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Pseudomonas aeruginosa USM-AR2 culture containing biosurfactant facilitates crude oil distillation process

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Abstract. Petroleum supplies a large portion of the world's energy, thereby driving industrial civilization. It remains an important factor in dictating market prices of diverse products and services. Distillation of crude oil is a common fractionation method of petroleum producing desirable products, such as automotive fuel and kerosene. Conventional distillation has been the current fractionation process for decades, producing distillates of interest and is conducted without any involvement of biological materials. The process involves tremendous amount of thermal energy and requiring a long time. This research offers an improved distillation performance and yield. A small amount of rhamnolipid produced from a microbial culture *Pseudomonas aeruginosa* USM-AR2, is added and mixed thoroughly with the crude oil prior to distillation. This results in a drastic reduction in distillation time without affecting or reducing the quality of the distillate. The pretreatment accelerates the emulsification and biodegradation process by decreasing surface tension and viscosity of oil, which in turn resulted in the shorter distillation times. Reducing the distillation times translates to energy and cost savings in producing petroleum products. Since oil prices affect many other industries, this discovery will have a great impact on the economy.

Keywords: rhamnolipid, distillation, crude oil, emulsification, *Pseudomonas aeruginosa*

1. Introduction

Petroleum microbiology is the study of interactions of microorganisms with petroleum, a complex chemical mixture that contains predominantly hydrocarbons. Beginning in the 1930s and extending through the late 1970s, Claude Zobell's research established that bacteria are important in a number of petroleum related processes. 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. It differs markedly in volatility, solubility, and susceptibility to biodegradation [1].

Isolating and identifying microorganisms responsible for hydrocarbon transformations have long been known as important from a fundamental and applied viewpoint, and lists of hydrocarbon-degrading organisms such as bacteria, yeasts, fungi and algae are available. These microorganisms are equipped with the metabolic machinery to use petroleum as a carbon and energy source [2,3,4].

It is widely agreed that the metabolic attack by microorganisms on a hydrocarbon substrate involves the production of surface-active compounds typically present in the culture medium, called biosurfactants [5,6]. They are amphiphilic compounds that reduce surface and interfacial tensions by accumulating at the interface of immiscible fluids or of a fluid and solid, consequently, increase the surface areas of insoluble compounds leading to increased mobility, bioavailability and subsequent biodegradation and emulsification. It can be accumulated as cell-bound or as an extracellular product [7,8]. Among the known surfactant-producing bacteria, *Pseudomonas* species is well known for its capability to produce rhamnolipid biosurfactant when grown on different carbon substrates [9,10].

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In this study, *P. aeruginosa* USM-AR2, identified as hydrocarbon-utilizing and biosurfactant-producing bacterium was explored to assist the conventional distillation process. Distillation is a basic and widely used separation process for the fractionation of petroleum used in the laboratory as well as in refineries, producing desirable products, such as automotive fuel and kerosene [11]. It has become the process of choice for petroleum refining. 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.

2. Materials and methods

2.1. Orcinol assay

The orcinol assay was used for direct assess of the amount of glycolipids in the culture sample. The quantification of the rhamnolipids was carried through an indirect way, using the rhamnose as reference as the rhamnose is a byproduct of the acid hydrolysis of the rhamnolipids [12]. Extracellular glycolipids concentration was evaluated by measuring the concentration of rhamnose: To 0.1 ml of each sample 0.9 ml of a solution containing 0.19% orcinol (in 53% H_2SO_4) was added. After heating for 40 min at 70 ºC the samples were cooled at room temperature and the OD_{421} nm was measured. The rhamnolipid concentrations were calculated from a standard curves prepared with L-rhamnose by comparing the data with those of rhamnose standards between 0 and 0.5 g/L and expressed as rhamnose equivalents (RE) (g/L).

2.2. Distillation study: Treatment of crude oil with P. aeruginosa USM-AR2 culture

A simple distillation apparatus was set up in laboratory. The crude oil used in this research was crude oil Angsi given by Petronas, Malaysia. The control crude oil sample (without bacterial culture) or a mixture of crude oil and bacterial culture was filled into the round bottom flask and added with a few anti-bumping granules to ensure even boiling. The flask containing samples was heated slowly by adjusting temperature through the heating mantle. The increased of temperature was observed and the distillate was collected through the distillation output.

Five mL of *P. aeruginosa* USM-AR2 culture at stationary phase was inoculated into 40 mL of crude oil Angsi and mixed for 15 minutes. The mixture was distilled to compare the performance between the crude oil which was treated with the microbial culture and the untreated crude oil (control). The performance was observed by comparing the distillation times. Distillation times are period when the distillation process starts (0 min) until distillate is collected at 200 ºC. Every experiment was carried out in duplicates. The crude oil was inoculated with cell pellet suspension, supernatant or the whole culture to determine if biosurfactants were released into the culture medium. Pellet and supernatant were obtained after the centrifugation of the culture.

2.2.1 Distillate analysis : Heat of combustion by bomb calorimetry

A calorimeter is a device used to measure heat of 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 was 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:

Heat absorbed (cal) = mass of water × temp. change × 1.00 cal/g °C

2.2.2 Distillate analysis : Gas Chromatography Mass Spectrometry

The distillates were also analyzed by Gas Chromatography Mass Spectrometry (GC-MS) for components identification. GC-MS analysis was performed using Agilent 6890N with Agilent Technologies 123-5032 DB-5 (30 m × 0.25 mm ×0.25 µm) capillary column and SGE 10µL syringe. 1 µL of distillate samples were

injected and oven temperatures was programmed from 50 ºC to 230 ºC at the rate of 4 ºC/min and then isothermally held for 10 min until the analysis was completed.

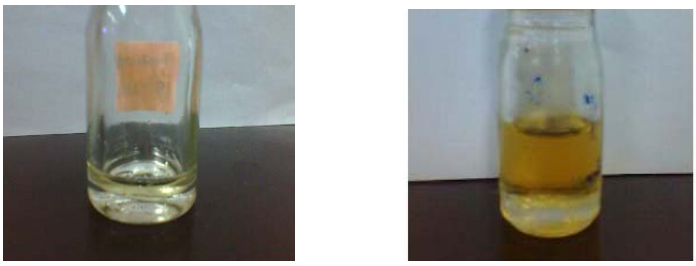
2.3. Viscosity performance study

Fourty mL of the culture of *P. aeruginosa* USM-AR2 were obtained and added into 10 g of engine oil to test whether it can reduce the viscosity of the oil [13], from 1200 mPa.s. Sterile medium added to the engine oil represents the control. Viscosity readings of the engine oil were taken after the treatment in duplicates at 25-30°C with a Vibro Viscometer, Model SV 10. Viscosity was not measured for the crude oil samples as we were lacking of the crude oils provided.

3. Results and Discussion

The shake flask culture was obtained after 120 hours cultivation during stationary phase. At this point, the cell biomass was about 9 g/L, while rhamnolipid concentration was around 2.4 g/L. The pellet suspension contains 0.25 g/L of rhamnolipid, while no significant difference in rhamnolipid concentration between supernatant and the culture. Crude oil Angsi was treated with five mL of the improved culture of *P. aeruginosa* USM-AR2 with either pellet suspension, culture or supernatant.

No obvious difference was observed among treatment with cell pellet, supernatant or the culture. Figure 1 showed the distillates collected after distillation experiment. Treatment with the improved culture of *P. aeruginosa* USM-AR2 resulted in 2 to 3-fold decrease of distillation times.



(a) (b)

Fig. 1: Distillate of crude oil Angsi collected after distillation (a) without treatment and (b) with treatment of *P. aeruginosa* USM-AR2 culture.

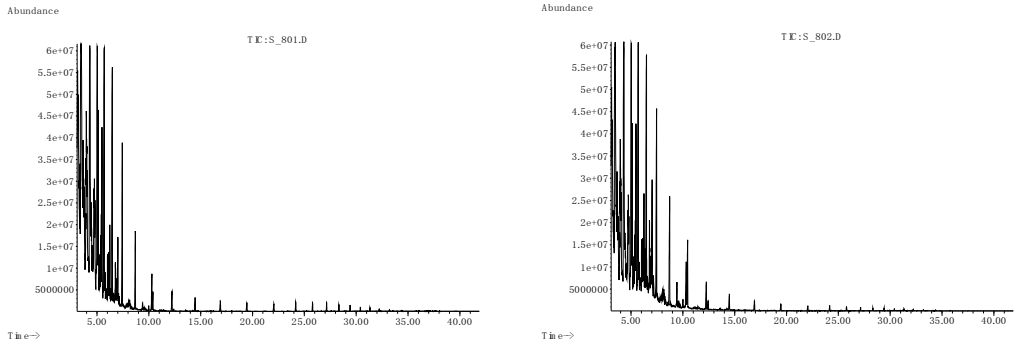
Since all ways of treatments resulted in almost the same reduction of distillation times, which were between 8 to 10 minutes compared to 20 to 25 minutes of control, it is more cost and energy effective to use the culture directly onto the oil without separating the cell and supernatant (data not shown). The ability to collect distillate at lower temperatures compared to that of the control, shortens the distillation times, from 25 min to 8 min (Table 1).

Table 1. Comparison of distillation performance between treated and untreated crude oil Angsi 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 (Refer Figure 2)	No difference (Refer Figure 2)

In addition, distillate from untreated crude oil can be collected when temperature reached 200 °C until 210 °C only. This was a very narrow range of temperature for obtaining distillate through conventional way. Thus, this resulted in a small volume of distillate (≤ 0.5 mL). By treating with bacterial culture, the volume of distillate collected can increase up to 20 times, which was between 7 to 10 mL. The lower and wider temperature range (100-200 °C) made it possible to collect higher amount of distillate. 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 results where the same compounds were identified by GC-MS but were collected at different temperatures (Figure 2). In addition, calorimetric tests showed parallel results, where the calories value of the distillate obtained remained the same after treatment (Table 1). The value between control and all treated samples showed no significant difference. Therefore, these two factors confirmed that the quality of the distillate was not compromised.



(a) (b)

Fig. 2: The same chromatogram of distillate of (a) untreated (801) and (b) treated crude oil Angsi (802)

It is necessary to recall the ability of this microorganism to produce biosurfactants (rhamnolipid), which accelerate the emulsification and biodegradation process. As this bacteria grow 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 [14].

Microorganisms are grouped to endo- and exo-type ones, due to biosurfactant accumulation. The ability to produce emulsifying surfactant compounds at the cell membrane is one of the determining characteristics of hydrocarbon assimilation [15]. Endotype biosurfactants are bound up with the wall surface of the microorganism cell and, as a rule, constitute components liposomally active. While, exo-type biosurfactants are excreted into medium by cell to provide substrate access to cell surface, due to emulsion or suspension production in liquid medium [16]. Reduced distillation times for treatment with pellet showed that the biosurfactants may exist as cell bound. Instead of biosurfactants production, bacterial cell membrane can also affect distillation performance in a positive way. The hydrophobicity of an organism is due to the presence of lipophilic cell wall and cell membrane components, some of which are surface active. Therefore, the cell itself can demonstrate significant emulsifying activity and act as a biosurfactant agent [17].

Furthermore, the treatment helped to reduce oil viscosity by breaking down the molecular structure of oil, making it more fluid. This was supported by the viscosity measurement of engine oil where culture treatment showed a significant reduction of the oil, where the treatment of engine oil with the culture of *P. aeruginosa* USM-AR2 resulted in lower viscosity (from 1200 mPa.s to 10 mPa.s). All above factors may contribute to the enhanced distillation performance.

From the results, *P. aeruginosa* USM-AR2 was shown to produce biosurfactants both extracellularly and cell bound, where hydrocarbon uptake by microorganisms was facilitated by hydrophobization of the cell

envelope with biosurfactants or by emulsifying hydrocarbons with extracellular surfactants [14, 17]. It was proven that *P. aeruginosa* USM-AR2 culture containing rhamnolipid helped to:

- shorten distillation times by shortening the period to obtain distillate (from 25 to 10 min)
- shorten distillation times by lowering the temperature where distillate can be obtained earlier
- increase the volume of distillate (from 0.5 to up to 10 mL)
- maintain the high energy/calorie value of distillate
- maintain the standard quality of distillate (proven by GC-MS analysis)

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