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ANNEX V - MICROBIAL ENHANCED OIL RECOVERY RESEARCH

Summary Annual Report for the Period
1990-1991

By
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Work performed under Contract No.DE-FG07-89BC14445

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SUMMARY ANNUAL REPORT

TITLE: Annex V - Microbial Enhanced Oil Recovery Research

CONTRACT NUMBER: DE-FG07-89BC14445

CONTRACT ORGANIZATION: State of Texas

PRINCIPAL INVESTIGATORS: Mukul M. Sharma and George Georgiou

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(i) the production, isolation, chemical characterization and study of the physical properties of microbially produced surfactants,

(ii) development of simulators for MEOR and

(iii) model studies in sandstone cores for the characterization of the interactions between growing microbially cultures and oil reservoirs,

(iv) design of operation strategies for the sequential injection of microorganisms and nutrient in reservoirs.

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surfactant on the rock surface. Generally, the interfacial tension must be lowered to 10^{-2} to 10^{-3} mN/m, corresponding to a capillary number $N_{Ca} = 10^{-3}$ to 10^{-4}, before significant oil recovery is achieved. Some biosurfactants are capable of reducing the interfacial tension to such values.

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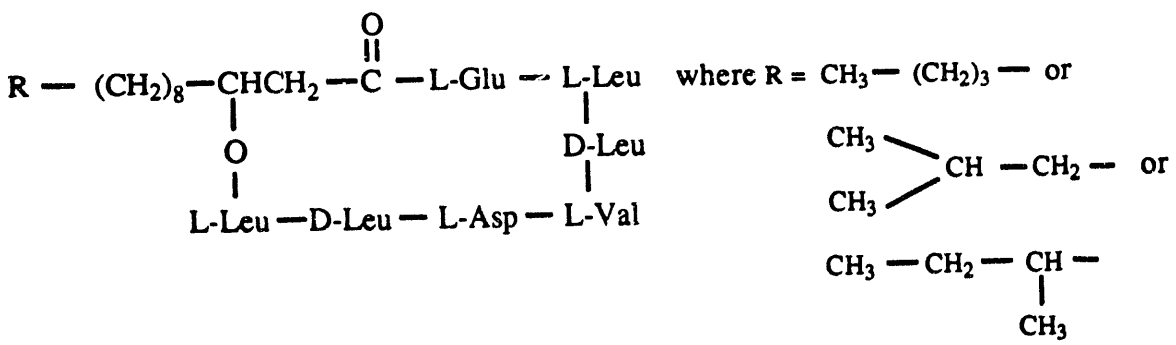
To characterize the chemical structure of the biosurfactant, infrared and mass spectroscopies were performed. The infrared spectrum indicated the biosurfactant was a lipopeptide. The presence of an peptide was demonstrated by the bands with wavenumber 3430 corresponding to amide NH, wavenumber 1655 corresponding to amide CO, and wavenumber 1534 corresponding to CNH. The presence of an aliphatic chain was also suggested by the bands with wavenumber 2800 - 3000. As ester carbonyl group (wavenumber 1733) suggesting the presence of a lactone ring was also observed. The fast atom bombardment mass spectrum (FAB/MS) indicated the biosurfactant was a homogeneous preparation with a molecular weight of 1036 Daltons.

Based on the spectral data described in previous reports and the various NMR experiments the following structure was proposed.



The biosurfactant consists of approximately equal amounts of iso- and anitiso-lipopeptides and trace amounts of normal-lipopeptide.

Interfacial Properties

The ability of the biosurfactant in reducing the interfacial tension of the fermentation broth at different pH was investigated. The biosurfactant had an optimal activity at pH 6.0. The interfacial tension measured at pH 6.0 was at least 20 times lower than that measured at pH 7.0. It was also observed that the biosurfactant had a higher

activity in the presence of higher concentration of NaCl. At pH 6.0, the interfacial tension of the broth in the presence of 5% NaCl was at least 2 times lower than that of the broth in the presence of 0.5% NaCl.

The critical micelle concentration (CMC) was determined by measuring the interfacial tensions of biosurfactant solutions prepared by serial dilution. The CMC was determined to be approximately 10 mg/l. The interfacial tension of the aqueous phase was still lower than 1 mN/m at a concentration as low as 5 mg/l. These results indicate that the biosurfactant produced by *B. licheniformis* JF-2 is one of the most effective biosurfactants known to date.

Mechanism of deactivation

Samples from three different stages of microbial growth were taken. Each sample was divided into three fractions, designated as A, B, and C. Fraction A was centrifugated to remove cells. To fraction B, 50 μ molar of carbonyl cyanide m-chlorophenylhydrazone (CCCP), an effective uncoupler of oxidative phosphorylation in mitochondrial systems, was added. To all fractions 50 - 70 mg/l of biosurfactant was added. These fractions were then incubated at 42°C with shaking. Samples from each fraction were taken periodically and analyzed with HPLC. In all three stages the concentrations of biosurfactant remained essentially constant for cell-free culture, (fraction A) whereas the concentrations of biosurfactant decreased significantly for control cell culture (fraction C) from 60 - 100 mg/l to 5 - 30 mg/l. On the other hand, the concentration profiles of biosurfactant for cell culture with CCCP, fraction B, varied. The concentration of biosurfactant for fraction B sampled at the mid-exponential phase remained constant during incubation, while the biosurfactant concentrations of those sampled at the late-exponential phase and stationary phase decreased significantly (fraction C). These results suggest that (1) the deactivation is independent of

extracellular compounds; (2) the deactivation is energy independent; and (3) the deactivation only occurs in the presence of cells sampled beyond the late-exponential phase.

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From laboratory experiments of bacteria transport and growth in porous media, the important parameters that control these processes are identified. Experiments were conducted with the facultative species, *Bacillus Licheniformis* (JF-2), in sandpacks for single - and two - phase flow. The effects of flow velocity injected concentration, degree of dispersion, salinity, temperature, ad the presence of a residual phase were investigated. The retention of bacteria and permeability reduction is significantly higher in the first few centimeters (~ 2.5 cm) of the porous medium as compared to the downstream sections. The log - jam effect seems to be the primary mechanism for bacteria retention and permeability reduction in the upstream section, but for downstream sections size exclusion may be the dominant mechanism. Added dispersants (polyvalent anions) low salinities, and higher temperatures enhanced bacteria transport. This clearly shows the importance of coagulation and bridging in the retention of bacteria. In contrast to the results from flow in a single - phase system, the presence of a residual oil phase increases bacteria retention.

Task 3. Simulation of MEOR Processes

A compositional numerical simulator is developed for simulating the transport and growth of bacteria and oil recovery in MEOR processes. The simulator can be used for predicting the transport and growth of bacteria in porous media, reduction of permeability from the retention of bacteria, consumption of nutrient, production of

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It is found that the permeability, the permeability contrast between layers, ratio of bacteria size to mean pore size, injected concentration, retention and growth parameters, and effective radius parameters are important in the transport and growth of bacteria in porous media. During bacterial suspension injection in a two - layer system, the low permeability layer is damaged more than the high permeability layer. These results show that for the selective plugging process to be effective, it is necessary to ensure zonal isolation to prevent bacteria injection into the low permeability. layer.

SIGNIFICANT ACCOMPLISHMENTS

1. We have obtained ultra low interfacial tensions (0.003 mN/M) between decane and 5% NaCl brine using biosurfactants obtained from *Bacillus Licheniformis*, JF-2. This is the lowest IFT ever reported for biosurfactants.
2. We have developed a method to isolate the biosurfactant from the growth medium.
3. The structure of the isolated biosurfactant has been determined.
4. Several techniques have been proposed to increase the yield of the surfactant.
5. An MEOR simulator has been completed.

OTHER ACCOMPLISHMENTS

1. Systematic discussion of the mechanisms important in MEOR processes.
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SIGNIFICANCE TO EOR RESEARCH PLANS

We have shown that biosurfactants hold the greatest promise for enhancing oil recovery through microbial treatments. Furthermore, we have demonstrated that biosurfactants can reduce interfacial tensions sufficiently to affect oil recovery. We are currently working on better understanding of the nature of these biosurfactants so that they can be optimally extracted and used. We are also actively pursuing the question of how these biosurfactants can be used in an in - situ process. This problem is intimately tied to the problem of bacteria transport and growth in rocks.

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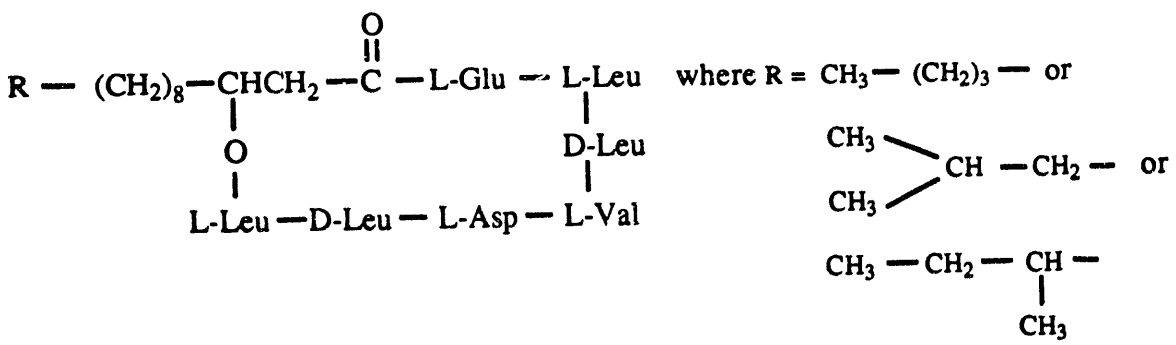

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