application to tertiary oil recovery.
2. A preliminary core flood evaluation of the biosurfactant solution to establish performance criteria did not result in significant oil displacement by biosurfactant over control floods lacking biosurfactant.
3. Although significant tertiary oil recovery was not realized in this initial study, we were successful in defining specific operational parameters which may influence improved performance with respect to oil displacement.
4. A major operational parameter identified was loss of pH control within the core matrix. In addition, the biosurfactant slug, as injected, was retarded by approximately 1.5-2 pore volumes. The data indicates at least a 2-fold dilution of the initial biosurfactant slug with loss of effective biosurfactant concentration. The possibility of biosurfactant fractionation within the core exists. In general, a combination of poor mobility, adsorption/fraction of biosurfactant and loss of pH control appears to have contributed to the performance properties of the biosurfactant solution in oil displacement.

5. It was determined that approximately 95% of the biosurfactant slug injected was recovered from the core, indicating insignificant loss due to adsorption.
6. The biosurfactant solution yielded interfacial tension values lower than that obtained with many synthetic commercial products.
7. Future core-flood evaluations should evaluate static and dynamic adsorption of biosurfactant onto various types of reservoir cores, injection-flow characteristics of biosurfactant in reservoir cores of varying permeabilities (linear core-floods), chromatographic fractionation of biosurfactant during flow through porous media, and effect on interfacial tension reduction capacity due to surfactant type, pH, alkali used for pH adjustment, cosurfactant use, surfactant concentration, water salinity, oil type, temperature, time and added mobility control agents.

A further extension of this study was the assessment of resistance factors present during the tertiary portion of the core flood. Pressure measurement during the tertiary portion of the core flood offer indications of difficulties that arise with the injection of a slug. These values are translated as resistance factor ratios. The pressure at the onset of the tertiary portion of the flood is taken as a baseline. Doubling the pressure drop from injection port to production port at the same rate of injection doubles the resistance factor. The incremental resistance factor ratios for the tertiary portion of the core flood are shown in Figures 5 and 6. The resistance factors for the biosurfactant slug never decreased to a value approaching the starting point as did the control slug, indicating the biosurfactant slug caused a reduction in core permeability. The level of permeability reduction were not severe and could be the result of numerous factors, including biosurfactant adsorption, precipitation or resaturation of the core with oil. The residual resistance factor of 2.9 indicates very little permanent permeability reduction occurred with the injection of the biosurfactant slug.

#### OIL DISPLACEMENT FROM TAR SANDS

A series of experiments were conducted to evaluate the effectiveness of biosurfactant solution in the release of oil from Athabasca tar sands and Kentucky tar sands.

Tar sands (100 gms) were incubated with biosurfactant solution at 30°C with shaking at 250 RPM in a 500 ml Erlenmeyer flask. Parallel controls were run concurrently lacking biosurfactant solution. The total amount of oil associated with 100 gms of tar sands was determined by direct solvent extraction with benzene, removal of solvent and direct weighing of the recovered oil. Oil release by biosurfactant was determined by benzene extraction of the aqueous layer and direct weighing of the recovered oil. Biosurfactant solution released 42% of Athabasca tar sand oil and 65% of Kentucky tar sand oil.

#### CONCLUSIONS

The objectives of this research were to evaluate roles for microbial processes that would effectively reduce the viscosity of heavy crude oils. These objectives relate to applications such as enhanced oil recovery, transportation of heavy oil and tar sand or oil shale displacement technology. The research concerned the elucidation of the chemical nature and physical properties of biosurfactant in relationship to specific characteristics for application in oil production, processing and transportation.

The accomplishments of this research program have been the isolation of microorganisms with the ability to reduce the viscosity of heavy oils 95% or

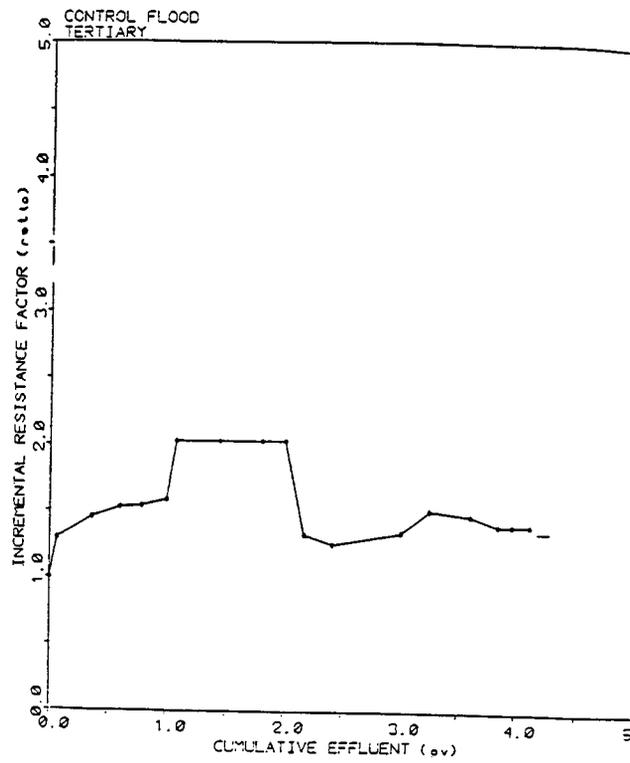


Fig. 5. Incremental Resistance Factor-Control

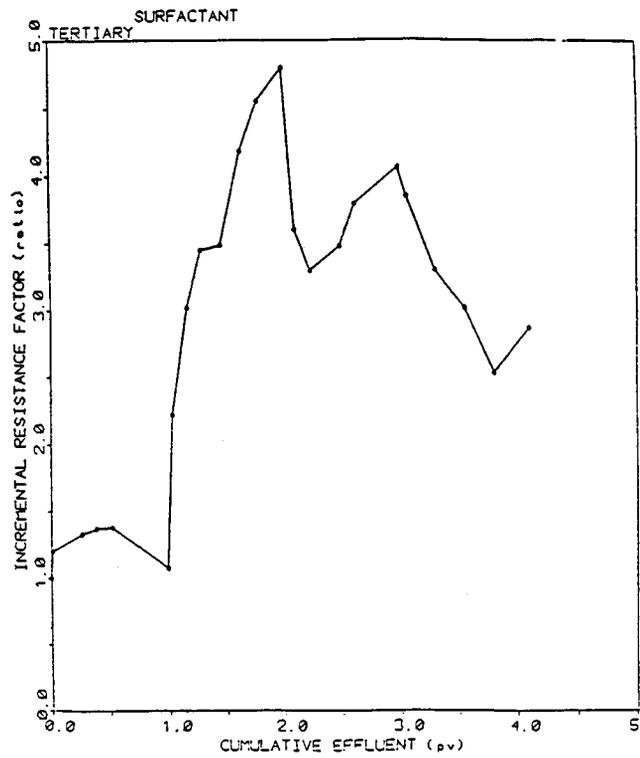


Fig. 6. Incremental Resistance Factor-Biosurfactant

greater through the formation of stable oil-in-water emulsions by extracellular biosurfactants. These microorganisms produce surface active products (biosurfactants) that are equal to, if not superior, to many synthetic surfactants. The biosurfactants are extracellular products which exhibit effective physical properties.

One bacterial isolate (H-13A) was studied in detail concerning the general physiology of biosurfactant production, the chemical nature and physical properties of the biosurfactant. H-13A biosurfactant is an extracellular product with excellent surface-active characteristics as either a purified product or as a component of the spent medium. H-13A biosurfactant is a glycolipid with a molecular weight of approximately 1500. Fatty acyl groups are covalently linked to specific hydroxyl groups of the oligosaccharide backbone. Deacylation of fatty acids destroys the surface-active properties of the molecule. Chemical composition consists of glycerol, trehalose, glucose, glucuronic acid plus normal saturated and 2-hydroxy fatty acids.

Physical properties of the biosurfactant are interfacial tension values of  $2 \times 10^{-2}$  mN/m at a CMC of 1.5 mg/ml to  $6 \times 10^{-5}$  mN/m with an alcohol coagent. The effective alkane carbon number is  $C_{10}$  or  $C_{11}$ . The biosurfactant exhibits good surface-active properties in the presence of brine and divalent cations, is active between pH 3.5-12.0, is stable to 150°C, forms stable oil-in-water emulsions, and exhibits phase behavior with formation of a middle phase.

Biosurfactant applications studies have demonstrated 95-98% viscosity reduction of heavy oils, oil displacement from sand packs and tar sands, and a potential for oil displacement from rock matrices.

Various technical problems exist in the oil industry with respect to cost-effective enhanced oil recovery technologies (particularly heavy oil), the pipelining of heavy oils and oil recovery from tar sands and oil shales. This research program has opened a potentially new biotechnology that offers promise as a significant CEOR technology for the production of heavy oils plus the pipeline transportation of heavy oils. This biotechnology promises to be cost-effective by being amenable to large scale fermentation technology with the production of spent culture media possessing appropriate physical properties. The application of spent culture media as the biosurfactant solution eliminates downstream process costs associated with the recovery of biosurfactant. Further, strain improvement through genetic and physiological engineering will offer substantial improvements to the cost-effectiveness of this biosurfactant. The continuing development and application of such new biotechnology would serve to improve the overall economics and accessibility of heavy oil, representing a further step towards greater energy independence.

#### ACKNOWLEDGEMENT

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