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## Laboratory simulation of microbial enhanced oil recovery using *Geobacillus toebii* R-32639 isolated from Handil Reservoir

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### Abstract

Microbial enhanced oil recovery (MEOR) is a biological based technology to enhance crude oil (CO) recovery at old oil wells by manipulating function or structure or both of microbial environments existing in oil reservoirs. Even if many biosurfactant-producing microbes can be found in different environments, the ability of *Geobacillus toebii* R-32639 to enhance CO recovery needs to be verified. In this study, the artificial cores (ACs) are used to simulate the application of MEOR by three scenarios of injecting the nutrients, microbial culture and bioproducts. The use of *Geobacillus toebii* R-32639 as biosurfactant producers is able of lowering a 25.3% interfacial tension and a 14.1% CO viscosity and is also able of degrading a 7.4-28.8% fraction of (C<sub>12</sub>-C<sub>34</sub>) hydrocarbons after 7 days of incubation. The injection of microbial culture into a laboratory-scale CO reservoir of the AC for the desirable purpose of enhanced oil recovery has the feasibility to achieve an average recovery factor of 14.27% but lower a 5.50% AC porosity and a 91.0% AC permeability; this is the best-case scenario of the microbial core flooding (MCF) experiments. The simulation of MEOR of using the bacterial strains of *Geobacillus toebii* R-32639 provides valuable insight into the use MCF experiments for getting more oil production of existing reservoirs.

**Keywords:** biosurfactant; *Geobacillus toebii* R-32639; Handil Reservoir; microbial core flooding treatment; microbial enhanced oil recovery

### 1. Introduction

Because of there have been no significant worldwide discoveries of new big reserves of oil since 2002, but the crude oil (CO) daily commodity is now depleting fast as the consumption is four times faster than its discovery,<sup>1-2</sup> in support of this determination of whether reasonable efforts need to be made to increase CO production. A decline in the production from major crude fields such as for Indonesia has encouraged the refiners to start mobilising the researches interested in opportunities for collaborating with local universities. For example, the Handil Field of Kutei Basin in East Kalimantan is anticline structural traps with oil reservoir sandstones between 450

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and 2,900 m.<sup>3</sup> The share of Indonesian CO production on that Borneo's land has fallen to a 90% low, according to the statistical data published by the Total Oil Company (TOC), from 200,000 barrels per day (BPD) in 1997 to only 20,000 BPD in 2009, even though there was only 12,500 BPD recorded in 2003. Since 2006, there have been several efforts made by the TOC as operator for Mahakam Block in East Kalimantan in collaborating with the Bandung Institute of Technology, including the petroleum microbiology researches with application in CO recovery.<sup>4</sup> The range of enhanced oil recovery techniques could be broadly classified into either thermal or non-thermal methods; the application of these methods has been proven effective for the extraction of heavy oils and bitumens.<sup>5</sup> It is recognised that using microbial enhanced oil recovery (MEOR) provides new challenges to get more oil out of existing reservoirs.<sup>6</sup> MEOR represents the use of microorganisms to extract the remaining CO from reservoirs and is a biological based technology of using biomass, metabolic activities of microorganisms and their metabolites, and transformation products.<sup>6-7</sup> Note that metabolites are the intermediates and products of bacterial metabolism such as biosurfactant, biogas, bioacid, biosolvent and biopolymer. The potential biological CO recovery technique could be cost-efficient in the extraction of CO remained trapped in capillary pores of the formation rock or in areas not swept by classical or modern enhanced CO recovery methods.<sup>7</sup>

Biosurfactant is a unique class of organic compounds and has been proven to have a variety of the potential applications, including the utilisation of such metabolic products for petroleum industries. Biosurfactants produced by certain bacteria are surface-active compounds involved in the degradation of such as a fraction of C<sub>12</sub>-C<sub>34</sub> hydrocarbons.<sup>6, 8</sup> Such a compound is commonly called amphiphilic or amphipathic molecules. The amphiphilic compounds contain a hydrophobic or hydrophilic group with a large hydrocarbon moiety and have the ability of their surfactant properties to reduce interfacial tension (IFT) between two media of either oil/water or oil/air systems.<sup>9-10</sup> Possibilities and challenges for biosurfactants use in petroleum industry offer advantages with regard to environment protection due to low toxicity and high biodegradability of these compounds make them better than synthetic surfactants.<sup>11</sup> The use of biosurfactants could be effective in increasing efficiency or selectivity when the MEOR operates at the combined conditions of extreme salinity, alkaline pH and elevated temperature.<sup>12-13</sup> Biosurfactants can reduce IFT between oil and water and induce a micelle to facilitate mobilisation of entrapped oil in mature reservoirs.<sup>14-15</sup> The presence of certain microorganisms can produce biosurfactants that would lead to increased rates of substrate bioavailability for microbial growth.<sup>11-12</sup> The ability of bacteria to adhere to hydrocarbons could be a characteristic feature of biosurfactant-producing microbes.<sup>16</sup> Anaerobic bacterial metabolism of hydrocarbons in a reservoir can be made by direct contact between cells and organic solvent<sup>17</sup> and activates the oxygenase activity of the enzyme resulted at the cell membrane capable of degrading hydrocarbons, lowering the CO viscosity.<sup>12, 18-19</sup> The increase of recovery factor (RF) from mature fields under suitable MEOR conditions will be critical to meet the increasing amount of CO production from existing reservoirs.

The objectives of this study are: (1) to perform activation of the *Geobacillus toebii* R-32639 strains and to make the artificial cores (ACs) and then saturate them for supporting the laboratory-scale MEOR experiments; (2) to assess the application of MEOR in conjunction with the microbial core flooding (MCF) experiments of injecting the nutrients, bacterial culture and

bioproducts; and (3) to verify the change in AC properties and to determine the values of RF yielded from three scenarios of MEOR simulation.

## 2. Materials and Methods

### 2.1. Activation of *Geobacillus toebii* R-32639 strains

The activation of *Geobacillus toebii* R-32639 strains was carried out at a stone mineral salt solution (SMSS) medium to which certain amounts of CO originally coming from the Handil Reservoir and NPK fertilizer as nutrients source for biosurfactant production were added gradually. The SMSS medium consisting of 0.25 % (w/v)  $\text{NH}_4\text{NO}_3$ , 0.1 % (w/v)  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 0.05 % (w/v)  $\text{KH}_2\text{PO}_4$ , 0.05 % (w/v)  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.02 % (w/v)  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  and 0.05 % (w/v)  $\text{CaCO}_3$  was diluted with distilled water in an Erlenmeyer flask. During the activation process, increasing the NPK concentration in SMSS solution was made step-by-step from 0.0125 to 0.025 to 0.0375 and to 0.05% (w/v) NPK in accordance with increasing CO concentration from 5 to 10 to 15 and to 20% (v/v) CO, respectively. Using sterile technique for minimising the contamination of the Erlenmeyer flask containing the SMSS medium, the inoculation with the *Geobacillus toebii* R-32639 strains in the SMSS medium was carried out periodically at any increase in the concentrations of CO and NPK. For each inoculation, the flask was incubated at 60°C in a rotary incubator shaker shaking at 200 rpm for 72 hours. Then 0.1 mL of *Geobacillus toebii* R-32639 strains was isolated streaked on a nutrient agar (NA) plate (Merck, Darmstadt Germany) for obtaining a pure culture. The bacterial strains were carefully counted by visual observation and should be more than 300 colony-forming units per NA plate of which would be categorised as active isolates. The micelle formation and the emulsion of oil droplets in water were confirmed based on visual observations.

### 2.2. Measurements of interfacial tension, viscosity and hydrocarbons degradation

The measurements of IFT were carried using a Du Nouy tensiometer (Fischer Surface Tensiomat, Model 21). Place sample solution in a 50 mL beaker and raise the platform until the Du Nouy ring was immersed approximately 0.5 cm in the sample. Adjust the beaker until the ring was at the interface and the lever arm was in the zero position. Increase the tension of the wire by lowering the beaker, while keeping the lever arm at zero. Read the tensiometer, when the film at the interface breaks, was the apparent IFT. The measurements of CO viscosity were carried out using a viscometer (Cannon-Fenske Ostwald Viscometer, Type 350 Akram 469). Both the measurements of IFT and CO viscosity, for treated CO sample consisting of SMSS medium, 20% (v/v) CO, 0.05% (w/v) NPK and *Geobacillus toebii* R-32639 strains and for control CO sample consisting of SMSS medium, 20% (v/v) CO and 0.05% (w/v) NPK, were performed at 60°C for every 24 hours during 7 days. The CO viscosity can be calculated using the following equation:

$$\eta = \eta_o \left( \frac{t \times \rho}{t_o \times \rho_o} \right) \quad (1)$$

where  $\eta$  is the viscosity of the CO sample (in cP),  $\eta_0$  is the viscosity of water (in cP),  $t$  is the time of the CO sample flow (in s),  $t_0$  is the time of water flow (in s),  $\rho$  is the density of the CO sample (in g mL<sup>-1</sup>) and  $\rho_0$  is the density of water (in g mL<sup>-1</sup>).

The degradation of hydrocarbons was determined based on the experimental data analysed using a gas chromatography mass spectrometer (Shimadzu GCMS-QP2010 Ultra Gas Chromatograph-Mass spectrophotometry). The fraction of hydrocarbons was identified by comparing the pristane (n-C<sub>17</sub>)/hydrocarbon ratio measured at zero-day to that measured at 7<sup>th</sup> day. It is recognized that the ratios of n-C17 to pristane and n-C18 to phytane are convenient biodegradation indicators.<sup>20</sup> Use of pristane (n-C<sub>17</sub>)/phytane (n-C<sub>18</sub>) ratio as a biodegradation indicator was also measured at zero- and 7-day as control.<sup>21</sup>

### 2.3. Formation and saturation of the artificial cores

A curing procedure was used to make AC, firstly blended Portland cement and quartz sand (mesh size 50  $\mu$ m) in a ratio of 1/4 in dry condition and then added a 10% (v/w) distilled water to make slurry such thing as conventional concrete mixture. Such concrete slurry was slowly added into a PVC pipe in which a formation of AC did work at constant pressure and then dried at room temperature during 3 days to make AC with a diameter of 2.54 cm and a length of 5 cm. The hardest AC was pulled out from the PVC pipe using a motor cub and then cut with a core cutter to have a dimension of 2.54 cm diameter and 4 cm length. Before using, the AC was washed with distilled water to remove soluble impurities adsorbed on its outer surface and dried in an oven at 60°C for 24 hours. Such a treatment was performed before the applications for the simulation of MEOR and has no effect on the MEOR performance even though the swollen quartz sand may reduce the permeability of AC. The porosity of AC was determined based on the experimental data measured using a gas porosimeter of PORG-200. The following equations can be used to calculate the porosity of the AC, such that:

$$V_g = -4.7813 \times \frac{P_u}{P_d} + 52.558 \quad (2)$$

where  $V_g$  is the volume of stored granular materials in the AC (in m<sup>3</sup>),  $P_u$  and  $P_d$  are the values of upstream and downstream pressure in the AC recorded at the porosimeter (in kPa).

$$\phi = \frac{V_b - V_g}{V_b} \times 100\% \quad (3)$$

where  $\phi$  is the porosity of the AC (in %),  $V_b$  is the volume of the bulk AC (in m<sup>3</sup>) and  $V_g$  is the volume of stored granular materials in the AC (in m<sup>3</sup>).

The AC sample was seated in the matrix cup. Helium was isothermally expanded into the matrix cup from the reference cell. The upstream and the downstream pressure in the AC were measured using the digital transducer and visually displayed. The volume of the bulk AC (the

matrix cup) was known. The only unknown was the volume of stored granular materials in the AC and this was computed using Eq. (2). Then the porosity of the AC can be determined using Eq. (3).

Permeability of the AC was determined based on the experimental data measured using a gas permeameter of PERG-200. The following equation can be used to calculate the permeability of the AC, such that:

$$k = \frac{1000 \times D_c \times \eta \times Q_f \times L}{A \times (P_u - P_d) \times \frac{(P_u - P_d)}{2}} \quad (4)$$

where  $k$  is the permeability of the AC (in mD),  $D_c$  is the pressure constant for each tested fluid rate (in mD kPa<sup>2</sup> s cP<sup>-1</sup> cm<sup>2</sup>),  $\eta$  is the viscosity of the CO sample (in cP),  $Q_f$  is the fluid rate (in cm<sup>3</sup> s<sup>-1</sup>),  $L$  is the length of the AC (in cm),  $A$  is the cross-sectional area of the AC (in cm<sup>2</sup>),  $P_u$  is the upstream pressure of the AC (in kPa),  $P_d$  is the downstream pressure of the AC (in kPa).

Value of the saturated oil residue (Sor) was determined together with formation water (FW) saturation and formation oil (FO) saturation. After the crude has been produced FW, it must be removed by treatment systems. This study used the FW and FO samples originally coming from the Handil Field of Kutei Basin, East Kalimantan. Before the saturation of AC with FW, it was necessary to calculate dry weight of the AC. Then the AC sample was placed in an Erlenmeyer that has been linked to a vacuum pump of being connected to the separator cone. The vacuum pump was used to remove air bubbles trapped in pores of the AC for 2-3 hours. The FW was then injected into an Erlenmeyer until the AC fully soaking in water and thus chocking the air in the AC by FW was removed using the vacuum pump for 2-3 hours. Wet weight of the AC was measured to calculate the mass of water, which can be converted to the volume of saturated water (with mass density of water ( $\rho_w$ ) equals 1,001 g L<sup>-1</sup>). Using a slim tube apparatus and Hessler core holder, the CO was injected into the AC at a rate of 0.3 cm<sup>3</sup> min<sup>-1</sup> under overburden pressure of 689.5 kPa until all the AC pores empty FW to have the FO saturation. Since CO entered the pores and forced FW out, the volume of FW that has been removed should be equal to the volume of oil entered the pores. Such a volume is defined as volume of the original oil in place (OOIP). Then the FO was displaced with FW at a constant flow rate of 0.6 cm<sup>3</sup> min<sup>-1</sup> under overburden pressure of 689.5 kPa until the Sor was reached and thus the OOIP can be determined.

#### 2.4. Simulation of microbial enhanced oil recovery

For each scenario in this work used five ACs with a porosity range of 20-35% and a permeability range of 20-80 mD. The characteristics of each AC could represent the physical features on the Handil Reservoir of Kutei Basin in East Kalimantan characterising with a porosity range of 5-36% and a permeability of less than 100 mD.<sup>3</sup> The application of simulation involved four consecutive steps i.e., waterflooding step-1 (WF-1), MCF, soaking and waterflooding step-2 (WF-2) to mimic real life operations in the oil industry in order for the simulation study to be successful; the temperature was maintained at 70°C during simulation. The simulation proposed

in this study can be expected to having a good system optimisation process with different factor values and considerations.<sup>22-23</sup> The WF-1 represents the primary and secondary sates of CO production, while the MCF, soaking and WF-2 represent the tertiary stage of CO production. The extraction of CO during the WF-1 is the process by which CO is extracted and removed from the AC. The FW was injected into the AC using a slim tube apparatus and Hessler core holder at a rate of  $0.6 \text{ cm}^3 \text{ min}^{-1}$  under overburden pressure of 689.5 kPa to force CO out of the AC that until actually cannot be forced out it again. Ratio of the volume of CO extracted during the WF-1 to the volume of OOIP could be the percentage of WF-1 recovery fraction. The MCF experiments consisting of three scenarios of core-flooding test were conducted using the best mounting SMSS medium of added a 20% (v/v) CO and 0.05% (w/v) NPK fertilizer and over 72 hours of age by injecting the nutrients, microbial culture and bioproducts, respectively. This determines whether there can have a measurable effect of flooding with *Geobacillus toebii* R-32639 used as biosurfactant producers. The CO was extracted from each AC by the injection of FW using a slim tube apparatus and Hessler core holder at a rate of  $0.6 \text{ cm}^3 \text{ min}^{-1}$  under overburden pressure of 689.5 kPa to force CO out until it cannot be seen again as the injection of FW cannot make it out from the AC. Ratio of the volume of CO extracted during the MCF to the volume of OOIP could be the percentage of MCF recovery fraction. The ACs were soaked in the FW and incubated at  $60^\circ\text{C}$  for 7 days; a small volume of CO can then be extracted during the soaking process of the AC and must be accounted for in the accumulation of the MCF recovery fraction. The extraction of CO during the WF-2 has the same process as the WF-1. Ratio of the volume of oil extracted during the WF-2 to the volume of OOIP must a covered entity account for the percentage of WF-2 recovery fraction. The percentage of MEOR recovery fraction could be the sum of percentage of MCF recovery fraction and percentage of WF-2 recovery fraction.

## 2.5. Analysis of geomicrobiological interactions

Before the analysis of geomicrobiological interactions, the AC sample was washed with toluene to remove any extracted ignitable liquid residues in the debris to the sample using a soxhlet extraction apparatus; the extraction was performed for 3-5 hours. Then the AC sample was dried at  $60^\circ\text{C}$  for 24 hours. The analysis of geomicrobiological interactions including the characterisations of final porosity and permeability of the AC sample can be made based on the data and photograph recorded from a scanning electron microscope (SEM) coupled with energy dispersive spectrometer (EDS) of JEOL JSM-6510LV (JEOL, Tokyo, Japan). The AC sample was broken down by physical mechanisms into smaller particles and soaked with a 2-3% (v/v) glutaraldehyde of 0.1M buffer solution with a pH range of 6.5-7.0. The AC sample was then dried in a vacuum desiccator for 24 hours. The assessment of geomicrobiological interactions by SEM-EDS analysis was performed only for the AC samples of injected *Geobacillus toebii* R-32639 culture to observe the images of zero-day and after 7<sup>th</sup> day soaking.

## 3. Results and Discussion

### 3.1. Ability of *Geobacillus toebii* R-32639 to affect the change in properties of crude oil

The use of *Geobacillus toebii* R-32639 strains as biosurfactant producers was able of reducing the IFT between oil and water to be approximately 25.3%, from an IFT of  $16.6 \text{ dyne cm}^{-1}$  measured at zero-day to that of  $12.4 \text{ dyne cm}^{-1}$  after 7 days of incubation at  $60^\circ\text{C}$ . The control

CO sample without affected by the presence of *Geobacillus toebii* R-32639 has a viscosity of 1.59 cP measured at zero-day and could be higher than the viscosity of the treated CO sample when affected by *Geobacillus toebii* R-32639 with a value of 1.51 cP, due to the real impact of biosurfactant-based MEOR techniques immediately reduces the CO viscosity. In addition to having an effect on oil production rates to enhance CO desorption and mobilisation in a laboratory AC model system, the use of such bacterial strains can reduce the viscosity of CO to be approximately 14.1%, from a viscosity of 1.51 cP measured at zero-day to that of 1.30 cP at 7<sup>th</sup> day (see Table 1). The reduction of IFT occurs due to the emulsification activity of biosurfactant is needed to saturate interfaces and would reach a critical micelle concentration (CMC), above which micelles are spontaneously formed.<sup>12, 24-27</sup> Moreover, the formation of micelles which are the simplest form of amphiphilic assemblies<sup>28</sup> may facilitate the microbial assimilation of hydrocarbons;<sup>29</sup> therefore, the bacterial growth rate increases. This can cause the increased hydrocarbon biodegradation rate and lowers the fraction of CO hydrocarbons,<sup>6, 19</sup> the viscosity of CO reduces. Figure 1a shows that both the reductions of IFT and CO viscosity occurred at a relatively constant rate might indicate the production of biosurfactants in a continuously operated incubation of the AC samples.<sup>30</sup> The production of biosurfactants commonly occurs at the end of log (or exponential) phase of bacterial growth followed by the stationary phase, in which the size of a population of bacteria remains constant, even though some cells continue to divide and others begin to die, due to the availability of the nutrients decreases.<sup>31</sup> Because of the activation of *Geobacillus toebii* R-32639 has been adapted to the SMSS medium environments to having a short time of the lag phase, the production of biosurfactants would be occurred from the start of incubation to the emergence of the young varies from 7 days. An optimisation-based framework<sup>32</sup> for thermo-biochemical route to degrade CO hydrocarbon can be proposed to offer empirical evidence due to the effect exerted by the biosurfactants on nature of the fluids could be evident in the AC. A plot (Fig. 1b) of IFT versus CO viscosity gives a slope-intercept form:  $Y = a \times X - b$ , where  $a$  is the empirical constant (in  $\text{dyne cm}^{-1} \text{ cP}^{-1}$ ) and  $b$  is the IFT reduction index relating to viscosity of the CO sample (in  $\text{dyne cm}^{-1}$ ), because  $a$  is the slope and  $b$  gives the  $Y$ -intercept.<sup>33-34</sup> Correlation between the reductions of IFT and CO viscosity is good ( $R^2 = 0.9312$ ; see Fig. 1b caption), indicating that the use of *Geobacillus toebii* R-32639 as biosurfactant producers is able of changing the nature of the fluids gradually.

**Table 1** Interfacial tension between oil and water and crude oil viscosity

Sample	IFT ( $\text{dyne cm}^{-1}$ )		CO viscosity (cP)	
	0-day	7-day	0-day	7-day
Treated CO sample	16.6	12.4	1.51	1.30
Control CO sample	16.9	15.6	1.59	1.54

Remarks that IFT means the interfacial tension (in  $\text{dyne cm}^{-1}$ ) and CO viscosity means the crude oil viscosity (in cP)

(Fig. 1 should be here)

The stability of CO emulsions decreased by increasing the CO content while increasing the biosurfactant concentration enhanced the emulsion stability.<sup>35</sup> Microbial degradation of the CO hydrocarbons affected by *Geobacillus toebii* R-32639 might reduce the CO viscosity; high viscosity of CO could be due to high molecular weight hydrocarbons. The results (Fig. 2) of GCMS analysis showed that isoprene biomarkers of pristane ( $C_{17}$ ) and phytane ( $C_{18}$ ) in CO



hydrocarbons degraded obviously after 7 days of incubation at 60°C (see the distribution of pristane and phytane relative to their neighboring n-C17 and n-C18 peaks of high and low red circle, respectively). The GCMS chromatogram of CO hydrocarbons recorded at zero-day (Fig. 2a) would look very different from the chromatogram of those recorded in GCMS after 7 days of incubation (Fig. 2b). According to the GCMS analysis, the degradation of (C<sub>12</sub>-C<sub>34</sub>) hydrocarbons has been in a range of 7.4-28.4% after 7 days of incubation at 60°C. The ability of *Geobacillus toebii* R-32639 to reduce IFT and CO viscosity and to degrade hydrocarbons could be important to enhance the mobilisation of CO in a reservoir and to increase the production of CO such as in the Handil Reservoir, in order to meet growing demands for energy in the coming decades.

(Fig. 2 should be here)

### 3.2. Microbial core flooding experiments to increase crude oil production

It is recognised that MEOR is a revolutionary tertiary CO recovery program, engineered to provide fast and comprehensive results on underperforming well sites that have ceased to be financially viable.<sup>4</sup> Analysis of financial ratio suggested that the use of MEOR is technically feasible and could be an economically competitive system for CO production.<sup>36</sup> The simulation of MEOR by three MCF scenarios of injected the nutrients, microbial culture and bioproducts would be expected to improve CO production increased to approximately 3.09, 14.27 and 8.48% of RF, respectively (see Table 2). The injection of nutrients can be expected to maximise the CO potential of MCF outputs by internally capturing and recycling carbon and nutrients.<sup>37</sup> A low RF value of 3.09% obtained during the MCF experiments of injected nutrients may be related to the development of a laboratory conditioning simulation module with the indigenous *Geobacillus toebii* R-32639 colonies; however, it was not the optimal response. The increase in RF value for the application of MEOR by injecting bacterial culture, both the MCF experiments with a 6.84% RF and WF-2 with a 7.43% RF, could be relatively impartial, while that by injecting bioproducts, the MCF experiments with a 7.43% RF and WF-2 with a 1.05% RF, could be very different. The difference in RF value between these two techniques should be due to the oil recovery mechanisms involved with MCF experiments fractured chalk blocks could be dependent on the wettability of the chalk, as the wettability had great impact on the fracture/matrix hydrocarbon exchange,<sup>38-39</sup> while the mechanisms of CO recovery involved with WF-2 could be mainly dependent on the IFT reduction.

**Table 2** Recovery factors for primary, secondary and tertiary states of CO production

Scenario	Sor (% of pore volume)	Primary and secondary sates of CO production	Tertiary stage of CO production			Total CO production
			RF <sub>WF-1</sub> (%)	RF <sub>MCF</sub> (%)	RF <sub>WF-2</sub> (%)	
NI	36.16	35.24	1.99	1.10	3.09	38.33
BCI	36.37	28.80	6.84	7.43	14.27	43.07
BPI	44.43	35.27	7.43	1.05	8.48	43.75

Remarks that NI is the abbreviation of nutrients injection, BCI is the abbreviation of bacterial culture injection, BPI is the abbreviation of bioproducts injection, CO is the abbreviation of crude oil, Sor is the abbreviation of saturated

oil residue,  $RF_{WF-1}$  is the abbreviation of recovery factor for waterflooding step-1,  $RF_{MCF}$  is the abbreviation of recovery factor for microbial core flooding,  $RF_{WF-2}$  is the abbreviation of recovery factor for waterflooding step-2,  $RF_{MEOR}$  is the abbreviation of recovery factor for microbial enhanced oil recovery, and TRF is the abbreviation of total recovery factor.

Understanding formation wettability and its management is very important for optimising CO recovery. In this work, wettability can be defined as the relative ability of a fluid (see either FW or FO) to spread on the AC surface in the presence of other fluid (see either FO or FW).<sup>40</sup> The adsorption of biosurfactant and the formation of biofilm on the AC surface regarding the bioadhesion of *Geobacillus toebii* R-32639 may constitute a new and effective means of changing the formation of wettability during MCF experiments.<sup>6-7, 41-43</sup> The use of bioproducts (or biosurfactants) in drilling FO during the MCF experiments may change the formation wettability from oil-wet to water-wet AC.<sup>44</sup> FO flowing can enhance the activity of *Geobacillus toebii* R-32639 and simulates the natural laboratory (ex-situ) biodegradation of CO hydrocarbons by inducing FW to flow toward the AC surface during the soaking process. The application of MEOR by the injection of bacterial culture can have a good tertiary CO recovery due to a constant IFT reduction occurs during the incubation period (see Fig. 1a). A pattern of the distinct oil composition changes could be due to the biodegradation of CO hydrocarbons might cause the change in wettability of the AC surface from oil-wet to water-wet.<sup>45-46</sup> The application of MEOR by the MCF experiments of injected bacterial culture and bioproducts that have already the presence of biosurfactants can expect to earn approximately 6.84 and 7.43% of RF, respectively (see Table 2). A slightly higher RF for the injection of bioproducts is due to the fact that insoluble particles can be removed through centrifugation to avoid the blocked pores for the injected bioproduct MCF operations. The images (Fig. 3) of the AC observed after soaking process for the application of MEOR with three scenarios of the MCF experiments could be different from each other. Figure 3b shows that the presence of bacterial activities for the MCF experiment of injected bacterial culture can be seen as easily visually. The figure indicated that when the production of gas may occur due to cell metabolisms, the various forces borne by degradation of hydrocarbons in the AC; therefore, the CO compounds can move through the porous rocks.<sup>47</sup>

(Fig. 3 should be here)

### 3.3. Geomicrobiological interactions

The decreases in porosity of the AC were verified as high as 6.85, 5.50 and 0.23% for the application of MEOR in conjunction with the MCF experiments of injecting the nutrients, bacterial culture and bioproducts, respectively (see Table. 3). A decrease in AC porosity of 6.85% for the injection of nutrients could be due to the accumulation of minerals that has not been used from the SMSS medium since the service of these minerals for bacterial metabolisms has not been started, but still used of such elements to form crusts on the rock pores. A 5.50% decrease in AC porosity should be due to the application of MEOR of injected bacterial culture can have the over production of microbial biomass, which may result in plugged rock pores. A very low decreased porosity of 0.23% could be caused by the injection of bioproducts with very low impurity content into the AC has very little impact on the change of porosity. The results (Table 3) show that the decreases in permeability for all the scenarios could be very important. Interestingly, the levels of permeability were significantly decreased to approximately 10% of

control (at zero-day) from 39.42 to 3.68 mD, from 41.16 to 3.70 mD and from 40.01 to 3.43 mD and therefore the permeability reduced should be 35.74, 37.46 and 36.58 mD for the MEOR experiments of injecting the nutrients, bacterial culture and bioproducts, respectively. Suggesting that increased exopolysaccharide production leads to the resistance to selectively clogging of fluid flow paths in the AC.<sup>48</sup> According to this suggestion, if the production of exopolysaccharide in the pores exceeds a certain critical value, the alternating motion of the CO driven by the clogging of the AC takes place in the pores. These results indicated that interfacial interactions between bacterial cells and minerals in the AC pores significantly affected the biodegradation of hydrocarbons. In order for CO to move thru a rock, the rock must have both good porosity and permeability; there must be some interconnection from pore to pore so that CO can move to migrate. It is recognised that the rock that has the porosity can be permeable or not permeable. As the pore fluid content increases, chemical changes mediated through the pore fluids will speed up as well. As the CO percolates through rocks, a complex series of physical and chemical reactions between CO and rocks of the AC drastically changes the chemical composition of both the CO and the rocks to probably clogging the fluid flow paths to cause the rock becomes less or not permeable.

**Table 3** The reductions of porosity and permeability of the AC

Scenario	Porosity (%)			Permeability (mD)		
	0-day	7-day	Reduction of $\phi$	0-day	7-day	Reduction of $k$
NI	26.78	19.93	6.85	39.42	3.68	35.74
BCI	28.99	23.49	5.50	41.16	3.70	37.46
BPI	26.20	25.97	0.23	40.01	3.43	36.58

Remarks that NI is the abbreviation of nutrients injection, BCI is the abbreviation of bacterial culture injection, BPI is the abbreviation of bioproducts injection,  $\phi$  is the porosity of the AC and  $k$  is the permeability of the AC.

Figure 4 shows the images of SEM photomicrograph for the AC sample scanned at zero-day (Fig. 4a) and after 7 days of incubation (Fig. 4b) for the injection of *Geobacillus toebii* R-32639 culture. As the natural capital upon which bacterial growth depends is limited in nutrients and oxygen supply and other extreme conditions during 7 days of incubation, the soaking process can cause a reduction in the *Geobacillus toebii* R-32639 cell size (see Figs. 4a, b; green arrows). The appearance and distribution of intermediate filament exopolysaccharides during incubation of the AC attach to the surface of the rocks and develop biofilms (see Fig. 4b; red arrow) and enable systematic understanding of diverse physiological processes of cells and their interactions.<sup>49</sup> The bioweathering action of *Geobacillus toebii* R-32639 on rocks of the AC might increase the availability of nutrients in the SMSS medium from dissolved minerals of calcium silicate as it consists of the biosurfactants. This condition typically corresponds to a lower IFT between oil and water (see Fig. 1), which produces a smooth surface. The effect of salinity on the MEOR by spontaneous imbibition could be due to higher salinity of the SMSS medium can reduce the attractions between oppositely charged sites on the heterogeneous interfaces.<sup>50</sup> These results help to understand the remarkable differences in AC smoothing of correlated and uncorrelated surfaces (see Figs. 4a, b), and the approach may be extended to sudden changes between other growth dynamics. Table 4 shows the EDS analysis of the AC sample materials consisting of C, N, SiO<sub>3</sub>, CaO and Al<sub>2</sub>O<sub>3</sub> components. These results indicate the remarkable differences in percentage of the components SiO<sub>3</sub> and CaO; the percentage of SiO<sub>3</sub> decreased approximately 41% (from 44.43% observed at 0-day to 3.46% observed at 7<sup>th</sup> day), while the percentage of CaO

increased approximately 18.1% (from 1.94% observed at 0-day to 20.02% observed at 7<sup>th</sup> day). The decay of crystalline SiO<sub>3</sub> occurred during the bioweathering process of the AC is due to the assimilation of SiO<sub>3</sub> by metabolism of *Geobacillus toebii* R-32639 could be difficult; the acidic environment of the AC is beneficial for the dissolution of SiO<sub>3</sub> in the SMSS medium from rocks of the AC. The increase in percentage of CaO in the rock matrix of the AC is due to the presence of CaO as cement based adhesive cannot be removed by bioweathering process. It is possible to further enhance MEOR performance by reducing salinity and to control bacterial growth rate by increasing and decreasing salinity.

**Table 4** Chemical compositions of the AC components during the bioweathering process

Component	Chemical composition of the AC components (%)	
	0-day	7-day
C	46.01 ± 0.06	45.18 ± 0.06
N	7.62 ± 0.37	9.03 ± 0.54
SiO <sub>3</sub>	44.43 ± 0.35	3.46 ± 0.33
CaO	1.94 ± 0.70	20.02 ± 0.83
Al <sub>2</sub> O <sub>3</sub>	-	3.46 ± 0.33

(Fig. 4 should be here)

#### 4. Conclusions

This study simulated the application of MEOR by three scenarios of the MCF experiments with using indigenous *Geobacillus toebii* R-32639 isolated from the Handil Reservoir. The use of *Geobacillus toebii* R-32639 strains as biosurfactant producers was able of reducing both IFT between oil and water and CO viscosity and would be also able of degrading the (C<sub>12</sub>-C<sub>34</sub>) total aliphatic hydrocarbons after 7 days of incubation. The application of MEOR in conjunction with the MCF experiments of injected the nutrients, bacterial culture and biosurfactants can improve the recovery of various fractions of CO trapped in the porous media. The assessment of geomicrobiological interactions by SEM/EDS analysis of the AC sample showed that the formation of biofilms was quite easy to be observed visually, and the increased percentage of CaO and the decreased percentage of SiO<sub>3</sub> in the rock matrix of the AC were identified. Porosity and permeability reduction as a result of selectively plugging due to the persistence of enhanced oil recovery was verified to contribute to the future of CO industry.

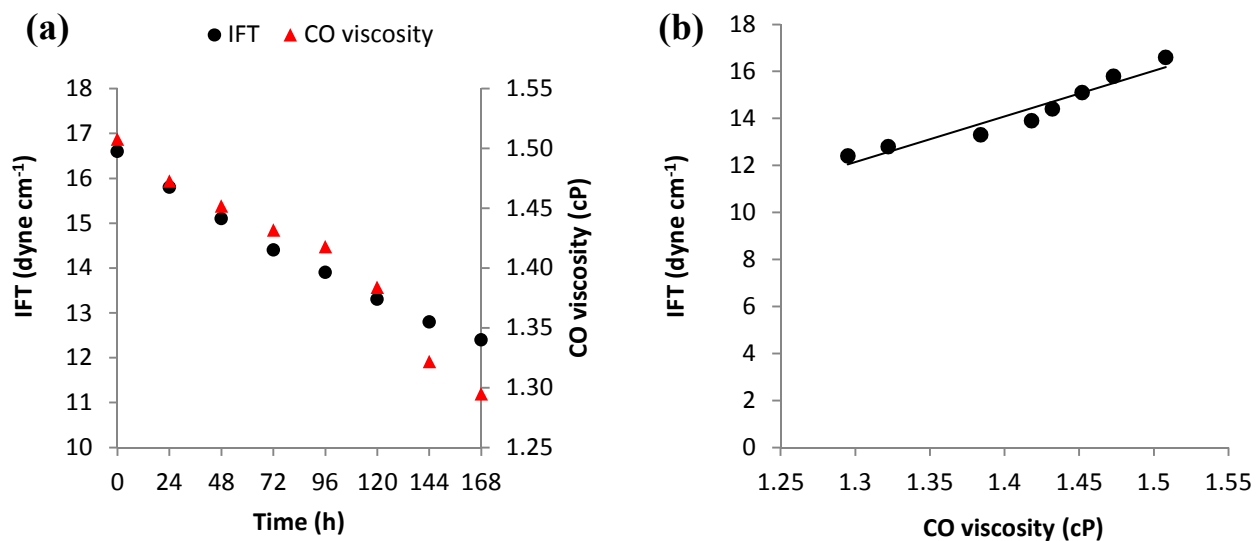
**Acknowledgments** The study used the financial supports from the Oil and Gas Recovery for Indonesia (OGRINDO) of the Bandung Institute of Technology. The financial supports provided by OGRINDO were greatly appreciated.

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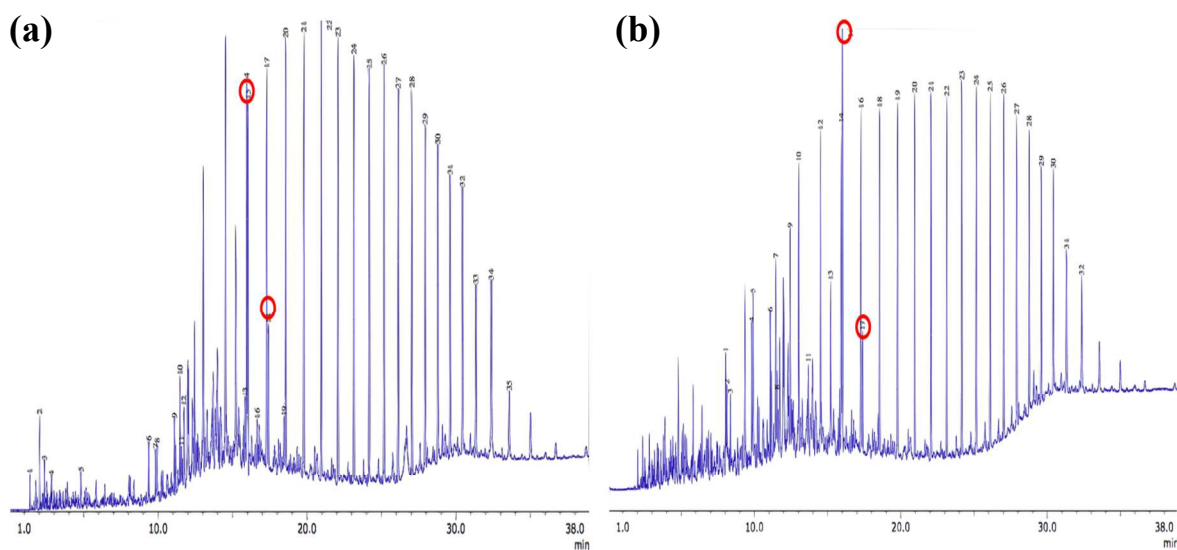
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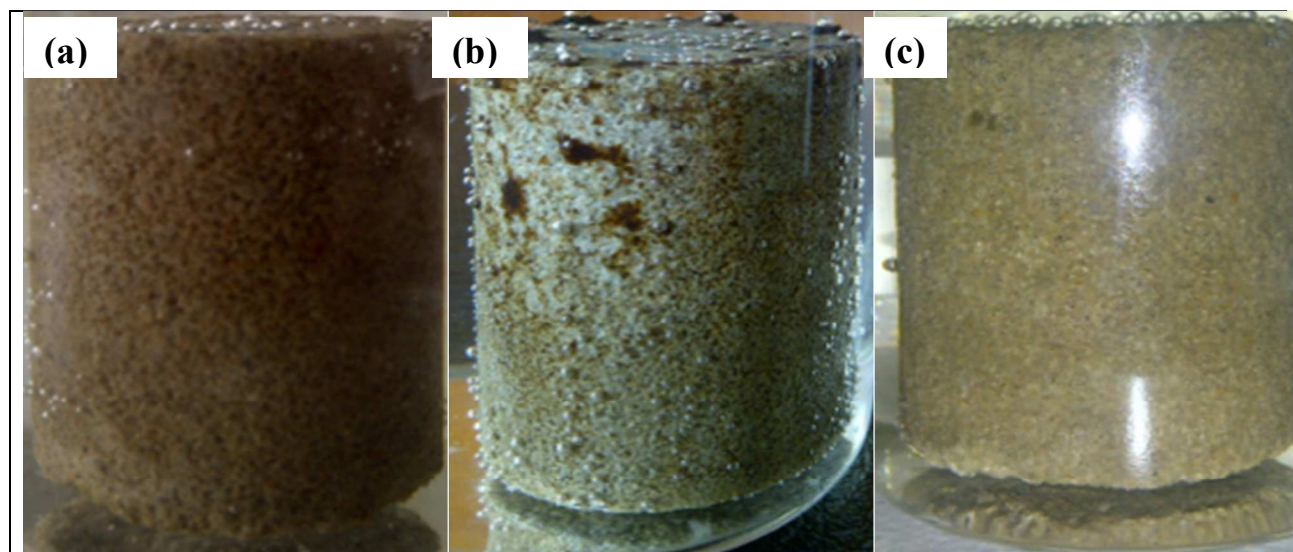


**Figure 1** The reductions of IFT and CO viscosity observed during seven days of incubation; where (a) the variations of IFT and CO viscosity pursuant to the time and (b) the linear regression analysis for correlation between IFT and CO viscosity, with  $a = 19.43 \text{ dyne cm}^{-1} \text{ cP}^{-1}$  and  $b = -13.1 \text{ dyne cm}^{-1}$  and  $R^2 = 0.9312$

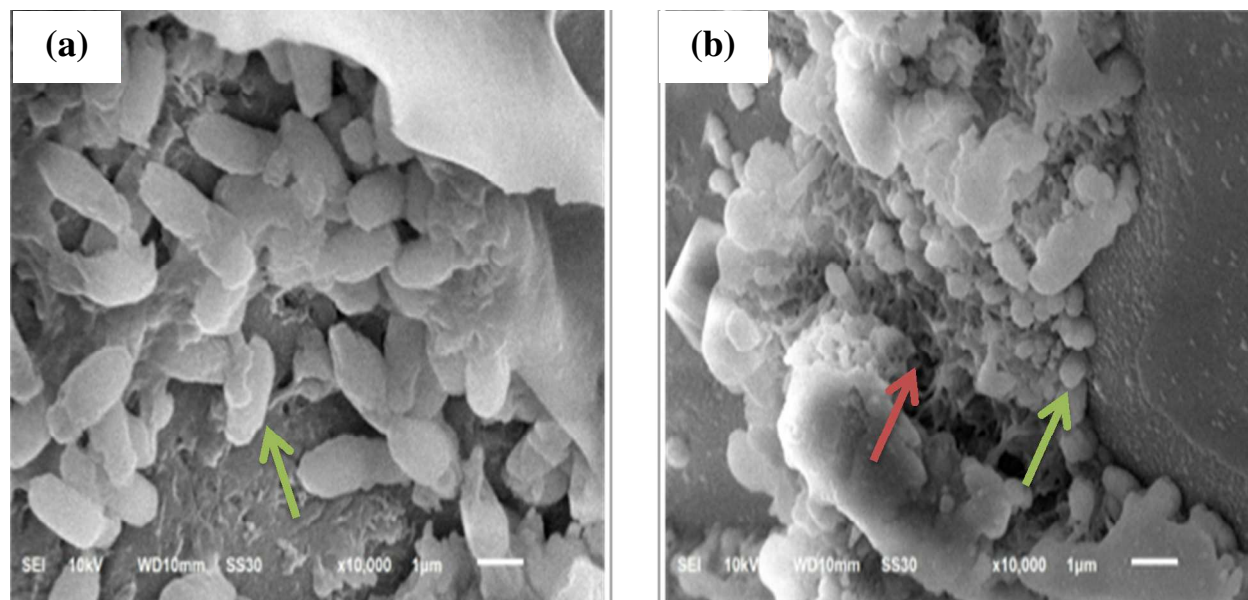


**Figure 2** The GCMS chromatogram for the CO samples with pristane (C<sub>17</sub>) and phytane (C<sub>18</sub>) as isoprene biomarkers (see the high point and the low point of the red circles, respectively), recorded **(a)** at zero-day and **(b)** after 7 days of incubation





**Figure 3** Images of the AC samples during the soaking process; with **(a)** the MCF experiment of injected nutrients, **(b)** the MCF experiment of injected bacterial culture and **(c)** the MCF experiment of injected bioproducts



**Figure 4** Images of SEM photomicrograph for the AC sample; with **(a)** the SEM photomicrograph scanned at zero-day and **(b)** the SEM photomicrograph scanned at seventh-day