

POTENTIALS OF *BACILLUS STEAROTHERMOPHILLUS* FOR ENHANCED OIL RECOVERY A LABORATORY EXPERIMENT*

M. Udiharto, S. Kadarwati, E.H. Legowo, M. Rahman, E. Jasjfi

ABSTRACT

B. stearothermophilus was isolated from among microbes recovered from a formation water from an oil field in Indonesia, where the temperature was 55°C.

Laboratory experiment was conducted to measure the growth, and to study the effects of its activity on the physical characteristics of reservoir rock and crude oil in an oil well.

The bacteria activities were found to increase the porosity of reservoir rock and decrease the interfacial tension of oil. An increase of 3,5% and 12,5% was observed in the porosity of silicate and carbonate rock, respectively, while the interfacial tension of crude oil was decreased by 29% within 28 day period. These properties are important for enhanced oil recovery (EOR).

I. INTRODUCTION

From a number of works reported on Microbial Enhanced Oil Recovery (MEOR) it was evident that many bacterial species have potential for development and subsequent application in improving the recovery of oil from its reservoir formation (Cooper and Goldenberg, 1987; Zajic et al., 1982; Wang, 1982; Matsuyama et al., 1992; Yakimov and Fredrickson, 1992). This is of especial importance in the age of decreasing oil reserves and increasing energy requirement, particularly liquid fuels for transportation.

MEOR techniques depend largely on the selection of a single or mixed culture of microorganism capable of producing biochemical products such as gases (CO₂, H₂, H₂S, CH₄), or carboxylic acids (acetic, formic, valeric, or propionic acids), solvents (alcohols, aldehydes, or ketons), or surfactants; each of which can be used to aid the flow and removal of oil from the porous formation rocks.

More than seventy genera of microorganisms are currently known to produce extracellular polysaccharides from carbohydrate as the source of carbon for the bacteria. Others such as *Brevibacterium viscosus* is known to produce them from other sources of carbons, such as crude oil or heavy paraffin liquids (Wang, 1982).

Rhodococcus sp., a bacteria isolated from Kuwait soil and cultured in hydrocarbon media such as kerosene or paraffin is found to produce biosurfactants, (Ruwaida et al., 1991). *Bacillus subtilis* ATCC 21332, a mutant prepared by ultraviolet treatment, produces biosurfactants at three times the activity of its parent strain (Mulligan et al., 1989).

Ramana and Karanth (1989) reported that *Pseudomonas aeruginosa* CFTR-6 is capable of producing biosurfactants with high emulsifying power in glucose or hydrocarbon containing media.

The works of Mc Innerney et al. (1985, 1990) indicated that *Bacillus licheniformis* strain JF-2 in anaero-

*Paper presented on ACHEMASIA 95 - International Meeting on Chemical Engineering and Biotechnology - 3rd Exhibition and Congress, 19 May 1995 in Beijing, China.

bic environment can produce a strong biosurfactant, capable of reducing interfacial tension of medium to less than 30 mN/m. This bacteria can also produce other types of biosurfactants at aerobic conditions.

Gula et al. (1982) isolated *Clostridia* and in subsequent screening found that certain isolates are capable of producing gases, acids, or solvents, which have potentials for application in enhancing oil recovery.

All such works indicated that many microorganisms have the capability of producing biochemical products which are useful for enhancing oil recovery. However, it is necessary to first isolate such microorganisms, then select the most potential for further improvement of its activities so that it can be employed to increase oil production and recovery by cost effective and environment friendly biotechnology.

The present work is an attempt to formulate the microbial strains from Indonesian soil. Such work is seen to have potential for success in view of the richness of the country in biodiversity, and the importance of oil resources in the country's development and economy.

II. MATERIAL AND METHODS

The microbe used in this study, *Bacillus stearothermophilus*, is isolated from formation water of an Indonesian oil field. This bacteria is a thermophilic aerobic microbe, active at about 55°C.

B. stearothermophilus is isolated and activated in dextrose casein pepton (DCP) media of pH 6.8 ± 0.2. Such media had been successfully used for thermophilic microbe by Williams (1936) in his study of identification and population counts of *Bacillus* species.

B. stearothermophilus culture was incubated in this study in an incubator at a temperature of about 55°C and maintained in static condition for a certain period of time. Each treatment was made in duplicate.

Observation was first made on the growth of *B. stearothermophilus* in 100 ml DCP media. Incubation was carried out for 12 hours. One milliliter sample was

drawn every three hours from 0 to 12 hours for which a population count was made.

The next step was to observe the activities of *B. stearothermophilus* in increasing the porosities of core samples. Two types of cores were used, namely a carbonate and a silicate type. Each core sample was initially sterilized and its porosity was measured.

B. stearothermophilus was cultured in 200 ml of DCP media and each core sample was immersed in the culture, incubated for a period of time. Population count of the microorganism, pH measurement of the media, and porosity measurement of the core were made at day 0, 14, and 28.

Test was also made on the effect of *B. stearothermophilus* activities in reducing oil-water interfacial tension. For this test, a 150 ml DCP culture was mixed with 150 ml of crude oil sample and allowed for a period of time. At day 0, 7, 14, 21, and 28, samples were drawn and population count, pH reduction and interfacial tension measurement were made.

Population counts were carried out as plate count (in cells/ml). Helium porosimeter was used for porosity measurement (as % void), and spinning drop tensiometer was the instrument employed for interfacial tension measurement (in mN/m).

III. RESULTS AND DISCUSSION

Figure 1 shows the growth of *B. stearothermophilus* in DCP media at about 55°C during 12 hours. High growth rate was observed after 9 hours.

The effect of *B. stearothermophilus* on pH of the medium where silicate and carbonate core sample is immersed is shown respectively in Figure 2 and Figure 3. As shown, *B. stearothermophilus* cultures in silicate as well as in carbonate cores produce bioacids. Atkinson et al. (1975) speculated that the carboxylic acids produced include acetic, propionic and isobutyric acids. Such bioacids is water soluble and cause reduction in the pH of the medium.

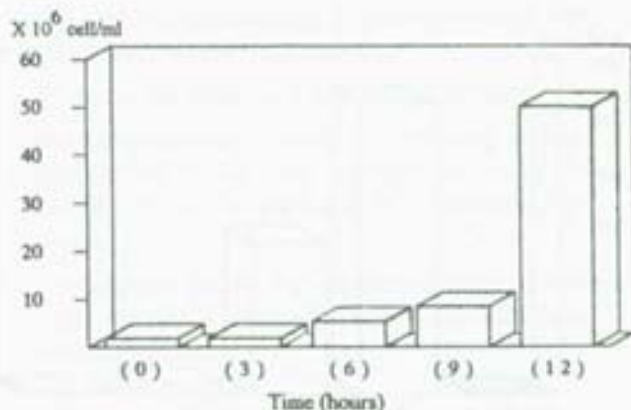


Figure 1
The growth *B. stearothermophilus* in DCP media at about 55°C during 12 hours incubation

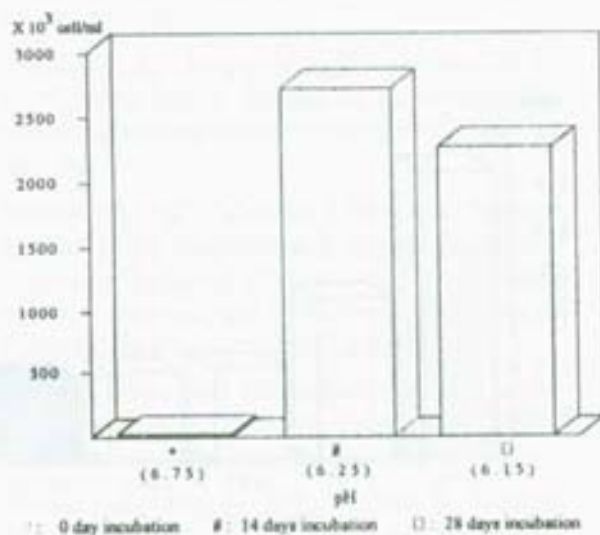
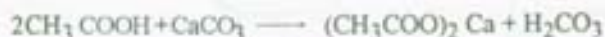


Figure 2
The effect of *B. stearothermophilus* on pH of during 28 days incubation the medium where silicate core sample is immersed

Acetic acid produced as the results of *B. stearothermophilus* metabolism effects the core by reacting with its carbonate components. The following reaction occurs:



In acid environment the carbonic acid formed reacts with CaCO_3 to produce calcium bicarbonate by the following reaction $\text{CaCO}_3 + \text{H}_2\text{CO}_3 \longrightarrow \text{Ca}(\text{HCO}_3)_2$.

It can be understood that the bioacids present gradually dissolve the carbonate and this enlarges and increases the porosity of the cores.

Both the silicate as well as the carbonate core samples experienced an increase in porosity when immersed in *B. stearothermophilus* culture, as shown in Figure 4. Higher increase in porosity was observed in carbonate core as compared to the silicate ones. A 28 day incubation resulted in 12.49% increase in porosity of carbonate core while a 3.57% increase was observed in the silicate one. This is consistent with the carbonate content of each type of cores, where in that the silicate type is relatively limited.

The observation on *B. stearothermophilus* activities in DCP media containing crude oil is demonstrated in Figures 5 and 6. Figure 5 relates the population growth the effect on the pH of the medium.

The growth of *B. stearothermophilus* affects the interfacial tension of the crude oil and water phases (Figure 6). A steady decrease is observed, from 3.170 mN/m to 0.945 mN/m, or a decrease to 29.81% in 28 days. Such decrease in interfacial tension is attributed to some biosurfactant produced in *B. stearothermophilus* metabolism.

As found in this work, *B. stearothermophilus* activities have all the effects that can be useful in enhancing oil recovery. These are increasing the porosity of the core as well as reducing the oil-water interfacial tension. As indicated by Bubela (1970), *B. stearothermophilus* cultured at high pressure (higher than 20,000 kPa) and high temperature (higher than 60°C) experienced a change in form, namely rod-shaped to a spherical form.

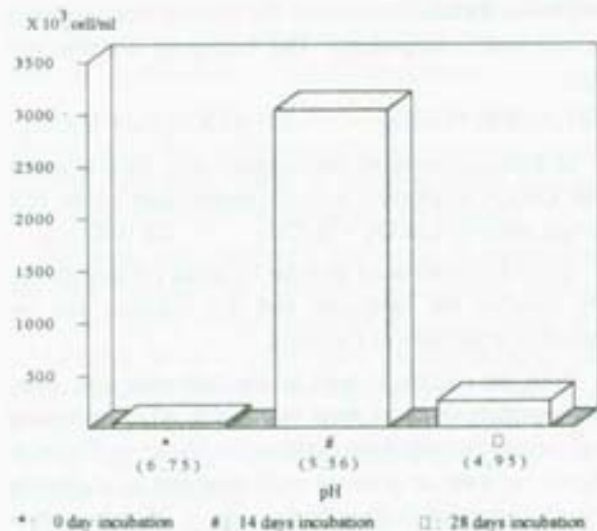


Figure 3
The effect of *B. stearothermophilus* on pH of the medium where carbonate core sample is immersed during 28 days incubation

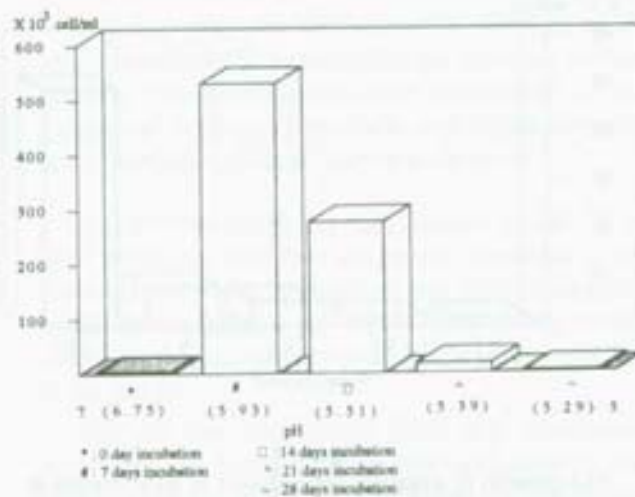


Figure 5
Relates the population growth of *B. stearothermophilus* the effect on the pH of the medium

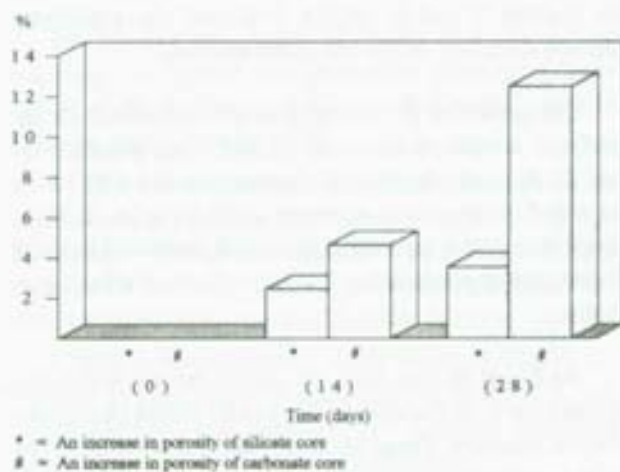


Figure 4
An increase in porosity of silicate and carbonate core when immersed in *B. stearothermophilus* culture

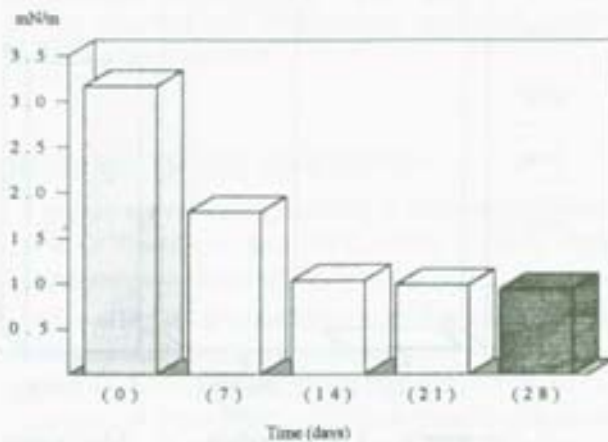


Figure 6
The growth of *B. stearothermophilus* and the effect on the interfacial tension of crude oil and water phase

IV. CONCLUSION

Our work demonstrated that *B. stearothermophilus* has the following useful characteristics:

- Thermophilic remains active at high pressure.
- Bioacid producing, capable of increasing silicate core porosity by 3.5% and carbonate one by 12.5% during a 28 days incubation at 55°C and static condition.
- Biosurfactant producing, capable of reducing oil-water interfacial tension by 29% at the incubation condition as above.

Such characteristics promise application in microbial enhanced oil recovery (MEOR).

REFERENCES

1. Abu-Ruwaidh, A.S., Banat, I.M., Haditirto, S., Salem, A., Kadri, M., 1991, "Isolation of Biosurfactant Producing Bacteria Product Characterization, and Evaluation", *Acta Biotechnologica*, 11, (4), 315-324.
2. Atkinson, A., Evans, C.G.T. and Yeo, R.G., 1975, "Behaviour of *Bacillus stearothermophilus* Grown in Different Media", *J. of Applied Bact.*, 38, 301-304.
3. Bubela, B., 1982, "Combined Effects of Temperature and Other Environmental Stresses on Microbiologically Enhanced Oil Recovery," *Proceedings of 1982 International Conference on Microbial Enhanced of Oil Recovery*, Oklahoma.
4. Cooper, D.G. and Goldenberg, G., 1987, *Surface Active Agents from Two Bacillus sp.*, *Applied and Environmental Microbiology*, Feb., 224-229.
5. Grula, E.A., Russell, H.H., Bryant, D., Kenaga, M. and Hart M., 1982, "Isolation and Screening of *Clostridia* for Possible Use in Microbially Enhanced Oil Recovery", *Proceedings of 1982 International Conference on Microbial Enhancement of Oil Recovery*, Oklahoma.
6. Matsuyama, T., et al., 1992, "Surface Active Novel Glycolipid and Linked 3-Hydroxy Fatty Acids Produced by *Serratia rubidua*", *Journal of Bacteriology*, Mar., 1769-1776.
7. Mulligan, C.N., Chow, T.Y.K. and Gibbs, B.F., 1989, "Enhanced Biosurfactant Production by a Mutant *Bacillus subtilis* strain", *Appl. Microbial Biotechnol.*, 31, 486-489.
8. Remana, K.V. and Karanth, N.G., 1989, "Factors Affecting Biosurfactant Production Using *Pseudomonas aeruginosa* CFTR-6 under Submerged Conditions", *Chem. Tech. Biotechnol.*, 45, 245-257.
9. Wang Xiuyuan, 1982, "A Microbial Polysaccharide Produced from Crude Oil or Liquid Paraffin and Its Application in Petroleum Industry", *Proceedings of 1982 International Conference on Microbial Enhanced of Oil Recovery*, Oklahoma.
10. Yakimov, M.M. and Fredrickson, H.L., 1992, "Potential of *Bacillus licheniformis* for Insitu Enhanced Oil Recovery from Natural Reservoirs", GBF Department of Microbiology, Rascheroderweg, 1.3300, Braunschweig, Germany.
11. Zajic, J.E., Hostak, J. and Seffens, W., 1982, "The Effect of HLB on the Surface Activity and Bitumen Extraction Capability of *Corynebacterium fascians*", *Proceedings of 1982 International Conference on Microbial Enhanced of Oil Recovery*, Oklahoma. □