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**Full Paper**

# Rhamnolipid produced by *Pseudomonas aeruginosa* USM-AR2 facilitates crude oil distillation

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**A biosurfactant-producing and hydrocarbon-utilizing bacterium, *Pseudomonas aeruginosa* USM-AR2, was used to assist conventional distillation. Batch cultivation in a bioreactor gave a biomass of 9.4 g $L^{-1}$ and rhamnolipid concentration of 2.4 g $L^{-1}$ achieved after 72 h. Biosurfactant activity (rhamnolipid) was detected by the orcinol assay, emulsification index and drop collapse test. Pretreatment of crude oil TK-1 and AG-2 with a culture of *P. aeruginosa* USM-AR2 that contains rhamnolipid was proven to facilitate the distillation process by reducing the duration without reducing the quality of petroleum distillate. It showed a potential in reducing the duration of the distillation process, with at least 2- to 3-fold decreases in distillation time. This is supported by GC-MS analysis of the distillate where there was no difference between compounds detected in distillate obtained from treated or untreated crude oil. Calorimetric tests showed the calorie value of the distillate remained the same with or without treatment. These two factors confirmed that the quality of the distillate was not compromised and the incubation process by the microbial culture did not over-degrade the oil. The rhamnolipid produced by this culture was the main factor that enhanced the distillation performance, which is related to the emulsification of hydrocarbon chains in the crude oil. This biotreatment may play an important role to improve the existing conventional refinery and distillation process. Reducing the distillation times by pretreating the crude oil with a natural biosynthetic product translates to energy and cost savings in producing petroleum products.**

**Key Words**——crude oil; distillation; emulsification; *Pseudomonas aeruginosa*; rhamnolipid

## Introduction

Petroleum microbiology is the study of interactions of microorganisms with petroleum (Van Hamme et al., 2003). From the 1930s to the 1990s, research proved that bacteria are important in a number of petroleum-related processes. Many bacteria have the ability to utilize specific hydrocarbons as sole carbon and energy sources by nature (Hua et al., 2004; Korda et al., 1997; Rahman et al*.,* 2003). More than 100 species representing about thirty genera have been identified. These include mostly bacteria, actinomycetes, yeasts and filamentous fungi. However, bacteria play the central role in hydrocarbon degradation (Chaerun et al., 2004; Ilori et al., 2006). Examples of hydrocarbon-utilizing bacteria are *Acinetobacter, Pseudomonas, Nocardia, Rhodococcus,* and *Mycobacterium* (Ilori et al*.,* 2005; Kosaric, 1993).

It is widely agreed that the metabolic attack by mi-

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croorganisms on a hydrocarbon substrate involves the production of surface-active compounds typically present in the culture medium, called biosurfactants (Batista et al., 2005; Rashedi et al., 2005). They are amphiphilic compounds that reduce surface and interfacial tensions by accumulating at the interface of immiscible fluids or of a fluid and solid, and consequently, increase the surface areas of insoluble compounds leading to increased mobility, bioavailability and subsequent biodegradation and emulsification (Ron and Rosernberg, 2001; Vasileva-Tonkova and Gesheva, 2007). The biological function of this surface-active compound is related to hydrocarbon uptake, where spontaneous release occurs with hydrocarbons as substrates (Guerra-Santos et al., 1984; Makkar and Cameotra, 1998; Singh et al., 2006).

In regard to hydrocarbon compounds, petroleum or crude oil is a liquid found in formations in the earth consisting of a complex mixture of hydrocarbons which includes mostly alkanes of various lengths (cycloalkanes, aromatics, and polycyclic) together with varying quantities of nitrogen, sulfur, and oxygen-containing compounds. Crude oil is the term for "unprocessed" oil, while the word petroleum means "rock oil" (Speight, 2001). Petroleum or crude oils exhibit wide variations in composition and physical properties. They differs markedly in volatility, solubility, and susceptibility to biodegradation. The mixture of hydrocarbons is highly complex; paraffinic, naphthenic, and aromatic structures can occur in the same molecule, and the complexity increases with boiling range (O'Donnell, 1975).

The capacity of bacteria, especially *P. aeruginosa,* to metabolize aerobically heavy oil or aliphatic hydrocarbons and the ability to secrete large amounts of biosurfactant while growing on a hydrocarbon-rich medium is well known (Chayabutra and Ju, 2000; Hommel, 1990). Moreover, the ability of *Pseudomonas* species in producing a rhamnolipid biosurfactant, with potential surface active properties when grown on different carbon substrates makes it a promising candidate for the large-scale production of biosurfactants (Dehghan Noudeh et al., 2010; Ueno et al., 2006).

In this study, *Pseudomonas aeruginosa* USM-AR2, a hydrocarbon-utilizing and a biosurfactant-producing bacterium (Nur Asshifa, 2009), was used to assist the conventional crude oil distillation process. Distillation is a common method for the fractionation of petroleum used in the laboratory as well as in refineries, producing desirable petroleum-based products such as automotive fuel and kerosene (Kister, 1992). It is a basic and widely used separation process in the petroleum industry. This process has evolved from the simple distillation units to complex multiplate still used in the refining industry. Distillation of crude oil yields several fractions, which are then used for different properties, for example to produce different carbon products based on the carbon atoms. Since the chemical structure of petroleum is composed of hydrocarbon chains of different lengths, these different hydrocarbon chemicals are separated by distillation in oil refineries to produce gasoline, jet fuel, kerosene, diesel and other fuel oils, and liquefied petroleum gas (Speight, 2001). Research was carried out to explore the potential of this bacterium and the effects of its activity on crude oil biomodification, thus facilitating the distillation process.

## Materials and Methods

*Production of biomass and rhamnolipid in bioreactor.* Batch cultivation of *P. aeruginosa* USM-AR2 was carried out in a 2 L fermenter (B.Braun, Biotech International, Model Biostat B) with 1 L working volume at ambient temperature (27°C) by maintaining dissolved oxygen at 30% through cascade mode with agitation in improved growth media containing 7% (v/v) diesel, 0.6% (w/v) yeast extract, 0.05% (w/v) $MgSO_4$, and 0.05% (v/v) Tween 80. The cultivation system was equipped with Multi Function Control System (MFCS) software. During cultivation, culture broth was periodically collected and analyzed for growth and biosurfactant (rhamnolipid) production. Cells were harvested by centrifugation at 10,000 $\times$ *g* for 5 min, treated with acetone to remove residual hydrocarbon (diesel) and washed twice with distilled water. The cell pellet was suspended in 3 ml of distilled water and the biomass, expressed in dry weight (g $L^{-1}$), was obtained from a calibration curve. Bacterial growth was monitored by absorbance measurements at 540 nm with a spectrophotometer (Thermo Spectronic Genesys 20, Model 4001-04, USA).

*Rhamnolipid quantification: Orcinol assay.* The orcinol assay was used for direct assessment of the amount of glycolipids in the culture sample. A modified version from previous research (Jeong et al., 2004) was carried out to quantify the rhamnolipids in the culture. The quantification of the rhamnolipids was car-

ried out through an indirect way, using the rhamnose as reference, as the rhamnose is a byproduct of the acid hydrolysis of the rhamnolipids. Extracellular glycolipid concentration was evaluated by measuring the concentration of rhamnose: To 0.3 ml of each sample, 2.7 ml of a solution containing 0.19% orcinol (in 53% $H_2SO_4$) was added. After heating for 40 min at 70°C the samples were cooled at room temperature and the $OD_{421}$ nm was measured using a spectrophotometer (Thermo Spectronic Genesys 20, Model 4001-04, USA). The rhamnolipid concentrations were calculated from a standard curve prepared with L-rhamnose by comparing the data with those of rhamnose standards between 0 and 0.5 g $L^{-1}$ and expressed as rhamnose equivalents (RE) (g $L^{-1}$).

*Biosurfactants detection*

*Qualitative analysis: Drop collapse test.* Qualitative drop collapse tests were performed on *P. aeruginosa* USM-AR2 supernatant. Two droplets of engine oil (black oil) were delivered onto a glass slide, following by another two droplets of supernatant into the center of the oil drop. If the drop remained beaded, the result was scored as negative. If the drop spread and collapsed, the result was scored as positive for the presence of biosurfactant (Youssef et al., 2003). A sterile liquid medium was used as the control.

*Quantitative analysis: Emulsification activity.* The interfacial activity of rhamnolipid was assessed by determining its emulsification index on different crude oils. Emulsifying activity of rhamnolipid was determined by adding 2 ml of crude oil to the same volume of rhamnolipid in a test tube. The tube was vortexed vigorously for 2 min and left to stand for 24 h. A chemical surfactant, Tween 80, was used as a control and for comparison. The $E_{24}$ was measured in three replicates for each crude oil. The emulsification index ($E_{24}$%) was determined using the following equation (Cooper and Goldenberg, 1987):

$$E_{24}\ (\%) = \frac{\text{Height of emulsified layer (mm)}}{\text{Height of total liquid mixture (mm)}} \times 100$$

*Distillation performance study.* Five ml of *P. aeruginosa* USM-AR2 culture obtained at the early stationary phase, containing 2.4 g $L^{-1}$ of rhamnolipid, was inoculated into 45 ml of crude oil and mixed or incubated for 15 min at 200 rpm. A simple distillation apparatus was set up in a laboratory. The control crude oil sample (without bacterial culture) or a mixture of crude oil and bacterial culture was poured into the round-bottom flask and supplemended with a few anti-bumping granules to ensure even boiling. The flask containing samples was heated slowly by adjusting the temperature through the heating mantle to force components, which have different boiling points, into the gas phase. The gas was then condensed back into liquid form and collected through the distillation output. The increased of temperature was observed and recorded. The performance was observed by comparing the distillation times. Distillation times are the period when the distillation process starts (0 min) until distillate is collected at least at 200°C. Every experiment was carried out in duplicate. The crude oils used in this research were crude oil TK-1 and AG-2 donated by Petronas, Malaysia. The detail properties of the crude oils were not provided, except for the code only.

*Distillate analysis*

*Heat of combustion by bomb calorimetry.* Calorimetry is an important tool in evaluating the products of petroleum or crude oil. A calorimeter is a device used to measure the heat of a reaction. The heat of combustion of the distillates obtained in every distillation process was determined by a bomb calorimeter. The bomb calorimeter works by burning a known weight of the distillate (hydrocarbon) in high pressure oxygen in a stainless steel container, maintaining a constant volume. The stainless steel container is surrounded by water and the heat evolved by the reaction is absorbed by the surrounding water. By measuring the temperature change of the water, the heat evolved under constant volume ($q_v$) can be determined by measuring the temperature difference before and after the reaction. Since the temperature differences are very small, an extremely sensitive thermometer is required for these measurements. It takes exactly 1 calorie (cal) of heat to raise the temperature of 1 gram of liquid water by 1°C. The total amount of heat absorbed by the water can be calculated as follows:

$$\text{Heat absorbed (cal)} = \text{mass of water} \times \text{temperature change} \times 1.00\ \text{cal/g °C}$$

*Gas Chromatography Mass Spectrometry.* The distillates were also analyzed by Gas Chromatography Mass Spectrometry (GC-MS) for component identification. GC-MS analysis was performed using Agilent 6890N with an Agilent Technologies 123-5032 DB-5 (30 m × 0.25 mm ×0.25 μm) capillary column and SGE 10 μl syringe. One microliter of distillate samples were injected and oven temperature was programmed from 50°C to 230°C at the rate of 4°C/min and then isothermally held for 10 min until the analysis was

completed.

## Results

*Growth and rhamnolipid production by P. aeruginosa USM-AR2 culture*

*P. aeruginosa* USM-AR2 growth of 9.4 g $L^{-1}$ was accompanied with rhamnolipid production. The culture started to produce rhamnolipid just as the cells entered the exponential phase, and the rhamnolipid concentration reached a maximum of 2.4 g $L^{-1}$ after 72 h when cells were in stationary phase (Fig. 1). This stationary phase culture was used to be inoculated or mixed to the crude oil. Biosurfactant activity was detected by the drop collapse test, by comparing the oil droplet treated with sterile medium as the control (Fig. 2a) and oil droplet treated with culture (Fig. 2b). A drop of supernatant containing biosurfactant collapsed and spread completely over the surface of the engine oil. In addition, biosurfactant activity was also confirmed by the emulsifying activity of rhamnolipid in supernatant on crude oil TK-1 and AG-2 (Table 1). Compared to Tween 80, rhamnolipid displayed better performances or higher emulsifying activity for both crude oils, thus confirming its interfacial activity and enabling their use for emulsifying purposes (Cruz et al., 2010).

*Distillation performance of treated and untreated crude oil*

Tests on microbial activity in biomodification of crude oil TK-1 and AG-2 have shown very convincing results, decreasing the surface tension of crude oil, resulting in significant reductions, at least 2- to 3-fold decrease of distillation times (Tables 2 and 3). As stated in Table 2 (crude oil AG-2), in the control experiment, distillate only could be collected when temperature reached 200°C to 210°C. This was a very narrow range of temperature of distillate obtained through the conventional way. This resulted in a small volume of distillate ($\leq$ 0.5 ml). By treating the crude oil with rhamnolipid, the volume of distillate collected increased up to 20 times, which was up to 10 ml. The same result was shown by crude oil TK-1(Table 3) where it paralleled the results of crude oil AG-2. Figure 3 (a) and (b) showed the distillates collected after distillation.

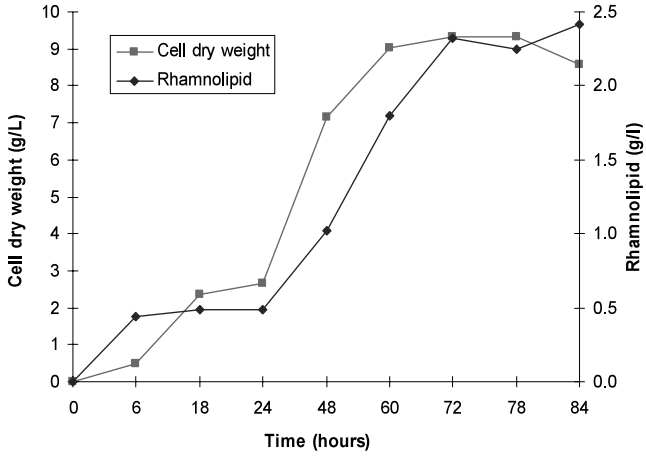
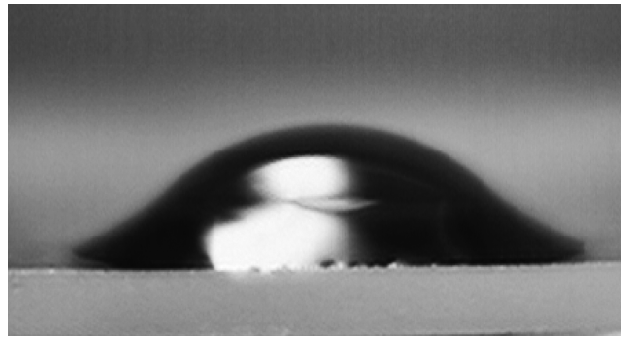
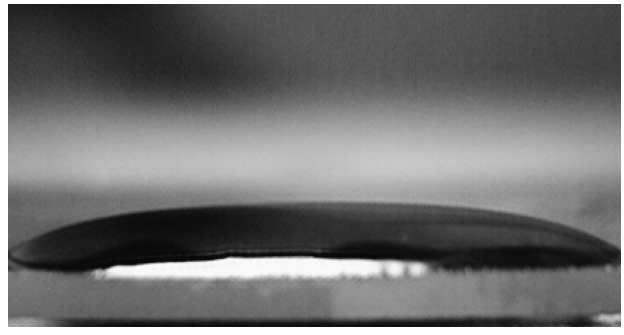



Fig. 1. Growth and rhamnolipid production of *P. aeruginosa* USM-AR2 culture in fermenter.



(a)



(b)

Fig. 2. (a) Oil droplet (black oil) remains beaded with sterile medium. (b) Oil droplet (black oil) collapses when treated with culture of *P. aeruginosa* USM-AR2.

Table 1. Emulsifying activity of rhamnolipid and Tween 80 (control) on crude oil TK-1 and AG-2 represented by emulsification index ($E_{24}$%).

| Crude oil | $E_{24}$ (%) Tween 80 | $E_{24}$ (%) Rhamnolipid |
|---|---|---|
| TK-1 | 10.5 | 56.6 |
| AG-2 | 2.1 | 76.8 |

Table 2. Comparison of distillation performance between treated and untreated crude oil AG-2 with *P. aeruginosa* USM-AR2 culture.

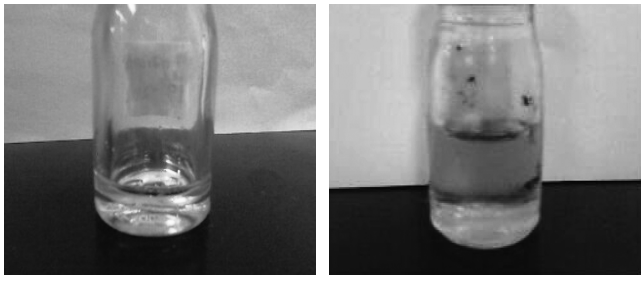
| Parameters | Control/Conventional way | With biotreatment |
|---|---|---|
| Time taken for obtaining distillate (min) | 25 | 8 |
| Temperature when distillate obtained | 200–210°C | 110–200°C |
| Volume of distillate (ml) | 0.5 | 10.0 |
| Calorie value (kcal/g) | 10.77 | 10.72 |
| GC-MS analysis | No difference[a] | No difference[a] |

[a] 'No difference' in GC-MS analysis means the same peaks representing the same components were identified in both distillates of treated and untreated crude oil AG-2 (control) (Refer Fig. 4).

Table 3. Comparison of distillation performance between treated and untreated crude oil TK-1 with *P. aeruginosa* USM-AR2 culture.

| Parameters | Control/Conventional way | With biotreatment |
|---|---|---|
| Time taken for obtaining distillate (min) | 22 | 11 |
| Temperature when distillate obtained | 160–210°C | 110–160°C |
| Volume of distillate (ml) | 0.5 | 5.0 |
| Calorie value (kcal/g) | 11.17 | 12.05 |
| GC-MS analysis | No difference[a] | No difference[a] |

[a] 'No difference' in GC-MS analysis means the same peaks representing the same components were identified in both distillates of treated and untreated crude oil TK-1 (control).



(a) (b)

Fig. 3. Distillate of crude oil AG-2 collected after distillation (a) without treatment and (b) with treatment of *P. aeruginosa* USM-AR2 culture.

*Distillate analysis*

The calorie value of the distillates remained the same after treatment (Tables 2 and 3). This showed that the energy value for the distillate of the control and treated sample stayed high and showed no significant difference. GC-MS analysis of distillate also showed parallel results, with no difference of compounds detected in the distillate of treated or untreated crude oil (control) (Fig. 4). These two factors confirmed that the quality of the distillate was not compromised and the incubation process with the microbial culture did not over-degrade the oil.

GC-MS analysis was used to identify components in the distillate from crude oil AG-2. More than 200 components were detected in the distillate for the control and treated sample. The same chromatograms were detected in both samples (Fig. 4 (a) and (b)). Eighteen components with the widest area and high percent matching were identified and characterized. Components that have been identified in the distillate of the control and treated crude oil AG-2 are listed (Table 4). Referring to that, it can be concluded that most of the compounds in the distillate of crude oil identified by GC-MS were the product of gasoline, diesel, kerosene and lubricating and motor oils (Potter and Simmons, 1998; Sheng et al*.,* 2006; Smith and Bruno, 2007).

## Discussion

Biosurfactant produced by this culture was a glycolipid type biosurfactant, named rhamnolipid. Most *Pseudomonas* sp., especially *P. aeruginosa,* produces rhamnolipid biosurfactant (Beal and Betts, 2000;

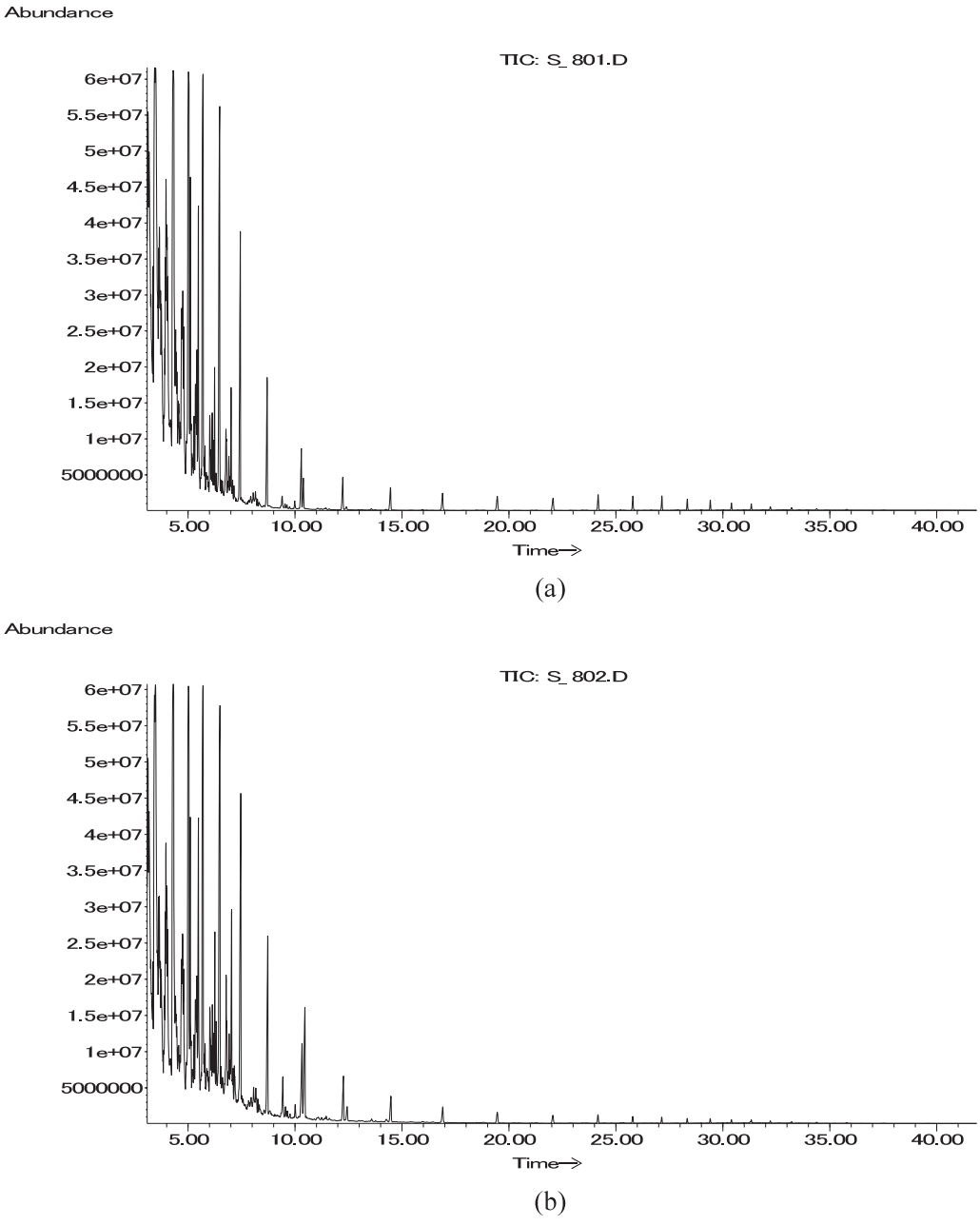



Fig. 4. The same chromatogram of distillate of (a) control (801) and (b) treated crude oil AG-2 (802).

Rashedi et al., 2005; Reiling et al., 1986). The screening of a biosurfactant producer is usually carried out by monitoring the properties that estimate surface activity, such as surface tension and the ability to emulsify oil as represented by the drop collapse test and emulsification index. The production of rhamnolipid by *P. aeruginosa* USM-AR2 culture using diesel as the hydrocarbon substrate showed that emulsification by biosurfactants is a cell density-dependent phenomenon, that is, the greater the number of cells, the higher the concentration of extracellular product (Fig. 1).

According to Ron and Rosenberg (2001), biosurfactants play various physiological roles and important characteristics include increasing the surface area of water-insoluble substrates by emulsification and increasing the bioavailability of hydrophobic substrates. As this bacteria grows at the oil-water interface, the production of biosurfactants when the density is high will increase the surface area of the drops, allowing more bacteria to feed (Ron and Rosernberg, 2001; Singh et al., 2006).The ability of *P. aeruginosa* USM-AR2 in facilitating crude oil distillation is also related to

Table 4. Characterization of compounds detected in distillate of treated and untreated crude oil AG-2 by GC-MS.

| No | MW | Time | % Matching | Compound | Molecular formula | Group/Product |
|---|---|---|---|---|---|---|
| 1. | 120 | 3.351 | 95 | 1,2,4-trimethyl-benzene | $C_9H_{12}$ | Gasoline, diesel, kerosene |
| 2. | 120 | 3.365 | 93 | 1,3,5-trimethyl- benzene | $C_9H_{12}$ | Gasoline, diesel, kerosene |
| 3. | 142 | 3.456 | 96 | Decane | $C_{10}H_{22}$ | Gasoline (petrol), diesel, kerosene |
| 4. | 156 | 4.051 | 94 | 3-methyl-decane | $C_{11}H_{24}$ | Kerosene |
| 5. | 156 | 4.260 | 94 | Undecane | $C_{11}H_{24}$ | Diesel, kerosene |
| 6. | 86 | 4.75 | 90 | 2-methyl-undecane | $C_{12}H_{26}$ | Kerosene |
| 7. | 170 | 5.009 | 95 | *n*-dodecane | $C_{12}H_{26}$ | Diesel, kerosene, lubricating and motor oils |
| 8. | 184 | 5.694 | 96 | *n*-tridecane | $C_{13}H_{28}$ | Diesel, kerosene, lubricating and motor oils |
| 9. | 198 | 6.464 | 98 | *n*-tetradecane | $C_{14}H_{30}$ | Diesel, kerosene, lubricating and motor oils |
| 10. | 212 | 7.429 | 98 | *n*-pentadecane | $C_{15}H_{32}$ | Diesel, kerosene, lubricating and motor oils |
| 11. | 240 | 10.297 | 98 | *n*-heptadecane | $C_{17}H_{36}$ | Diesel, kerosene, lubricating and motor oils |
| 12. | 268 | 10.388 | 99 | 2,6,10,14-tetramethyl-pentadecane (pristane) | $C_{19}H_{40}$ | Diesel, lubricating and motor oils |
| 13. | 254 | 12.235 | 99 | *n*-octadecane | $C_{18}H_{38}$ | Diesel, gasoline, kerosene, lubricating and motor oils |
| 14. | 282 | 12.403 | 99 | 2,6,10,14-tetramethyl-hexadecane (phytane) | $C_{20}H_{42}$ | Diesel, lubricating and motor oils |
| 15. | 268 | 14.46 | 98 | *n*-nonadecane | $C_{19}H_{40}$ | Diesel, kerosene, lubricating and motor oils |
| 16. | 282 | 16.901 | 99 | *n*-eicosane | $C_{20}H_{42}$ | Diesel, kerosene, lubricating and motor oils |
| 17. | 296 | 19.454 | 99 | *n*-heneicosane | $C_{21}H_{44}$ | Diesel, kerosene, lubricating and motor oils |
| 18. | 310 | 22.063 | 98 | *n*-docosane | $C_{22}H_{46}$ | Diesel |

the ability of this microorganism to produce rhamnolipid, which accelerates the emulsification process of the crude oil (Cybulski et al., 2003; Rashedi et al., 2005; Zhang and Miller, 1995). Rhamnolipid produced by *P. aeruginosa* USM-AR2 enhanced degradation by dispersing and emulsifying hydrocarbons in the crude oil (Biswas et al., 2005; Desai and Banat, 1997; Francy et al., 1991). This is supported by emulsification index ($E_{24}$) results, which recorded 76.8% emulsifying activity of rhamnolipid towards crude oil AG-2 and 56.6% towards crude oil TK-1 (Table 1). The emulsification index was an important parameter of surface-active agent properties through the course of producing a stable emulsion. The higher the emulsification index, the higher the emulsification activity (Dehghan Noudeh et al., 2010). The emulsifying activity of rhamnolipid produced by *P. aeruginosa* USM-AR2 revealed that it could be used as an emulsion-forming agent for hydrocarbons and crude oils.

Referring to Table 2 and 3, the lower and wider temperature range of distillate obtained from treated crude oil compared to the control made it possible to collect a higher amount of distillate and shorten the distillation times, from 25 min to 8 min (AG-2) and from 22 min to 11 min (TK-1). The complexity of petroleum chains may cause the same compounds of distillate to come out at different temperatures, as long as it is still in almost the same temperature range. This is observed in the results where the same compounds were identified by GC-MS but were collected at different temperatures.

Generally, the enrichment of a bacteria population results in a more rapid rate and a greater extent of hydrocarbon utilization (Dibble and Bartha, 1979; Noordman and Janssen, 2002). Bacteria are known to utilize certain hydrocarbons present in crude oil as an energy source, typically through a slow and incomplete attack. The breakdown of certain chains by bacterial cul-

ture is expected to result in an increase in the porosity and permeability of the hydrocarbon bonds, making oil emulsification easier and more complete. Thus, a higher volume of distillate can be collected as the degradation or emulsification loosens the complex trapped in the chains. The same reason can explain why the distillate of a treated crude oil was obtained at lower temperatures.

As for calorie value, calorimetry is the science of measuring the amount of heat, and hydrocarbons are among the most energy-rich and cleanest-burning fuels. Heats of combustion are of practical importance, as fuels are traded based on the calorific value of the material; for example liquid fuels such as gasoline, kerosene, and diesel are also valued by bomb calorimetry. The heat of combustion of the fuel will provide a measure of the energy available from a fuel.

Overall, from this research, it was proven that rhamnolipid produced by *P. aeruginosa* USM-AR2 culture helped to:

i. Shorten distillation times by shortening the period to obtain distillate
ii. Shorten distillation times by lowering the temperature where distillate could be obtained earlier
iii. Increase the volume of distillate obtained
iv. Maintain the high energy/calorie value of the distillate
v. Maintain the quality of petroleum distillate (no difference in compounds characterized by GC-MS).

In conclusion, the ability of rhamnolipid in facilitating the distillation process without affecting or reducing the quality of distillate obtained from the crude oil has been proven. Emulsification of hydrocarbon chains by rhamnolipid, a biosurfactant which is known to influence various processes related to hydrocarbon degradation, was the main factor that enhanced the distillation performance. Results showed that its application may play an important role to improve the existing conventional refinery and distillation process for the petroleum industry. Reducing the distillation times together with enhanced distillate volume translates to energy and cost savings in producing petroleum products. A patent related to this research paper entitled "Application of USM-AR2 (*Pseudomonas aeruginosa*) in facilitating hydrocarbon distillation process" (PI 20095437 and PCT/MY2010/000047) has been filed regarding a detailed method for pretreating crude oil using the microorganism. On-going studies are being carried out on the analysis of factors that may affect the distillation performance of different crude oils at different extents. At this moment, the production of rhamnolipid and its applicability in the crude oil distillation process was still carried out on a lab scale as there is a lack of large-scale facilities for testing large volumes of crude oil. However, attempts have been made in looking for a larger scale testing in the oil refinery field. By applying and realizing the technology on a larger scale, an accurate cost could be determined.

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