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variation of the nutrient for microbes affect the type and the quantity of bioproduct produced by bacteria. Every microorganism has different enzymatic systems, which are specific in breakingdown of certain nutrient compounds. Nutrient manipulation may provide a mechanism to increase bioproduct activities.

Crude oil is a carbon and energy resource, which is suitable for microbial growth but lacks of nitrogen and phosphor (Leahy and Colwell. 1990). Stone Mineral Salt Solution (SMSS) contains essential nutrient for microbe and has been used for hydrocarbonoclastic microbe studies (Astuti. 2003). The objective of this study is to examine the feasibility of Stone Medium Salt Solution (SMSS) as a potential nutrient for biosurfactant and biosolvent production on MEOR application.

II. MATERIALS AND METHODS

A. *Microbe Culture and Crude Oil Preparation*

The microbial mixed culture as consortia was isolated from formation water in nutrient broth (NB) as basal medium. The mixed culture was used because no single pure culture could completely degrade crude oil due its highly complex structure (Astuti. 2003). The identification showed that microbial consortia were dominated by *Bacillus* sp. and *Pseudomonas* sp.

Three crude oils obtained from Indonesian oil well were tested as sample identities of C1, C2, and C3. The properties of crude oils are described in Table 1.

These properties are still in the range of general screening parameter for MEOR application. For example, salinity is less than 10 %, temperature should be less than 170°F (76.7°C), depth is less than 8000 ft (2440 m), permeability is greater than 75 mD, and pressure should be less than 20,000 psi (NIPER.1986).

B. *SMSS Medium*

The composition of SMSS is described in Table 2. All the compounds of SMSS medium were added and mixed in distillate water. Finally, the solution was sterilized in autoclave at 121°C for 15 minutes.

The previous test was used in order to observe microbial growth in 0%, 25%, 50%, and 100% (v/v) of SMSS concentrations in Nutrient Broth (NB) medium, based on the total plate count. The variation of SMSS concentrations in NB is shown in Table 3. In erlenmeyer flask, 10% (v/v) of microbial mixed culture was added in 500 mL medium and incubated for 24 hours at room temperature.

C. *Tube Test*

25 mL of mixed culture and 10 mL of crude oil were added in 250 mL of sterile SMSS medium in 500 and 1000 mL erlenmeyer flasks as microaerophilic and aerophilic conditions. The culture was activated using aseptic technique and set in a shaker incubator for 7 days at 55°C. The 7-day incubation will give the possibility for certain complex-hydrocarbon-degrading-microbes to grow (Horowitz et al. 1975).

The microbial activities were determined by population, pH, interfacial tension, and viscosity under microaerophilic and aerophilic conditions, at 0-day and 7-day incubations. Microbial consortia population was determined by using total plate count, pH value was observed with Hanna pH meter, interfacial tension was measured using plate method from Processor Tensiometer Kruss, and viscosimeter

Table 1
Crude oil properties

Sample	Crude type	Rock type	Temp. (°F)	Depth (m)	Pressure (psi)	Water content (%)	Water salinity (ppm)
C1	Asphaltic	Sandstone	146	943 - 958	1600 (1961 C) 300 (1996 AB)	100	68,5
C2	Asphaltic	Sandstone	146	1089.5-1108.5	900 (C)	87	4017
C3	Asphaltic	Sandstone	146	1106 - 1121	600 (C) 150 (D)	94,5	4017

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Haake was used for viscosity measurement.

D. *Microbial Core Flooding (MCF) Test*

The best crude oil and SMSS concentration were examined in MCF test. 500 mL of medium with 10% v/v microbial consortia were activated in experiment temperature during 24 hours. The crude oil and formation water were filtered to avoid clogging by grains. The Classach S2 was used as core plug in this test. The core was 7.65 cm in length and 3.75 cm in diameter. The properties of the tested core are listed in Table 4. Before used, the core plug was washed with methanol and toluene respectively, dried in the oven, and measured the porosity and permeability (Ka).

The core flooding equipment used in this study is shown in Figure 1. The vessel was connected to the core plug inside the "Hassler" core holder. The oven was set at 64°C as reservoir temperature. A confining pressure at 500-1000 psi was main-

Table 2
Composition of Stone Mineral Salt Solution

No.	Compounds	mg/L stock
1	Ammonium nitrate (NH_3NO_3)	0,25
2	Sodium hydrogen phosphate (Na_2HPO_4 12 H_2O)	1
3	Manganese II chloride ($MnCl_2$ 4 H_2O)	0,2
4	Potassium hydrogen phosphate (K_2HPO_4)	0,5
5	Calcium carbonate ($CaCO_3$)	5
6	Magnesium sulfate ($MgSO_4$ 7 H_2O)	0,5
7	Yeast extract	0,1

(Astuti. 2003)

Table 3
Variation of SMSS Concentration

No.	SMSS Concentration (%)	NB : SMSS
1	0	100 % : 0 %
2	25	75 % : 25%
3	75	50 % : 50 %
4	100	0 % : 100 %

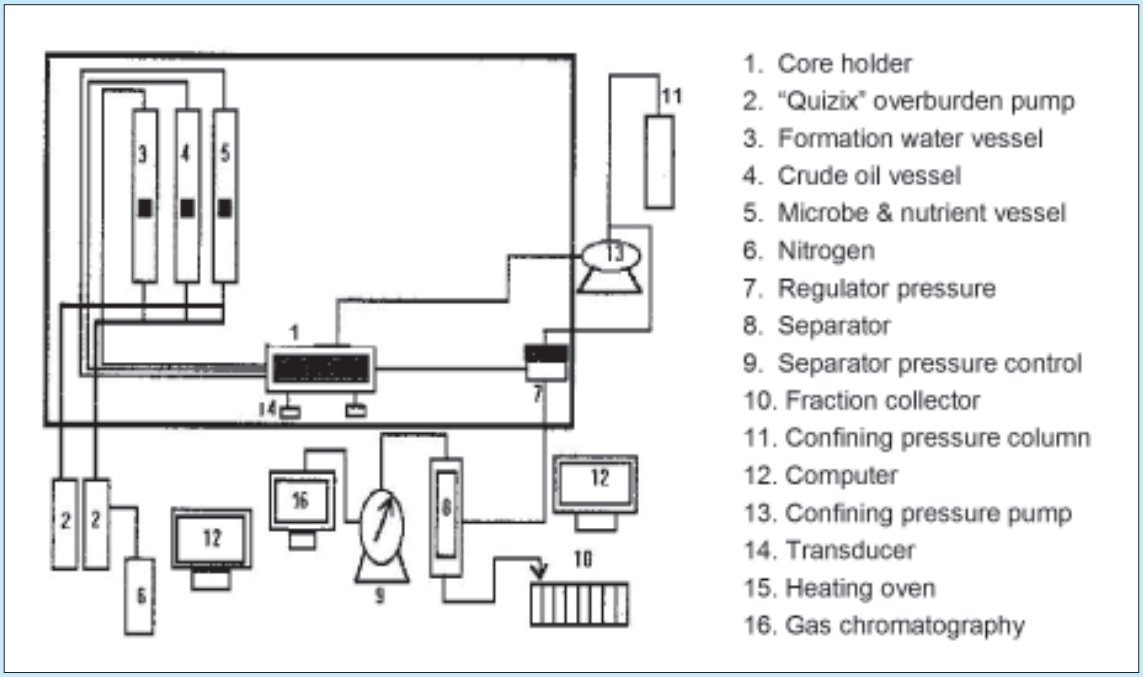

Figure 1
Schema of core-flooding equipment

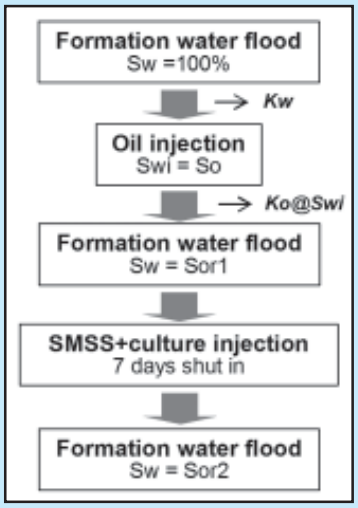

Figure 2
Flooding test sequences

Table 4
Basic properties of the core plug tested

Core No.	Length (cm)	Pore Volume (cc)	Porosity (%)	Ka (mD)
S2	7,65	14,90	17,6	502,80

Table 5
Basic properties of core flooding experiment

Core No.	Ka (mD)	Kw (mD)	Ko@Swi (mD)	Swi (%)	Sor (%)
S2	502,8	329,44	134,13	30,20	69,80

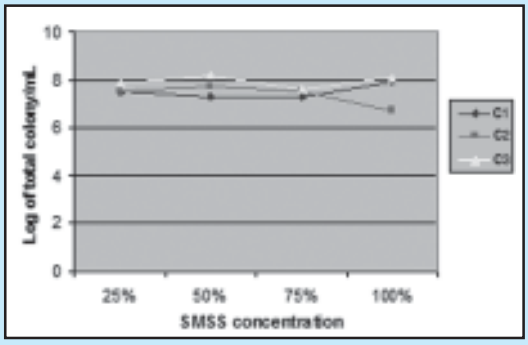

Figure 3
Microbial population in C1, C2, and C3 crude oil using several SMSS concentration

tained through the core plug. The core plug was flushed with formation water until saturated condition (Sw=100%) and measurement of permeability in saturated water (Kw) using Darcy's formula, followed by crude oil injection until irreducible water saturation (Swi) and measured the oil permeability at immobile water saturation (Ko@Swi). The core plug was flushed again with formation water until no oil came out or until there was a residual oil saturation (Sor1). The volumes of the out coming oil were calculated. The remaining oil in the core plug injected with SMSS nutrient and mixed culture microbes then shut-in for 7 days according to the flask test. After this aging, the core plug was flushed with formation water until a second residual oil saturation (Sor2). The last volume crude oil was determined as oil recovery enhancement. The core flooding test followed the sequence of step as shown in Figure 2.

III. RESULT AND DISCUSSION

A. *Effect of SMSS on microbial growth*

The total plate count in SMSS concentration test demonstrates that the increase of SMSS concentration does not significantly affect on the microbial growth in all 3 crude oils (Figure 3). It denotes that SMSS medium are acceptable for microbial consortia. The microbial consortia in all SMSS concentrations of C1, C2, and C3 crude oils exhibit good growth with population over log 6 (more than 10^6 total colony/mL). Therefore 100% SMSS concentration was used in the next test as microbial consortia medium.

B. *Microbial Activities*

The population of microbial consortia in 100% SMSS increases in both microaerophilic and aerophilic conditions (Figure 4). At 0 day, microaerophilic populations were less than aerophilic population, however after 7 days of incubation, microaerophilic populations were greater than aerophilic. Soriano and Periera (2002) revealed that in aerated bioprocesses, microbial activity is closely related to oxygen consumption. However, very high oxygen availability is potentially harmful to microbes because the incomplete reduction of oxygen generates reactive oxygen species

during respiration. It causes that the aerophilic populations are not greater than microaerophilic. In addition, at low oxygen concentrations, oxygen diffusion through the cell substance limits respiration rate (Johnson. 1967). The microbes can live by anaerobic system using SO_4^{2-}, NO_3^- Fe^{3+} and organic compounds as electron recipient due to the limited oxygen in the reservoir (Sublette. 1993). It clearly appears that SMSS nutrient supports the microbial growth in anaerobic system by providing the important nutrient.

The interfacial tensions of C1, C2, and C3 oils in 7 day declined both of microaerophilic and aerophilic condition (Figure 6). The best reduction occurred in C2 oil (51.61% in aerophilic and 47% in microaerophilic) followed by C1 in aerophilic 43.89% and C3 in microaerophilic. The decrease of interfacial tension in both conditions showed that the microbial consortia produced biosurfactant. The C2 oil in aerophilic condition had the highest reduction of IFT although it had the fewest microbial population. According to Soriano and Periera (2002), the production of surfactant occurs even after the growth of indigenous mixed populations

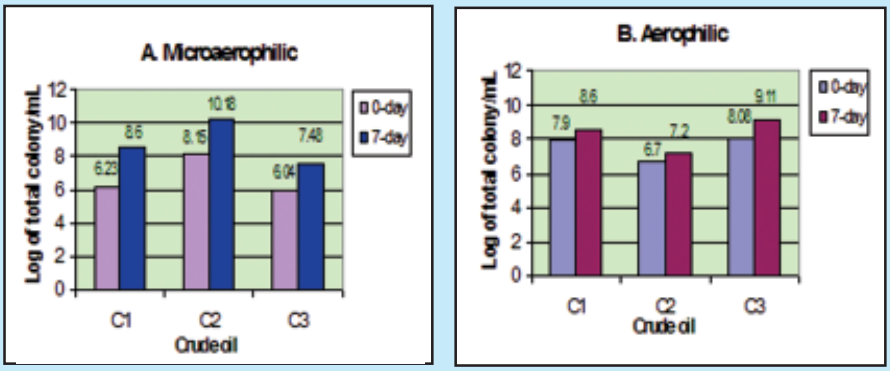

Figure 4
Microbial consortia population in
(a). microaerophilic and (b). aerophilic condition

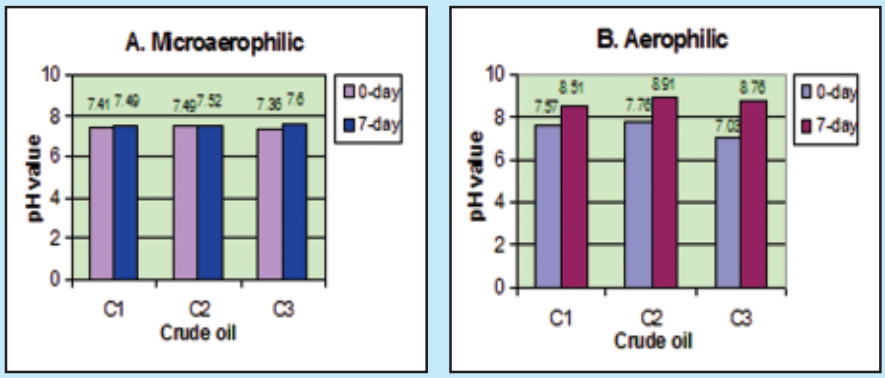

Figure 5
pH value in (a). microaerophilic and (b). aerophilic condition

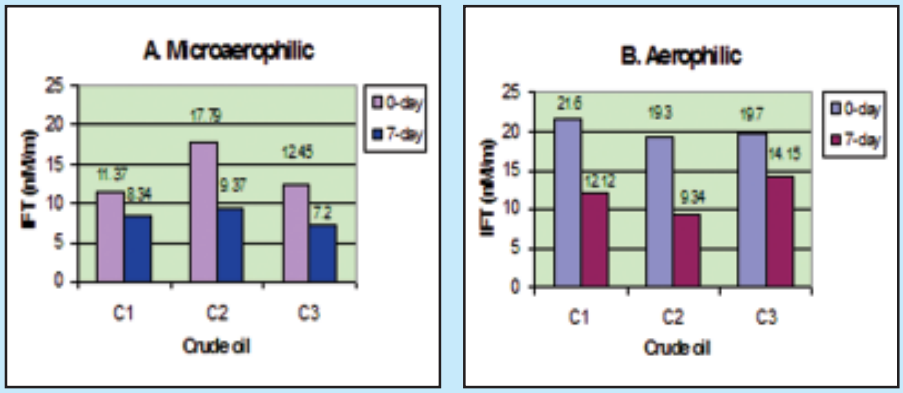

Figure 6
Interfacial tension of crude oil in
(a.) microaerophilic and (b). aerophilic condition

on hydrocarbon has ceased. It is because that the existence of microorganisms are able to produce associated surfactants.

The viscosities of C1, C2, and C3 oils decreased in 7 day except for C1 in microaerophilic (Figure 7). The significant decrease occurred on C2 in aerophilic (58.73%) followed by C3 in aerophilic (55.94%). The significant decreasing of viscosity in aerophilic indicated that biosolvent was produced. The increase of C1 viscosity in microaerophilic was probably caused by the remaining asphaltene, which could not reduce. Liu et al. (2003) denotes that asphaltene in crude oil causes the raising and abnormality of crude viscosity. Structure and character of asphaltic molecules make the molecules easily gather and associate with each other at the oil/water interface and finally form a viscoelastic interfacial film of great mechanical strength.

The best result of interfacial tension and viscosity reduction occurred in aerophilic condition. The aerobic condition is needed in hydrocarbon degradation process (Leahy and Colwell.1990).

After 7 days, pH in microaerophilic was relatively constant, compare to the aerophilic condition which increased about 1 point (Figure 5). The pH values in microaerophilic and aerophilic were in the range of 7.49 to 7.52 and 8.51 to 8.91 respectively. The pH values for microbial growth were between 7-9. The increase in aerophilic was probably caused by microbial activities in hydrocarbon conversion. Dibble and Bartha (1979) describes that the greatest extent of hydrocarbon conversion occurrs at the pH 7.8. Moreover, the pH value shows that microbial mixed

Core No.	OOIP (cc)	RF Waterflood		RF MEOR Flood		
		(cc)	(%)	(cc)	(%OOIP)	(%Sor)
S2	10,40	6,12	58,85	0,17	1,63	3,97

Table 6
Recovery Factor of Core flooding

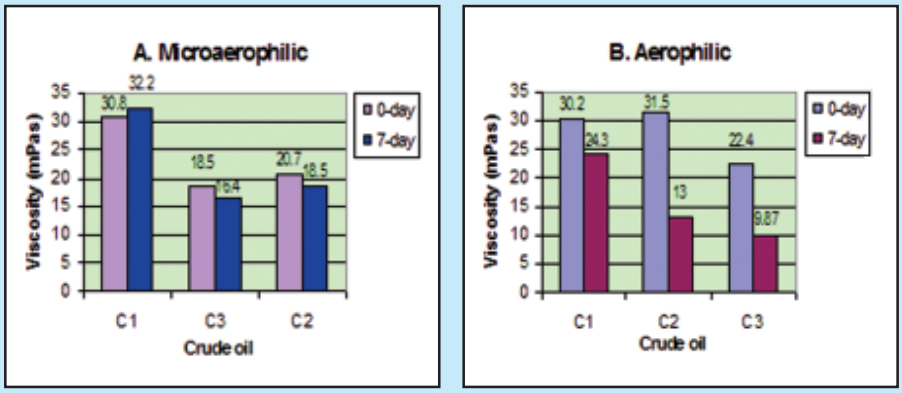

Figure 7
Crude oil viscosity in
(a). microaerophilic and (b). aerophilic condition

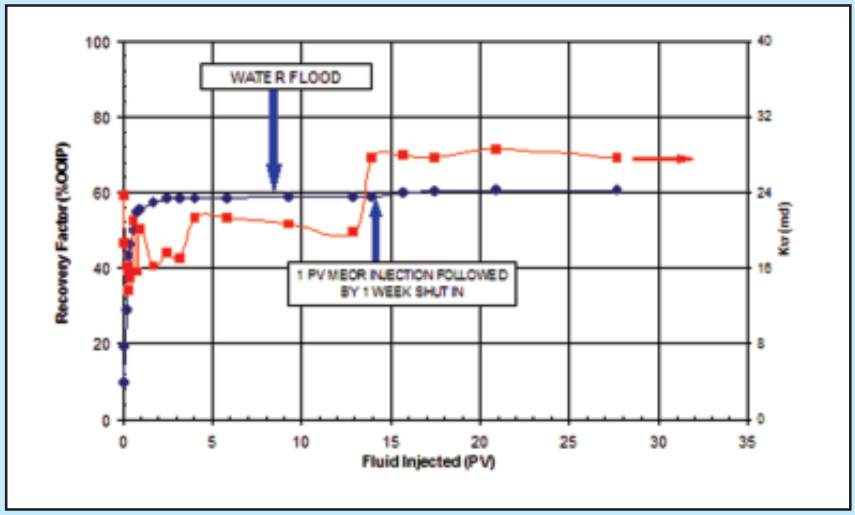

Figure 8
Recovery factor of MEOR injection

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culture does not produce bioacid since there is no decreasing pH value.

Based on the results of microbial activities test, there are obvious evidences that mixed culture bacteria as consortia can produce biosurfactant and biosolvent using SMSS nutrient.

C. *MCF Experiment*

In the MCF experiment, 100 % SMSS concentration is used as nutrient and C2 as crude oil. The viscosity of formation water at 50°C is 0.5707 and crude oil viscosity is 7.905141. Water permeability measurement at 100 % water saturation (Kw) is 329.44 mD, whereas after oil flood irreducible water saturation (SWi) is 30.20% and after flood water, residual oil saturation (Sor) in core plug is 69.80%. Basic properties of core flooding experiment are described in Table 5.

The remaining oil in core plug or OOIP (*original oil in place*) is found to be 10.40 cc. The recovery oil after SMSS and microbes injection are approx 0.17 cc. It means that the recovery factor is only 1.63% from OOIP (*original oil in place*) or 3.97% of Sor. On the other hand, the recovery factor of waterflood is 6.12 cc or 58.85% (Table 6.). The recovery factors of MEOR injection are illustrated in Figure 8.

The recovery factor of MEOR injection using SMSS nutrient shows that the tiny enhancement is probably caused by pore clogging of SMSS nutrient particles. Furthermore the non-homogenous medium in core plug at aging time will reduce microbial enzymatic activities. Direct application of SMSS in field test requires modification in calcium component to evade pore clogging. However this study proves that SMSS nutrient can be applied in MEOR research by ex situ biosurfactant and biosolvent production.

IV. CONCLUSIONS

SMSS nutrient is feasible to use in MEOR application by ex situ biosurfactant and biosolvent production. Furthermore those biosurfactant and biosolvent can be used for enhancing oil recovery. The MEOR application requires extensive studies due to the wide varieties of crude oils as well as microbial activities.

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