Isolation and Characterization of Oil-Degrading Bacteria from One of South Sumatera’s Oilfield

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Abstract. Microbial Enhanced Oil Recovery (MEOR) is a tertiary oil recovery method that utilizes microbes to enhance oil production. This research was focused on the isolation and characterization of indigenous bacteria from a South Sumatra’s oilfield which were able to degrade heavy crude oil and decrease oil viscosity. The total of 33 colonies were successfully isolated based on sequential isolation method and screened based on oil degradation activity and SARA analysis. Isolate G3, G7, and N6 were chosen as the best candidate as they were able to reduce oil viscosity up to 22.67%; 23.14%; and 24.36% respectively. Based on 16S rRNA analysis, isolate G3 which was able to degrade aromatic fraction (38.27%) and resin (29.26%) was identified as Pseudoxanthomonas taiwanensis. Isolate G7 which degraded aromatic fraction (61.14%) was identified as Brevibacillus agri while N6 which degraded asphaltene fraction (51.76%) was identified as Bacillus subtilis. In addition, the change in n-alkana fraction (C11 – C28) abundance relative to phytan showed that all of the bacterial isolates were able to change those fractions of crude oil. This study showed that three bacterial species isolated from South Sumatran Oilfield were able to degrade heavier fraction of crude oil and reduce its viscosity. This result suggests that those bacteria are highly potential to be applied for MEOR technology.

1. Introduction

Low API (19) and high water cut (98%) are some characteristics of South Sumatra’s oilfields. Low API indicates that the reservoir contains heavy crude oil. Heavy crude oil is known to have high viscosity and low mobility. Therefore, tertiary recovery method is needed to enhance oil production in reservoirs with this type of oil. MEOR (Microbial Enhanced Oil Recovery) is a tertiary recovery method that uses microorganisms or microbial metabolites to enhance residual oil production [1]. Technical mechanisms of MEOR include microbe or microbial product injection, nutrition injection, and the combination of both. This study chose MEOR to solve South Sumatra’s Oilfield recovery problem due to its less energy requirement and operational costs, as well as its relatively simple process [2].

Previous study on a South Sumatra’s oilfield which dealt with optimization of nutrition composition for MEOR injection reported that the oilfield was inhabited by indigenous bacterial consortium which can alter oil viscosity and interfacial tension, and degrade crude oil[3]. Based on that report, this research aims to isolate those indigenous bacteria and characterize their ability to degrade crude oil.

2. Methods

2.1. Sample

Crude oil was taken from one of South Sumatera’s Oilfield.
2.2 Sequential isolation

Bacterial isolation was conducted in two stages. For the first stage, crude oil sample (2%) was inoculated to SMSSse Medium (0.5 g of CaCO₃, 2.5 g of NH₄NO₃, 0.5 g of MgSO₄.7H₂O, 1.0 g of Na₂HPO₄.7H₂O, 0.5 g of KH₂PO₄, and 0.2g of MnCl₂.7H₂O per litter of aquadest) enriched with 0,1%(w/v) yeastextract [4]. The culture was incubated for a week at 70°C and 50°C with 120rpm agitation. Every day of the incubation period, 1 mL of each culture was inoculated to nutrient agar using pour plate method and incubated for 3 days at 50°C and 70°C to grow colonies for bacterial identification. After 7 days, the remaining residual oil from the culture was taken and inoculated to a new enriched SMSSse Medium for second isolation stage. The isolation method was conducted as stated in the first stage of isolation [4].

2.3 Identification of bacteria

Bacteria identification was performed based on morphology using Gram staining [5] and based on genotypes using 16S rRNA molecular identification. The sequencing results were analyzed using Mega 6.0 with Neighbor Joining methode.

2.4 Screening of oil-degrading bacteria

Screening of oil degrading bacteria was conducted based on viscosity reduction data and Emulsification index.

2.5 SARA analysis

Bacterial isolates were inoculated to SMSSse medium (enriched with 2% crude oil and 0.1%(w/v) extract yeast) and incubated for 7 days at 50°C. After 7 days, crude oil from each batch was analyzed using SARA method. Firstly, crude oil sample was extracted with n-hexane to obtain asphaltene fraction and then filtrated. After this process there are three remaining fraction (saturate, aromatic, and resin) in crude oil sample that are collectively called maltenes. Each fraction was separated using different solvent in chromatography column. Saturated fraction was eluted with n-hexane, aromatic fraction was eluted with toluen, and resin fraction was eluted with toluen:methanol (90:10). Gravimetry analysis was used to analyse the dry weight of each fraction [6].

2.6 Abundance of crude oil fraction analysis using GCMS

Crude oil fraction abundance was analysed in Puslabor Jakarta Pusat. The calculation was done based on abundance ratio of hydrocarbon target relative to phytan.

3 Results and discussion

3.1 Isolation of oil-degrading bacteria result

Based on sequential isolation, 19 isolates were obtained from first stage and 14 isolates were isolated from second stage. A decrease in number of isolates in second stage could be due to the limiting factor of carbon source. The first stage used crude oil as carbon source whereas the second stage used residual oil from the first stage which could not be utilized by some isolates [4]. The order of hydrocarbon from the most readily to the most difficult to be degraded is as follows:n-alkane > branched alkane > low molecular weight aromatic compound > high weight aromatic compound > polycyclic aromatic compound [7].

3.2 Morphological identification of bacteria isolates

Identification results indicated that isolates from first and second stages of sequential isolation were mostly Gram negative. Gram negative bacteria have periplasmic that can mediate transport nutrition and chemical metabolism, and inactivate harzard compound. This mechanism can aid to survival against extreme condition in reservoir [8]. Gram positive bacteria were also found among the isolates. Gram positive bacteria can also survive in extreme condition in the form of endospore[8].

3.4 Screening of oil-degrading bacteria

There are many factor that could affect oil degradation process including temperature [9], and bioavailability [1]. Bioavailability of carbon source can be improveby adding biosurfactant [1].
Biosurfactant is an amphipathic molecule that allows to reduce interfacial and facial tension when interacting with other compound [10].

Screening of oil degrading bacteria in this research was focused on viscosity reduction and emulsification index. Emulsification index screening yielded 9 isolates to be analyzed for their viscosity reduction ability. Based on emulsification index and viscosity reduction data (Table 1 and 2), there were three candidates showed high performance in both parameters i.e. isolate G3, G7 and N6 that were retrieved from second stage of isolation. Second stage of isolation yielded isolates with higher performance and ability to degrade oil due to the carbon source at this stage was residual oil from the first stage that contained heavier fraction of hydrocarbon.

| Isolation stages | Isolate | % Viscosity reduction |
|------------------|---------|-----------------------|
| 1                | Control | -                     |
| 2                | Control | 0.48%                 |
| 3                | D1      | 22.07%                |
| 4                | E3      | 23.05%                |
| 5                | T1      | 21.88%                |
| 6                | G3      | 22.67%                |
| 7                | G6      | 21.90%                |
| 8                | G7      | 23.17%                |
| 9                | M2      | 22.57%                |
| 10               | N6      | 24.36%                |
| 11               | N10     | 23.43%                |

| Isolation stages | Isolate | Emulsification index |
|------------------|---------|----------------------|
| 1                | Control | 0.00%                |
| 2                | Control | 53.65%               |
| 4                | E3      | 51.68%               |
| 5                | T1      | 44.66%               |
| 6                | G3      | 72.90%               |
| 7                | G6      | 66.72%               |
| 8                | G7      | 68.57%               |
| 9                | M2      | 58.10%               |
| 10               | N6      | 45.31%               |
| 11               | N10     | 51.43%               |

3.5 SARA (Saturate, Aromatic, Resin, and Asphaltenes) analysis result

Due to this complex composition of crude oil, characterization of each molecular type is not possible. Instead, analysis of hydrocarbon group type can be done using SARA (saturate, aromatic, resin, and asphaltenes) method [12].

Based on SARA analysis (Figure 1), each isolate candidate (G3, G7, and N6) showed different degrading activity on each fraction. Overall, asphaltenes, resin, and aromatic fraction showed reduction abundance compared to control. However, saturate fraction abundance was increased. This could be due to accumulation of intermediate compound resulted from high fraction degradation of crude oil. A previous study has also reported that degradation and biotransformation of crude oil from Oman oilfield resulted in significant increase to short carbon chain (C12 and C14) abundance [12]. It revealed that biodegradation and biotransformation could cause heavier fraction to change to lighter fraction resulted in the decrease of crude oil viscosity [12][13].
SARA result showed that isolate G3 had high ability to degrade aromatic fraction (38.27%) and resin fraction (29.26%), isolate G7 had high ability to degrade aromatic fraction (61.14%), and N6 isolate had high ability to degrade asphaltene fraction (51.76%), as depicted in Figure 1. Each bacteria has a unique mechanism to degrade crude oil compound, but overall cometabolism is the common mechanism involved in resin and asphaltene degradation. Other study in asphaltene degradation analysis using FTIR confirmed that asphaltene degradation could produce aromatic group, and aliphatic saturated fraction at 2,675 and 3,115 cm\(^{-1}\) [14]. These studies indicated that the increase of saturate fraction in SARA analysis could be caused by accumulation of asphaltene fraction degradation products.

![Figure 1. Distribution of SARA fraction (K: control; A: treatment using G; B: treatment using G7; C: treatment using N6)](image1)

3.6 Gas chromatography-mass spectrometry analysis result
The change in total crude oil composition was analyzed using GCMS. Resin and asphaltene fraction couldn’t be detected because both fraction have high boiling point which caused improper separation [15].

In this study, phytan was used as biomarker to analyze GCMS data because it shows stable abundance in control or in treatment condition. The ratio of phytan and target compound showed that the abundance of hydrocarbon chain from C\(_{11}\)-C\(_{18}\) decreased significantly (Figure 9). This confirmed that the three isolates could degrade C\(_{11}\)-C\(_{18}\) hydrocarbon. However, the abundance of C\(_{19}\)-C\(_{28}\) hydrocarbon for isolate G3, G7, and N6 treatment were increased. The total alkane abundance (Figure 3) for all treatment were increased and this could be due to accumulation of bioproduct from aromatic, resin, and asphaltene biodegradation process. This result also confirmed the SARA result.
3.7 Molecular identification of G3, G7, and N6 isolates using 16S rRNA

Phylogenetic result showed that isolate G3 was *Pseudoxhantomonas taiwanensis*, 100% identity match. *Pseudoxhantomonas* is a genus of bacteria that is commonly find in oil contaminated soil. Several study reported that *Pseudoxhantomonas* sp. could degrade n-tetradecane and n-hexadecane [16]. This characteristic of *Pseudoxhantomonas* sp. corresponded with its ability to degrade C_{11}-C_{18} hydrocarbon fraction in this study as indicated by GCMS result.

Isolate G7 showed 99% identity match with *Brevibacillus agri* (bootstrap 100). *Brevibacillus agri* has the ability to degrade tetradecane, hexadecane, and alkanesulfonate [17]. Moreover, this bacteria was reported to have enzymes that is involved in biodegradation process [17]. GCMS result confirmed that *Brevibacillus agri* could degrade C_{11}-C_{19} hydrocarbon fraction.

Isolate N6 showed 99% identity match with *Bacillus subtilis*. Several study reported that *Bacillus subtilis* has nahAc gen and nidA that code for dioxygenase enzyme that is involved in biodegradation process [18]. *Bacillus subtilis* could produce lipopeptide biosurfactant type and could degrade C_{19} alkane [19,20].

![Figure 3. Comparison alkanes hydrocarbon fraction abundance relative to phytan](image)

Figure 3. Comparison alkanes hydrocarbon fraction abundance relative to phytan

4. Conclusion

From this study, tree bacterial species (isolate G3, G7, and N6) were chosen for their ability to degrade crude oil. Molecular identification revealed that isolate G3 was *Pseudoxhantomonas taiwanensis* which could degrade aromatic and resin fraction, isolate G7 was *Brevibacillus agri* which could degrade resin, and isolate N6 was *Bacillus subtilis* which could degrade asphaltene. This result suggested that those bacteria were highly potential to be applied for MEOR technology.
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