Isolation and Optimization of Compound Bacteria for Wax Content and Particle Size

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ABSTRACT

For removing wax and reducing viscosity of wax, microbial technology can effectively be used, and thereby, by using this technology, the flow properties of crude oil are improved. In this study, optimization of compound bacteria isolated from oil-contaminated soil and its effect on wax content and particle size are investigated. Moreover, experimental results show that the optimized compound bacteria have the highest cell density value under the optimal conditions. After #11 and #12 compound bacteria are added, the wax content reduces from 21.46% to 8.3%, and the wax removal rate becomes 61.32%. In addition, the polarizing microscope analysis demonstrates that the paraffin is degraded with the bacteria, and its wax crystal micro-structure is changed with the bacteria. Moreover, the focused beam reflectance measurement (FBRM) shows that the proportion of particles of large diameter is significantly decreased, and that of small ones is increased. Consequently, the flow properties of waxy crude oil are improved. The viscosity changes at different temperatures after treatment with bacteria. Finally, it is found out that at the temperature which is equal to 40 to 45 ℃, the rate of viscosity reduction is higher than 60%. Therefore, the paraffinic hydrocarbons of crude oil can be degraded with compound bacteria, and their rheological properties are improved.

Keywords: Waxy Crude Oil, Compound Bacteria, Wax Content, Particle Size, Viscosity.

INTRODUCTION

The increase in the world energy demand and the decline in conventional crude oil have made waxy crude oil a future hydrocarbon resource [1] and have increased its development and utilization. In China, 80% of crude oil produced is waxy crude oil. Crude oil generally refers to a mixture of long-chain hydrocarbons-n-alkanes with the carbon chain length of 17 or higher [2-4]. The wax dissolves in petroleum under high temperature, and the wax content varies with temperature and can range as high as 20% [5, 6]. During transportation, the temperature of crude oil in the pipeline gradually decreases due to some factors such as buried pipeline depth, environmental conditions, and pipe cooling condition. Wax precipitates when the temperature is lower than the cloud point [7]. This condition reduces the radius during transportation

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and even can cause the tube to stop, and thereby the probability of accidents is increased, and casualties are caused. This phenomenon, called wax deposition, is increasingly attracting attention. Many researchers have carried out extensive experiments in laboratory settings, and some of these are applicable to the pipeline or well-zone. At present, the industrial methods are mainly electric heating and chemical agents [8]. However, these methods do not reduce the content of wax, and they only inhibits the precipitation of wax. Thus, the impact is only on the rheological property of crude oil. Therefore, microbial technology is important to improve the flow properties of crude oil.

The above-mentioned technology can greatly reduce the impact of paraffin on the rheological property of crude oil [9]. Since the 1980s, some western countries such as America and Canada started to study microbial anti-wax technology. The technology was applied to oil wells in Texas in 1986 and achieved good results [10]. In recent years, microbial anti-wax technology has been used to improve the fluidity of crude oil in the petroleum industry. It has been found out by Ohadi et al [11] that Acinetobacter junii B6 strain can produce biosurfactants and degrade nearly all aliphatic hydrocarbons in crude oil. The Nocardia farcinica strain obtained from the crude oil sample in the Gujarat well was isolated by Patel and Lakshmi [12]; in addition, Nocardia farcinica strain was used to reduce the pour point and fluidity by measuring the gas chromatogram of paraffin. Moreover, solid enzyme preparations were used by Zhang et al [13] to isolate six Aspergillus strains for degrading paraffin under laboratory conditions. Also, it has been found out by Etoumi et al [14] that treatment of Pseudomonas and Actinomyces may be an effective method to biodegrade heavy paraffins and improve the properties of crude oil.

Microbial anti-wax technology can have many effects on crude oil, such as decomposing aliphatic hydrocarbons, decreasing pour point, and improving flow performance. This technology is an efficient, economical, and versatile alternative for improving fluidity [15]. This study aims to obtain the best compound conditions (that is, the optimal compound ratio, inoculum, and culture period) and apply them to waxy crude oil to determine the change in wax content, particle size, and viscosity. Finally, the wax crystal structure is observed microscopically, and experimental data are used for subsequent analysis.

EXPERIMENTAL PROCEDURES

Materials and Methods

Microorganism

Valid strains were isolated from oil-contaminated soils. These soils were collected from the surrounding areas in Liaohe oilfields (located in the downstream of the Liaohe River and the shore of the Bohai Bay).

Source of the Crude Oil

The crude oil in the experiment was coupled with the contaminated soils from the same area. The oil sample was waxy crude oil. In Table 1, the basic properties of the oil are shown.

Table 1: The basic oil properties.

Density /(kg/m^3)	Wax content /%	Wax temperature /^{o}C	Wax peak temperature /^{o}C
914	21.46	42.57	25.15

Methods

Determination of Performance of Each Bacterium and Cultivation of Compound Bacteria

Two bacteria from the laboratory were used to verify the abilities to degrade paraffin and produce biosurfactants. The two bacteria were mixed in different proportions and inoculum levels, and then placed in an air shaker and shaken at 37 °C. The optical density (OD) value of the cells was determined by a microplate reader at a wavelength of 600 nm [16,17] to determine whether the effect of the compound bacteria on the crude oil is higher than that of the single one and ascertain the optimal growth conditions or not.

Effect of Compound Bacteria on the Wax Content, Particle Size Distribution, and Viscosity of Crude Oil

The wax content in crude oil was measured using the Q2000 Differential Scanning Calorimeter (Q2000 DSC) to assess the changes after suspension was added. The microscopic morphology of wax crystal structure was observed with a polarizing microscope. FBRM was performed to obtain the particle size distribution of crude oil samples [18]. After the change in particle size distribution was obtained, the mechanism of wax removal and the action of compound bacteria were examined microscopically. The change in viscosity in crude oil after treatment with microorganisms was measured by a HAAKE rheometer.

Determination of Bacteria Performance Capability of Isolated Bacteria to Decompose Crude Oil

Two parts of 2-gram paraffin wax were poured into a 100 mL Erlenmeyer flask after being melted at high temperature. After cooling, #11 and #12 bacteria in a ratio of 1:1 were inoculated at a 1% inoculum level in Erlenmeyer flask, which contained 30 mL of enrichment medium. After samples were incubated at 37 °C with the appropriate shaking rate for 5 days, they were filtered, washed, dried, and weighed. The wax removal rate of bacteria was calculated using Formula (1). The results in Table 2 show that the wax removal rate of #11 bacteria (23.5%) is higher than that of #12 bacteria (8.5%).

$$\text{Wax removal rate} = \frac{2-M}{2} \times 100\% \quad (1)$$

where, M is the mass of remaining paraffin wax after degradation.

Table 2: Wax removal rate of strains.

Strain	Paraffin quality/g					Wax removal rate
	1st	2nd	3rd	4th	5th	
11#	2	1.89	1.83	1.72	1.53	23.5%
12#	2	1.93	1.91	1.87	1.83	8.5%
Control group	2	2	2	2	2	0

Biosurfactant Producing Test

The capability of the bacteria to produce biosurfactant is usually determined by the size of the emulsified ring or the plaque formed by the bacteria. The oil displacement activity can reflect the surface activity of the sample intuitively [19]. In this method, the oil phase is often used in olive oil, machine diesel, and liquid paraffin [20]. The color difference between these oil phases and the water phase is insignificant, and the effect diagram is not evident. Thus, the current experiment improved the method of oil displacement. Crude oil was used as the oil phase. #11 and #12 bacteria were separately inoculated into the enrichment medium

at a 1% inoculum level and cultured for 3 days. Moreover, the experimental temperature was set from 25 to 50 °C with a gradient of 5 °C, and the centrifuge was set at 8000 r/min for 30 min. A total of 20 mL of distilled water was placed in a Petri dish and then added to 10 µL of crude oil (preheated) with 10 µL of bacteria suspension containing biosurfactant. A clear area was formed in the oil layer, and the area started to expand by increasing the biosurfactant concentration. In addition, the oil displacement area was measured in accordance with the area of clear zone in the shape of a circle. In Table 3, the diameter of the oil displacement area is shown

Table 3: Diameter of Oil Displacement Area at Different Temperatures.

Temperature/°C	diameter /cm	
	#11	#12
25	0.5	2.7
30	0.5	3.3
35	0.7	4.5
40	1.0	5.8
45	0.8	4.3
50	0.5	3.6
55	0.5	2.2

Determination of the Optimal Conditions for Compound Bacteria

The growth of strain affects the treatment of crude oil. The addition of less compound bacterial fermentation broth can rapidly emulsify crude oil and reduce viscosity. However, the amount of the strain that produces the biosurfactant needs to be well controlled. If the proportion of viable bacteria is too low, then the viscosity-reducing effect will not be achieved. If the amount of viable bacteria is too high, then the effect will be inhibited. Therefore, the ideal proportion and amount of inoculation of compound bacteria should be determined. First, the best proportion of compound bacteria should be tested. #11 and #12 bacteria were compounded in the ratios of 1:1, 1:2, 1:3, 2:1, 2:3, 3:1, and 3:2 and inoculated in the enrichment medium at a 1% inoculum level. They were placed in the air shaker for 7 days at 37 °C. The absorbance of compound bacteria in different ratios was measured periodically to create a growth curve, as shown in Figure 1a, for determining the optimal ratio.

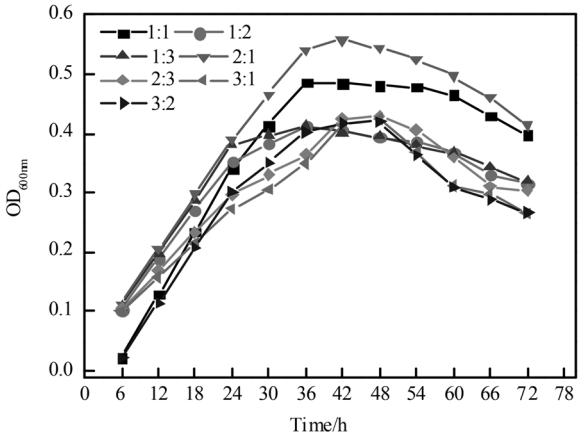

Figure 1a: Growth curves of #11 and #12 bacteria at different proportions.

The optical density (OD) is high, which indicates that the bacteria grow vigorously. When the compounding ratio is 2:1, the absorbance is higher, and the duration is longer than those in other ratios.

The optimal inoculum level was tested. After #11 and #12 bacteria were compounded in the ratio of 2:1, the suspension was inoculated in the enrichment medium at 1%, 2%, 3%, 4%, and 5% inoculum levels. The optical density was measured periodically to create a growth curve, as shown in Figure 1b. The figure shows that, when the suitable inoculum is 4%, the optical density is higher than those in the other levels.

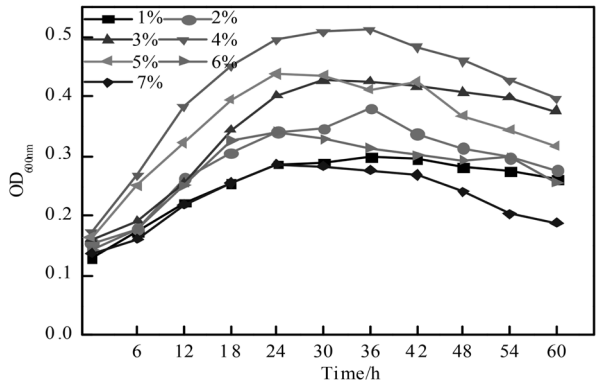

Figure 1b: Growth curves of #11 and #12 bacteria at different inoculum.

The optimal time for the cultivation of compound bacteria was also determined. After #11 and #12 bacteria were compounded in the ratio of 2:1, they were inoculated in the enrichment medium (with the pH value of 7) under the inoculation amount of 4% and shaken for 7 days. The OD values of #11 and #12 were measured. As shown in Figure 1c, the best growth condition is observed for the combination of #11 and #12 bacteria.

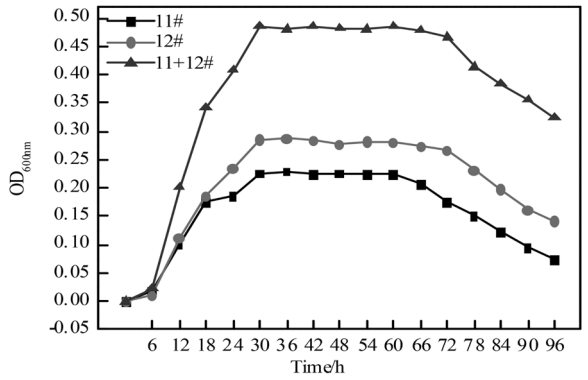

Figure 1c: Growth curves of #11, #12 bacteria and compound bacteria.

Determination of Wax Content

Wax content, wax precipitation point, and waxing peak were measured by using Q2000 DSC. The temperature control accuracy was ±0.1 ºC, and the heat flow accuracy was 0.1 µm. Before testing, 4 to 8 milligrams of the oil sample were weighed in aluminum crucible and then compacted. The resultant was placed in the crucible pool of the DSC, and heated at 80 ºC and then at −20 ºC at a rate of 5 ºC/min [21]. After the test was completed, the heat flow graph was processed using a software program.

After the thermal spectrum was obtained, it was processed as follows: the wax appearance temperature (WAT) points of the samples were determined, −20 ºC was considered the exothermic peak of baseline, and DSC curve interpolation was ascertained to determine the amount of heat released; the average temperature of wax was determined as 210J/gr [22]. The wax content is calculated as follows:

$$\text{Wax content} = \frac{\int_{T_c}^{-20} dQ}{\bar{Q}} \quad (2)$$

where, T_c is the temperature of the thermal line of the baseline, dQ is the amount of heat released by wax crystals in the temperature range, and $\bar{Q}$ is the average heat of crystallization of wax.

Experimental data were analyzed to determine WAT and content of wax in crude oil before and after treatment.

Observation of Wax in Crude Oil

The wax micro-structure in crude oil was observed with a polarizing microscope, and the reduction in wax content was examined microscopically. The selected magnification was 20×10, and the temperature (room temperature) was considerably lower than WAT. The microscope stage involved hot and cold stages to control temperature within a certain range. In addition, the temperature control accuracy was ±1 ºC.

Measurement of Particle Size in Crude Oil

FBRM was conducted to monitor the change in particle size distribution of crude oil. The experimental temperature was set to 58 ºC, which

was higher than the temperature of wax melting point (Table 1) and the temperature setting of the device, to eliminate the influence of temperature on the wax. The probe rotation speed was set to 2 m/s, and the stirring rate was 300 rpm. The online measurement time interval was set to 10 s to ensure minimum error of the original chord length distribution (CLD) data [23, 24].

Measurement of the Viscosity of Crude Oil

Crude oil treated with microorganisms generally exhibits change in viscosity. The viscosity of oil samples was measured with a HAAKE rheometer under different temperatures. The compound bacterial fermentation broth was mixed with 50 mL of crude oil in a conical flask under a volume fraction of 40%, and six such samples were prepared. The samples were placed in a water bath of constant temperatures of 30 ºC, 35 ºC, 40 ºC, 45 ºC, 50 ºC, 55 ºC. The rotational speed was maintained at 140 r/min.

RESULTS AND DISCUSSION

Changes in Wax Content

Figures 2a and 2b show the measurement results of wax content, WAT, and waxing peak of crude oil.

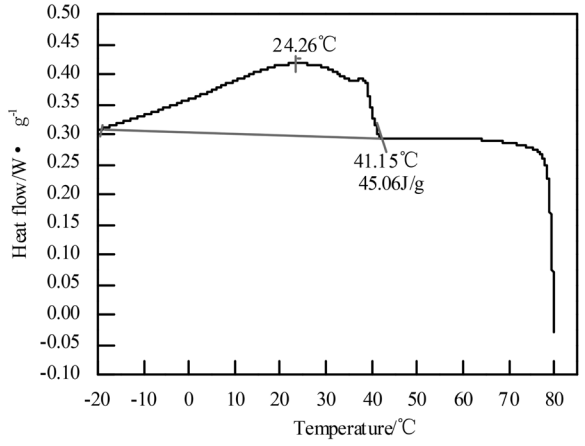

Figure 2a: Wax content of the oil sample before the treatment.

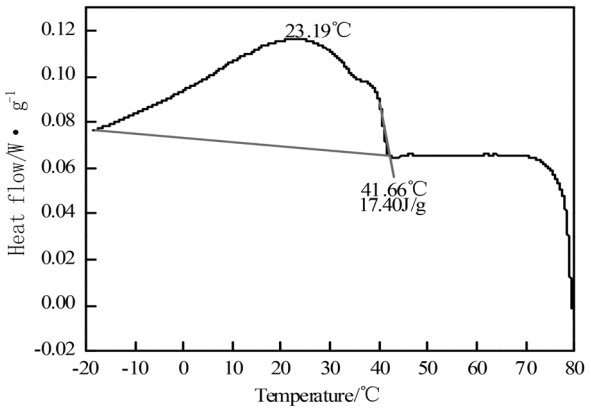

Figure 2b: Wax content of the oil sample after the treatment.

Comparison of the figures shows that the corresponding heat flow value and the crystallization heat of wax before and after the action of bacterial fermentation broth significantly decrease. The wax content of untreated crude oil is 21.46%, which decreases to 8.3% after 7 days. The rate of wax removal is 61.32%. WAT is also decreased. The main components of paraffinic alkanes are C_{16}–C_{28} saturated hydrocarbons [25]. Thus, the compound bacteria on C_{16}–C_{28} saturated hydrocarbons significantly degrade [26]. Therefore, the bacteria are suitable for wax removal in waxy crude oil.

Variation in Wax Micro-structure

The wax micro-structure, which was processed before and after the compound bacteria were added, were observed using a polarizing microscope. The results in Figures 3a and 3b show that the wax micro-structure significantly differs before and after treatment.

The wax structures untreated by bacteria are large and congregated, and the wax crystals are aggregated into a large 3D network structure [27].

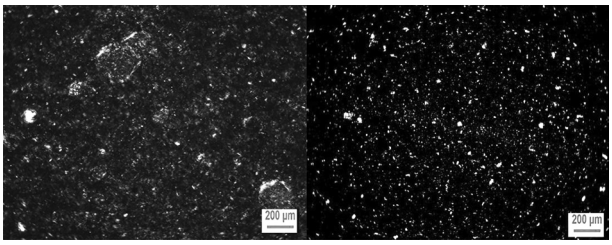

Figure 3: The microstructure of wax crystals: (a) Control group and (b) Test group.

In comparison with the flaky or needle-shaped wax crystals, the small-sized wax crystals are large in number and are relatively dispersed, and their configuration and shape hinder the formation of 3D network structure. In addition, the small-sized wax crystals maintain a certain distance among them, thereby delaying their aggregation [28]. After treatment, the wax crystals in the oil samples become significantly small and variable, and densely distributed.

Changes in Particle Size

FBRM was performed to monitor the particle size distribution of crude oil. The results in Figure 4 indicate that the size of the control oil sample is centered at 0-50 μm, and the size of this interval section accounts for the largest ratio.

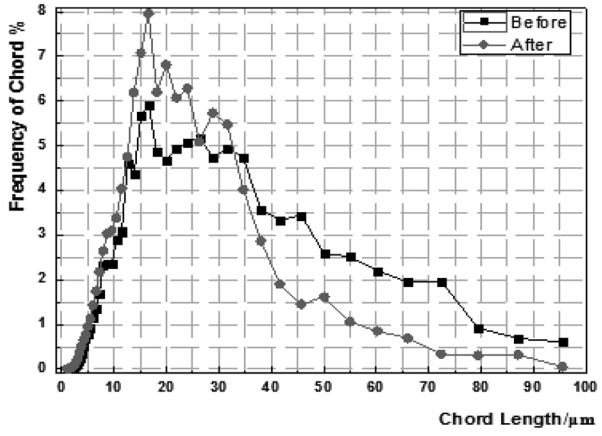

Figure 4: The chord length distribution (CLD) of the oil sample.

The ratio of particle size in the 40-60 μm section is higher than 2%. The particle size of oil sample in the interval of 0-30 μm increases significantly after treatment with compound bacteria. The highest ratio in this interval segment is 8%, which is considerably higher than that of the control oil sample. The proportion of 45 μm particle size is greatly decreased from 3.5% to 1.5%, which is the largest decrease. The particles of large sizes of 60-100 μm are completely degraded after treatment, and their proportion does not exceed 1%.

The changes in particle size are due to the following reasons: 1) the degradation of microorganisms degrades macro-molecular hydrocarbons in oil samples into small ones, thereby decreasing the particle size after treatment, and 2) the metabolism of microorganisms enable them to produce biosurfactant, which plays an important role in solubilizing and dispersing substances in petroleum hydrocarbons [21,29].(Yang et al. 2015) The biosurfactant can emulsify petroleum hydrocarbon substances into fine particles, which are conducive to the attachment and degradation of microorganisms.

Change in the Viscosity of Crude Oil

The viscosity of each crude oil sample was measured after 72 hours. The results in Figure 5 indicate that when the temperature is between 30 °C and 40 °C, the viscosity of crude oil treated with the compound bacteria significantly decreases with the increase in temperature. The growth characteristics of the compound bacteria are lower than 40 °C.

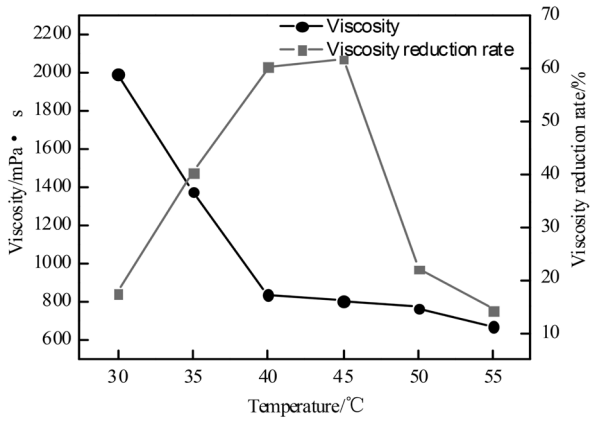

Figure 5: Effect of compound bacteria on viscosity of crude oil.

The growth and reproduction abilities increase with the increase in temperature, and the rate of viscosity reduction reaches 60.3% at 40 ℃. When the temperature is in the range of 40 to 45 ℃., the rate of viscosity decreases slowly with the increase in temperature. The viscosity of crude oil in the interval is less affected by temperature. After 72 hours incubation at 45 ℃., the viscosity of crude oil decreases to 810 mPa·s, and the rate of viscosity reduction reaches 61.8%. With the further increase in temperature, the viscosity of crude oil decreases significantly. Furthermore, excessively high inactivation of cells causes no significant change in viscosity before and after treatment with bacteria. When the temperature reaches 55 ℃., the viscosity changes slightly. In addition, the strain is inactivated, and the crude oil is lowered due to excessive temperature.

CONCLUSIONS

The results show that the content and micro-structure of wax are changed by #11 and #12 compound bacteria under certain conditions. The conclusions are listed as follows:

1) Two bacteria are screened from oil-contaminated soil and are compounded. The experiment shows that the optimal inoculum level is 4%, the best ratio is 2:1, and the optimal growth cycle is 7 days.

2) The compound bacteria considerably decrease the wax content in crude oil from 21.47% to 8.3%. The size of wax crystal is significantly changed after treatment as observed with a polarizing microscope. The bulk wax crystals disappear, and small ones increase in number. Apart from changing the wax content, the compound bacteria also break the bulk wax crystals by producing biosurfactants that can disperse wax crystals.

3) The analysis of the CLD image indicates that the proportion of particles of large sizes in waxy crude oil is decreased to less than 1%. By contrast, the proportion of small ones is significantly increased. The particle size ratio of 15 μm is increased to 8%. This result confirms the conclusion obtained using the polarizing microscope. Finally, specific performance of the compound bacteria can change the wax structure. The large particle size becomes small and spreads out, thereby changing the wax content.

Therefore, compound bacteria provide possibilities for the pipeline to prevent wax deposition and have great potentials in the transportation and mining of crude oil.

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NOMENCLATURES

OD: Optical Density

#: Number sign

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