ISSN: 2664-9640

Preparation and Performance Evaluation of Bio-Foam Oil Dispersant

Zhaokun Wang[1, a], Mingxuan Chen[1, b], Haodong Yin[1, c], Zhe Xv[2, d], Shixin Zhang[1,e]

[1] School of Shandong, Jiaotong University, Weihai 264210, China;

[2]School of Shandong, Yantai University, Yantai 264005, China.

[a]xiaokuntongxue2020@163.com, [b]1986866116@qq.com, [c]1290450002@qq.com, [d]845922975@qq.com, [e]2914806347@qq.com

Abstract

In order to improve the bioremediation efficiency of the oil spill, a bio-foam dispersing agent was prepared, which was mainly composed of dispersing agents and oil degrading microflora screened in the previous experiment. The optimum agent oil ratio was DOR, 25%, and the emulsification rate of the oil spilled under the ratio was 69.28%. The emulsification effect was good when the pH value was 8, the temperature was 30 degrees, and the salinity was 3%. By measuring the surface tension of the prepared dispersant and flora, the interaction between them was verified. By monitoring the growth of flora under different compounding ratios, it was determined that the optimal compounding ratio of dispersant and flora was 1:9, and the 7 d degradation rate of crude oil was 87.62%. The technology and index of the prepared bio-foam dispersing agent meet the requirements of the national standard GB18188.1-2000.

Keywords

Biosurfactant, bio-degradation, oil spill treatment, emulsification rate, surface tension.

1. Introduction

With the rapid development of social economy, the demand for oil in life and production activities is also increasing. At the same time, oil spills occur frequently in the process of oil exploitation, transportation, and utilization [1, 2]. As the main pollution problem of marine ecological environment, The oil spill has caused serious damage to the ecological balance of marine and coastal areas. Alkanes in petroleum components are a class of persistent organic pollutants with low biodegradability and stronger biological toxicity. The oil spill will pollute the ecological environment, destroy the balance of the ecosystem, directly endanger the organisms in the water area and its surrounding environment, and indirectly endanger human health and living environment [3, 4].

At present, there are three main methods to deal with oil spills: physical, chemical, and biological methods, but each has its advantages and disadvantages [5, 6]. Generally, it is first considered to use physical and mechanical devices to quickly remove the oil slick on the water surface. Although this method is economical and efficient, it has little effect on the oil film with a thickness of less than 5 mm. In the later stage of oil spill treatment, the thinner oil film on the water surface is usually dispersed by spraying dispersant. The use of dispersants to combat oil spills in marine environment is widely accepted all over the world and has been used as an effective tool to reduce the impact of oil spills [7]. However, most of these chemical dispersants have certain toxicity to marine organisms [8]. Therefore, many government organizations restrict the use of chemical dispersants. Generally, commercial dispersants are composed of volatile organic solvents and surfactants, which are incompatible with the marine environment. The potential persistence of dispersants (Corexit 9500A and Corexit 9527) used after the

ISSN: 2664-9640

explosion blowout of the Deepwater Horizon drilling platform in the Gulf of Mexico has also attracted attention, resulting in various environmental and toxicity problems [9, 10]. Corexit 9580, a dispersant used in the "Exxon Valdez" oil spill, contains toxic surfactants and carcinogenic ingredients ($C_6H_{14}O_2$), so it has long been discontinued [11, 12]. Therefore, strict government legislation and higher environmental awareness urge researchers to develop environmentally friendly oil spill dispersants with high efficiency, non-toxicity, and high biodegradability. It is the key to seek surfactants and solvents with high emulsifying activity and low biological toxicity. It is also the main research direction and focus of researchers at home and abroad in the application field of oil spill dispersants.

At present, the most promising environment-friendly dispersant is biosurfactant [13]. Biosurfactant molecules contain both lipophilic and hydrophilic groups, which have a good affinity for oil and water; When acting on the water -oil interface, biosurfactants can change the interaction of the water -oil interface, greatly reduce the water-oil interface tension, emulsify the spilled oil, and disperse the spilled oil into one with a diameter less than 70 μm small oil droplets [14]. Secondly, biosurfactants have low toxicity and high biodegradability. In addition, they have the ability to improve the solubility and fluidity of petroleum hydrocarbons, which makes them have a strong attraction to the bioremediation of crude oil. Hajibagheri et al. [15] found that the binary mixture of chemical surfactant (SDBS) and surfactant produced by Enterobacter cloacae showed better interfacial activity and foaming characteristics than the single component. Mansoor UI Hassan Shah et al. [16] combined the surfactant lauric acid with the biosurfactant Sophora sugar lipid to prepare a green dispersant for oil spill repair, with a maximum dispersion rate of 83% for crude oil.

Although dispersant can emulsify and disperse the spilled oil well, it can not degrade and eliminate the oil pollution; Although biological treatment of spilled oil can not cause secondary pollution to the environment, it takes a long time and can not treat the spilled oil in time. Therefore, a green dispersant was prepared in this experiment and combined with biological method to repair the spilled oil pollution. Through the blending of biosurfactant rhamnolipids and other surfactants, *Pseudomonas sp*. was prepared to prepare environment-friendly dispersants and explore and combine with oil degrading microflora. Because of its good foaming properties, it is vividly called "bio-foam oil dispersant".

2. Materials and methods

2.1. Materials and reagents

The strain comes from swamp oily soil and oily bilge water near the Bohai Sea. It is identified as *Alcaligenes*, *Pseudomonas,* and *Proteus* by physiological and biochemical characteristics and molecular biological methods. It is preserved by Shandong Key Laboratory of ship safety and pollution prevention.

Nutrient broth(NB): Deionized water 1000 mL, Peptone 10 g, Beef dip powder 3g, NaCl 5 g;

Inorganic salt medium: Deionized water 1000 mL, NH_4Cl 1 g, NaCl 5 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, $NaNO_3$ 2 g, KH_2PO_4 4 g, $K_2HPO_4 \cdot 3H_2O$ 10 g;

Crude oil medium: Add 1 g of crude oil to every 100 ml of inorganic salt medium.

Adjust the pH of the above medium to neutral, and sterilize in a high-pressure steam sterilizer at 121℃ for 15 minutes for standby.

Main equipment and instruments: UV-1800 ultraviolet-visible spectrophotometer, BBS-H1500 double person single side super clean worktable, Vortex-2 vortex mixer, ZWY-2012C double-layer constant temperature culture oscillator, BKQ-B120 II automatic sterilizer, 101-A electric blast drying oven, SHP-150 constant temperature incubator, H1805 medical centrifuge, BZY-2

ISSN: 2664-9640

surface tension instrument, SYD-265B viscosity tester SYD-261D open flash point automatic tester

2.2. Experimental method

2.1.1 Preparation of green environmental friendly dispersant

(1) Weigh natural soap powder 25 g, rhamnolipid 20 g, Alcohol ethoxylate 20 g, polyethylene glycol 8 g, ethanol 25 g, purified water 140 g, ethylene glycol 5 g, coconut diethanolamide 5 g.

(2) Mix the weighed natural soap powder, rhamnolipid, alcohol ethoxylate and 1/2 polyethylene glycol, 1/2 ethanol, 1/2 purified water, 1/2 ethylene glycol and 1/2 coconut diethanolamide, stir and heat to 40 ~ 60℃, stir for 20-30 min, and cool to room temperature.

(3) Mix the mixture obtained in step (2) with the remaining 1/2 polyethylene glycol, 1/2 ethanol, 1/2 purified water, and 1/2 ethylene glycol. The purpose of mixing solvents, additives, and surfactants step by step is to make the mixing more uniform.

2.2.2 Characterization of dispersant

(1) Determination of emulsification rate

Add crude oil and dispersant into 50ml artificial seawater according to different agent oil ratio, oscillate and emulsify at a certain frequency for 3min, stand for 30s and 10min respectively, take out the lower emulsion and extract it with petroleum ether, dilute the extract to an appropriate concentration, place it on the UV spectrophotometer and measure its absorbance value at 255 nm, calculate the emulsification rate after different standing time, and conduct three parallel experiments in each group, and take the average value. The emulsification rate after standing for 30 s represents the emulsifying capacity of the dispersant; The emulsification rate after standing for 10 min represents the emulsification stability of the dispersant.

$$T = \frac{51.2Cn}{m \times 10^6} \times 100\% \quad (1)$$

T: emulsification rate, %;

C: oil concentration calculated from the absorbance value of the extract, mg/L;

n: dilution ratio of extract;

51.2: artificial seawater, The value of the sum of oil and dispersant volume (mL);

m: addition of crude oil, g.

(2) Measurement of surface tension of dispersant

a. Surface tension of emulsion under different DOR: Add 50 mL artificial seawater and 1 g crude oil into five cylindrical funnels respectively. Add dispersant according to different DOR. Replace dispersant with equal amount of deionized water in blank samples. After uniform oscillation, stand still. Take the lower emulsion and place it on the surface tensiometer to measure its surface tension.

b. Surface tension of dispersant at different concentrations: 0, 50, 100, 150, 250, 500, 1000, 1500, and 2000 mg/L dispersant solutions were prepared with dispersant and distilled water respectively, and their surface tension was measured.

c. Surface tension of microflora: The dominant oil degrading microflora were inoculated into NB medium, and the surface tension of the medium was measured every 12 h.

d. Surface tension after compounding dispersant and microflora: Add an appropriate amount of dispersant to the NB medium inoculated with oil degrading microflora, and measure the surface tension of the medium every 12 h.

(3) Ignition point test

Put the dispersant sample into the crucible to the specified score line. Firstly, rapidly raise the temperature of the sample, and then slowly raise the temperature. When it is close to the flash point, raise the temperature at a constant speed. At the specified temperature interval, use a

ISSN: 2664-9640

small igniter flame to pass through the sample surface according to the regulations, and use the lowest temperature of the igniter flame to flash the steam on the sample surface as the flash point of the open cup method. Continue the test until the minimum temperature when the sample is ignited with the igniter flame and burned for at least 5 s is used as the ignition point of the open cup method.

(4) Viscosity test

Put the dispersant sample into a clean and dry pincer capillary viscometer with a pore diameter of 1.0 mm, suck the sample to the mark b with a rubber ball, pay attention not to generate bubbles in the process, adjust the viscometer to a vertical state, adjust the constant temperature bath to the specified temperature, and then immerse the viscometer with the sample into the constant temperature bath to observe the flow of the sample in the tube, When the liquid level just reaches the mark a, start the stopwatch. When it reaches the mark b, stop the stopwatch. Take the arithmetic average value obtained from the flow time of no less than three times as the average flow time of the sample. The kinematic viscosity formula is as follows:

$$\nu_t = c \times \tau_t \tag{2}$$

ν_t: Kinematic viscosity of sample, mm^2/s;

c: Pincer viscometer constant, 0.074920 mm^2/s^2;

τ_t: Average flow time of sample, s.

(5) Acute toxicity test of dispersant

The toxicity of oil spill dispersants to aquatic organisms is usually evaluated by the test results of acute toxicity to fish. Put the test fish in different concentrations of oil spill dispersant for 24 h or 96 h, and obtain the concentration of the test solution for half death of the test fish, that is, the half lethal concentration for 24 h or 96 h. In this experiment, the half lethal time was measured at the concentration of 3000 mg/L specified in standard GB18188.1-2000 [17].

Prepare a certain concentration of dispersant test solution, add about 15 L of test water into the test cylinder, add the calculated amount of dispersant to make it reach the test concentration, and stir evenly. Pick 10 × 3 test fish and place them in three 1000 ml beakers, with 10 fish in each beaker. After the test preparation is completed, transfer the test fish to two beakers and a blank beaker. If you find a fish that doesn't move or breathe, gently touch its tail with a glass rod. If the fish doesn't respond, it will be judged as dead and fished out in time. If the mortality of test fish in the blank test exceeds 10%, the experimental results are not available.

(6) Biodegradability

Biodegradation refers to the process that organic matter is metabolized by organisms and finally completely transformed into inorganic matter under aerobic conditions. The whole process of biodegradation is very slow, so specific conditions and methods are usually used to evaluate the biodegradability of organic matter. This experiment adopted the method of comparing the correlation between five-day biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD).

Prepare a certain concentration of oil spill dispersant aqueous solution (300 mg/L), measure its BOD_5 and COD respectively, and calculate the evaluation index of biodegradability of oil spill dispersant. The calculation formula is as follows:

$$D = \frac{BOD_5}{COD} \times 100\% \tag{3}$$

D: Biodegradability evaluation index of oil spill dispersant, %;

BOD_5: Five-day biochemical oxygen demand, mg/L;

COD: Chemical oxygen demand, mg/L.

ISSN: 2664-9640

2.2.3 Influence of various factors on emulsification rate of dispersant

The emulsification rate of dispersant under different DOR, salinity, temperature and pH was investigated according to the agent oil ratio of 25%.

2.2.4 Combination of green dispersant and oil degrading microflora

The dispersant prepared in the experiment was compounded with petroleum degrading bacteria to prepare a biological oil remover. Theoretically, the higher the content of dispersant, the higher the emulsification rate of microbial oil spill dispersant. However, the dispersant contains organic surfactants and solvents. Although its toxicity is low, its effect on the activity of oil degrading microflora is uncertain. Therefore, different proportions of dispersant are set to mix with oil degrading microflora, and the compound ratio of the two is determined by taking the growth of microflora in the dispersant after mixing and the emulsification rate of dispersant as reference indexes.

Inoculate the compound microflora into the liquid medium, culture in a constant temperature oscillation incubator at 30℃ and 160 r/min for 12 ~ 24 h, prepare 100 ml of mixed culture medium respectively according to the volume ratio of dispersant to culture medium: 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 and 10:0, and transfer it to the honeycomb plate, Put it into the *Bioscreen C* automatic growth curve analyzer to monitor the growth of microflora under different compounding proportions. Set the detection wavelength of the automatic growth curve analyzer to 600 nm, the culture temperature to 30 ℃, the time interval of each measurement is 30 min, vibrate for 15 s before measurement, and the culture time is 168 h.

2.2.5 Study on emulsion degradation of dispersant and oil degrading microflora

Although dispersant can effectively disperse and emulsify crude oil, it has no degradation effect on it; The aging of crude oil degradation by microorganisms alone is low. Therefore, this test can achieve better degradation effect of crude oil by mixing the proportion of microflora and dispersant.

(1) Drawing of standard curve: extract crude oil with petroleum ether and dilute it to appropriate concentration, and conduct full wavelength scanning under ultraviolet spectrophotometer to determine the optimal absorption wavelength. Prepare a series of crude oil standard sample solutions with mass concentrations of 0 $mg \cdot L^{-1}$, 10 $mg \cdot L^{-1}$, 20 $mg \cdot L^{-1}$, 30 $mg \cdot L^{-1}$, 40 $mg \cdot L^{-1}$, 50 $mg \cdot L^{-1}$, 60 $mg \cdot L^{-1}$, 70 $mg \cdot L^{-1}$ and 80 $mg \cdot L^{-1}$, read the absorbance value at the optimal wavelength and draw the standard curve.

(2) In the crude oil culture medium, the dispersant is added according to a certain agent oil ratio, and the crude oil culture medium without dispersant is used as the control experiment. After sterilization, the oil degrading microflora are added according to 5% of the inoculation amount. After 7 d of culture in the shaking table, all the culture solution in the conical flask is poured into the separating funnel, the culture flask is cleaned with 50 mL petroleum ether, and the undegraded residual oil is extracted by full oscillation, After standing, the supernatant was collected and centrifuged in a centrifuge. Use a pipette to transfer an appropriate amount of extract into a constant volume bottle, fix the volume with petroleum ether, use an ultraviolet spectrophotometer to measure the absorbance at the wavelength of 255 nm, obtain the residual oil concentration in the culture medium through the crude oil standard curve, and calculate the crude oil degradation rate.

$$\eta = \frac{C_0 - C}{C_0} \times 100\% \tag{4}$$

η: Degradation rate;

C_0: Initial oil concentration;

C: Oil concentration after treatment.

ISSN: 2664-9640

3. Results and analysis

3.1. Suitable application conditions of green environmental protection dispersant

Dispersion rate refers to the ability of oil spill dispersant to emulsify and disperse oil into water, which is the fundamental index of dispersant. The factors affecting the emulsion dispersion rate and emulsion stability of oil spill dispersant are not only the formula composition of emulsifier itself, application method, sea energy and dosage, but also many other factors. In this paper, the effects of DOR, temperature, salinity and pH on the emulsion dispersion rate of the prepared green type preserving dispersant were investigated. The results are shown in Fig. 1 ~ 5.

(1) Comparison of emulsification rate under different DOR

Nowadays, in the treatment of oil spills, dispersant abuse is most common, and its excessive use will bring potential harm to the environment. Therefore, the emulsification rates at DOR of 15%, 20%, 25%, 30%, 35% and 40% were measured at room temperature. The results are shown in Fig. 1.

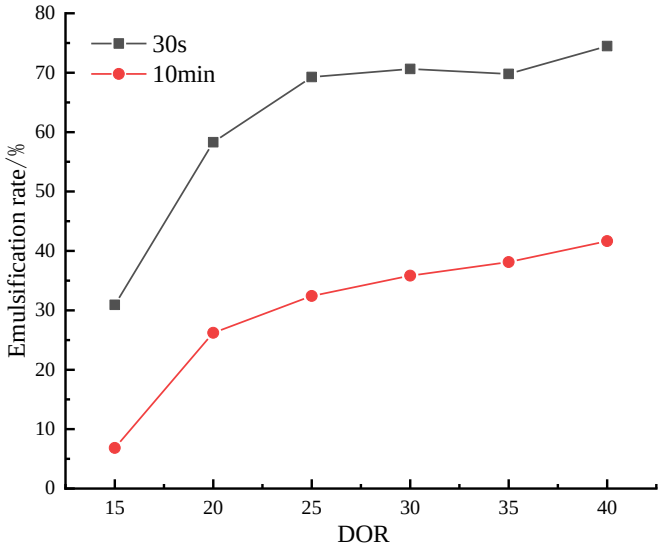

Fig. 1 emulsification rate of dispersant under different DOR

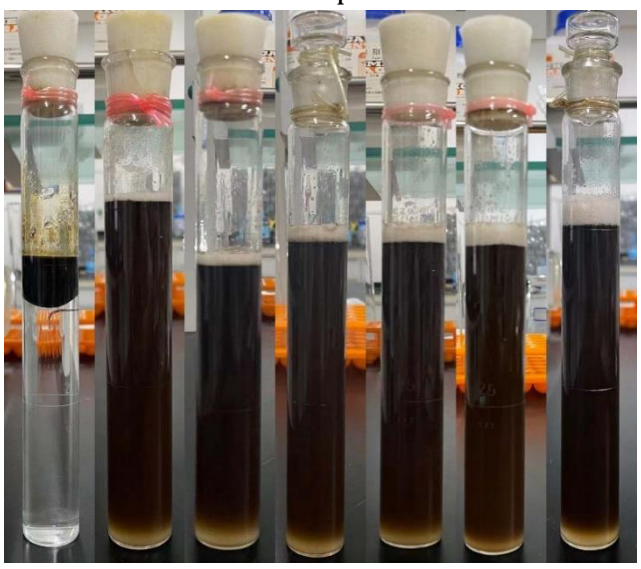

Fig. 2 Emulsification of crude oil under different agent oil ratio

It can be seen from Fig. 1 that when DOR is 15%, the maximum emulsification rate in 30 s can reach 30.92%, but the emulsification rate in 10 min is 6.84%. When DOR reaches 40%, the maximum emulsification rate reaches 74.48% in 30 s and 41.66% in 10 min. Therefore, the

ISSN: 2664-9640

emulsifying capacity and emulsifying stability are improved with the increase of DOR. It is easy to see from the figure that in the initial stage of DOR increase, the emulsification rate of crude oil increases rapidly, but when it reaches 25%, the effect of DOR increase on the emulsification rate is no longer so obvious. It can also be seen from Fig. 2 that with the increase of DOR, the color of crude oil emulsification becomes darker.

According to the research, the national standard for emulsification of oil remover is that the emulsification rate reaches more than 60% in 30 s, the emulsification rate reaches more than 20% in 10 min, and the use amount should be 20% ~ 30% of the oil spill. The more dispersant is added to the spilled oil surface, the easier the spilled oil is to form smaller particles and disperse in the water body. Of course, the increase of dispersant dosage will improve the emulsification rate, especially for heavy crude oil and weathered crude oil, but this is not unlimited. The treatment effect cannot be achieved if the dosage is insufficient, and the effect is not obvious if the dosage is too much. The use of too much oil remover will cause unnecessary waste and add an additional burden to the marine environment. Therefore, the amount of oil remover is not the more the better. Therefore, comprehensively considering that the best DOR of the green environmental protection dispersant prepared in this test is 25%.

(2) Comparison of emulsification rate at different temperatures

Temperature is an important factor affecting the emulsification effect of oil spill dispersants. Its influence is mainly reflected in the increase of oil viscosity and oil-water surface tension with the decrease of temperature, resulting in the decrease of dispersion rate. If the temperature is close to or lower than the freezing point of oil, the oil spill dispersant will almost lose its function. Under the condition that the pH, salinity and other conditions of the test water body remain unchanged, when the DOR is 25%, the emulsification and dispersion effect of the dispersant on crude oil at the water temperature in the range of 15 ~ 40℃ is investigated. The results are shown in Fig. 3.

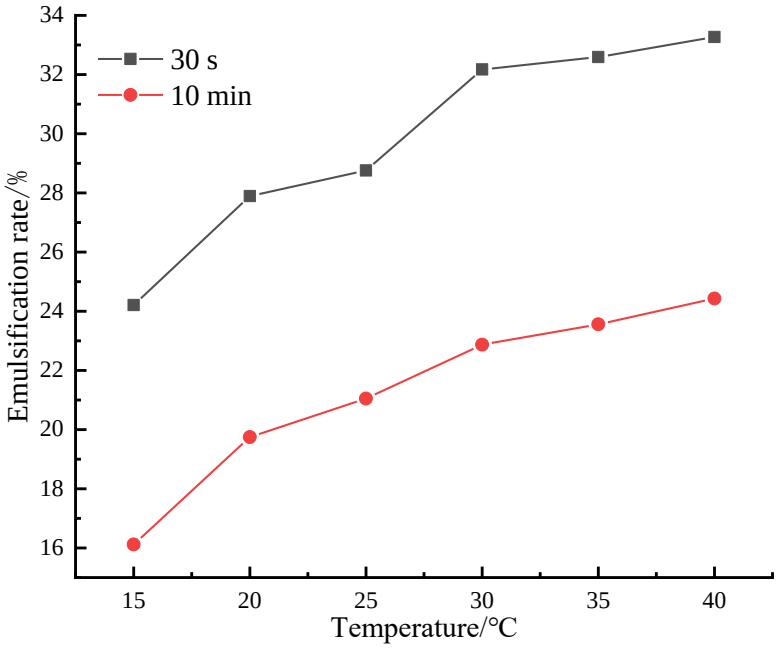

Fig. 3 Emulsification rate of dispersant at different temperatures

It can be seen from Fig. 3 that the emulsification rate of dispersant to crude oil increases with the increase of temperature. It can be seen from the figure that taking 30℃ as the dividing point, the emulsification rate increases greatly when it rises from 15 ℃ to 30 ℃. When the temperature rises from 30℃ to 40℃, although the emulsification rate is still increasing, the increase is slightly lower than before.

ISSN: 2664-9640

Because the temperature of the water body has a direct impact on the viscosity of crude oil, it changes the oil-water interfacial tension, that is, the lower the temperature of seawater, the greater the viscosity and oil-water interfacial tension of crude oil, and vice versa. Therefore, when the temperature is relatively low, the amount of dispersant can be appropriately increased; When the temperature is relatively high, blindly increasing the dosage of dispersant can not achieve the expected purpose, but also cause unnecessary economic losses.

(3) Comparison of emulsification rate under different salinity

Salinity is a sensitive factor affecting the emulsifying activity of oil spill dispersants. It mainly affects the hydrophilic-lipophilic equilibrium value (HLB) of surfactant and changes its surface activity. When the agent oil ratio is 25% and the salinity is in the range of 0 ~ 10%, the influence of dispersant on the emulsification and dispersion effect of crude oil under different salinity of the water is investigated. The results are shown in Fig. 4.

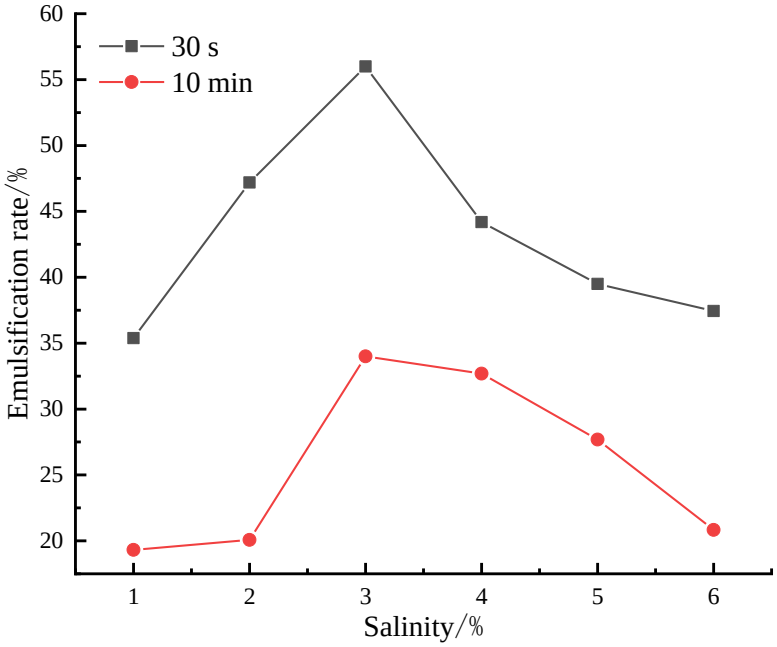

Fig. 4 Emulsification rate of dispersant under different salinity

It can be seen from Fig. 4 that the emulsification rate first increases and then decreases with the increase of salinity. When the salinity is 1%, the emulsification rate of spilled oil is very small, including 35.38% in 30 s and 19.32% in 10 min; With the increase of salinity, the 30 s / 10 min emulsification rate increased and reached the maximum when the salinity reached 3%, of which the 30 s emulsification rate reached 56% and the 10 min emulsification rate reached 34%; When the salinity continues to increase, the emulsification rate of the two crude oils decreases.

(4) Comparison of emulsification rates at different pH

The pH of the water will affect the micelle structure and shape formed by surfactants, and then affect their emulsification and solubilization ability. When the agent oil ratio is 25% and the pH is in the range of 4 ~ 9, the influence of dispersant on the emulsification and dispersion effect of crude oil at different pH of the water body is investigated. The results are shown in Fig. 5.

ISSN: 2664-9640

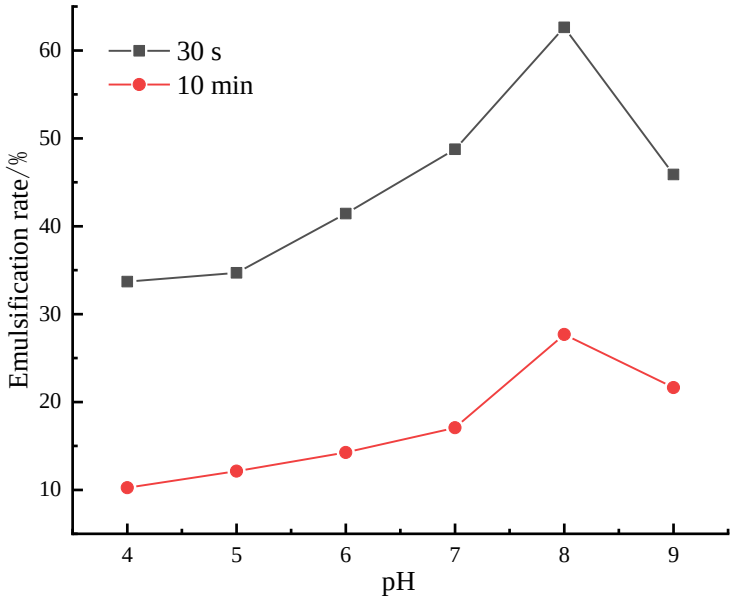

Fig. 5 Emulsification rate of dispersant at different pH

It can be seen from Fig. 5 that the emulsification rate of dispersant first increases and then decreases with the increase of pH. The pH increased from 4 to 8, in which the emulsification rate increased from 33.69% to 62.63% in 30 s and from 10.25% to 27.69% in 10 min. It can be easily seen from the figure that the emulsification rate in 10 min is less affected by the change of pH value than that in 30 s. The emulsification rate fluctuates greatly under acidic conditions. With the increase of pH value, the emulsification rate increases rapidly and reaches the maximum value when pH is 8. With the continuous increase of pH, the emulsification effect decreases slightly.

The pH value can affect the emulsification effect in two ways: first, it affects the acid-base characteristics of the dispersant itself; second, it changes the existing state of ions in the water, thus promoting the change of the chemical properties and charge characteristics of the dispersant and affecting the emulsification effect. Some surfactants have good acid resistance and are not easy to hydrolyze in acidic solution, but they lose their emulsifying ability due to hydrolysis under alkaline conditions, thus affecting the emulsifying rate of crude oil. Coconut oleic acid diethanolamide, a surfactant, has good alkali resistance and is easy to hydrolyze and lose its effectiveness under acidic conditions. The dispersant prepared in this experiment is weakly alkaline, relatively sensitive to acid, and the emulsification rate decreases slightly under alkaline conditions, while the emulsification effect is better under neutral and weakly alkaline conditions.

3.2. Changes of surface tension of dispersant and microflora under different conditions

The surfactant in the dispersant has amphiphilic properties, which can be adsorbed on the oil-water interface and reduce its distortion. Such substances will be produced during the growth and proliferation of the selected oil degrading microflora. Usually, the lower the surface tension, the easier the whole system is to be dispersed and emulsified. At the same time, in order to investigate whether the dispersant and flora cooperate with each other, the changes of surface tension of dispersant and microflora under different conditions are investigated. The test results are shown in Fig. 3 ~ 6.

ISSN: 2664-9640

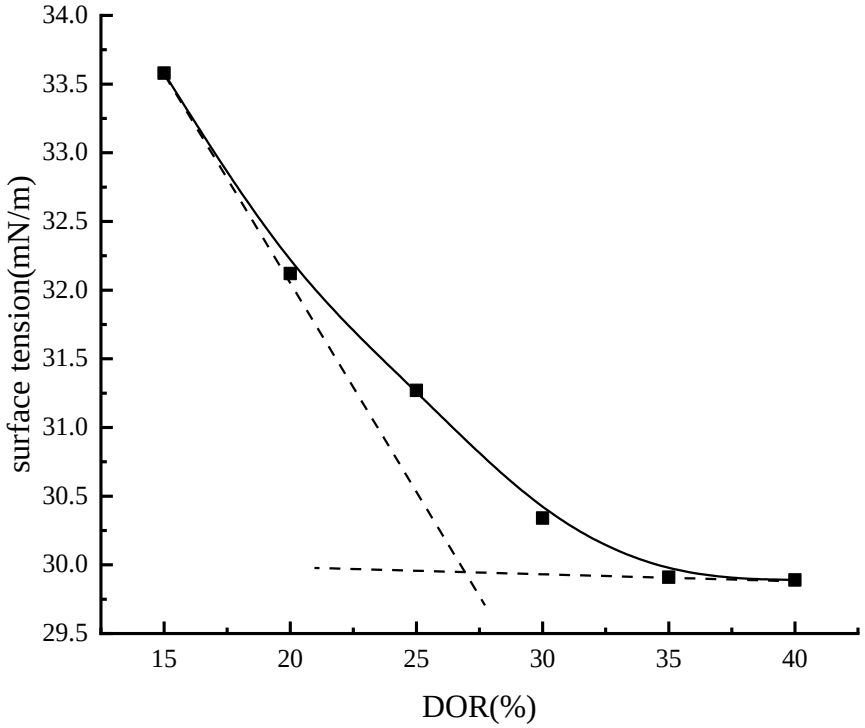

Fig. 6 Surface tension of emulsion under different agent oil ratio

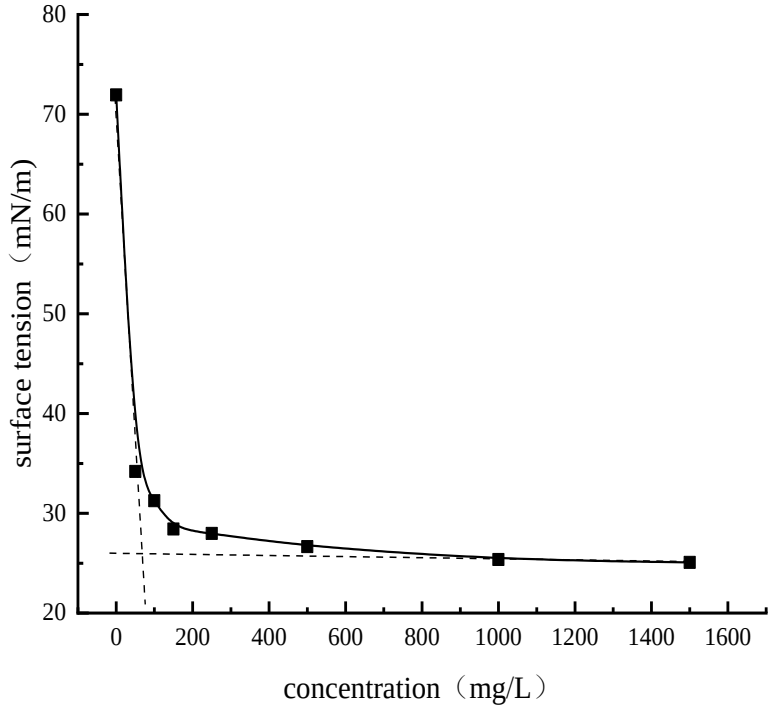

Fig. 7 Surface tension of dispersants with different concentrations

As can be seen in Fig. 6, with the increase of DOR, the surface tension value of the emulsion gradually decreases. As can be seen from Fig. 7, the surface tension of the dispersant solution can be reduced from 71.94 mN•m^{-1} to 28.42 mN•m^{-1}. Since the surfactant in the dispersant component is relatively stable, the surface tension is stable and there will be no large fluctuation up and down after the surface tension drops to the minimum value. It can be seen from the figure that the critical micelle concentration (CMC) of dispersant is less than 100 mg / L. Therefore, the concentration of dispersant specified in the national standard is much greater than the CMC of the dispersant, and the surface tension has reached the equilibrium value.

ISSN: 2664-9640

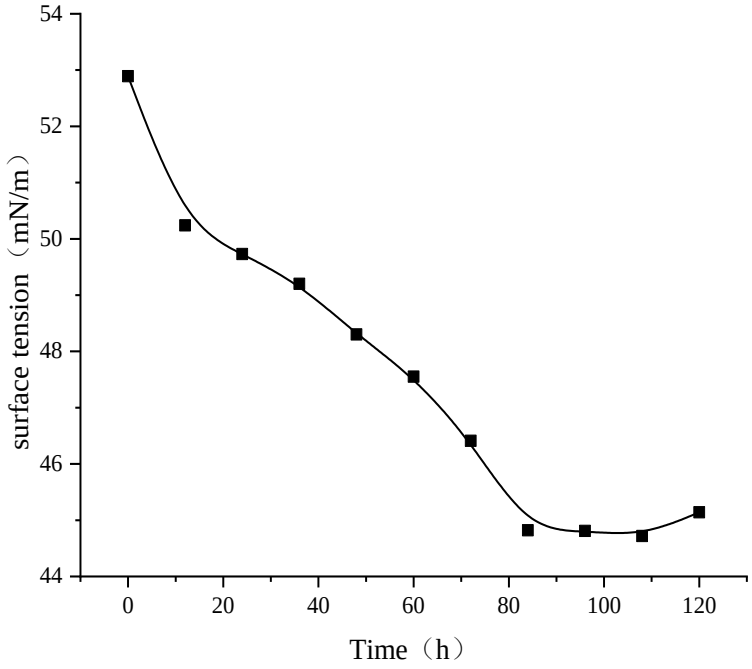

Fig. 8 Surface tension of flora at different time periods

It can be seen from Fig. 8 that after 84 h fermentation of the medium, the surface tension of the fermentation broth decreased significantly from 52.89 mN•m^{-1} to 44.82 mN•m^{-1}, and then decreased slowly. Until 96 h, the surface tension of the fermentation broth decreased to the lowest value of 44.72 mN•m^{-1}. However, with the continuous growth of fermentation time, the surface tension tended to increase. This may be because with the increase of fermentation time, after the growth of microflora enters the stable period, biosurfactants and other metabolites begin to be produced slowly, which reduces the surface tension of fermentation broth. When the fermentation reaches 108 h, the surface tension of the fermentation broth tends to increase. Therefore, it is not that the longer the fermentation time, the higher the yield of biosurfactant. As the growth of the strain enters the decline period, the metabolites of the strain will be toxic to the growth of the strain, affect the continuous growth of the strain, and reduce the yield of biosurfactant.

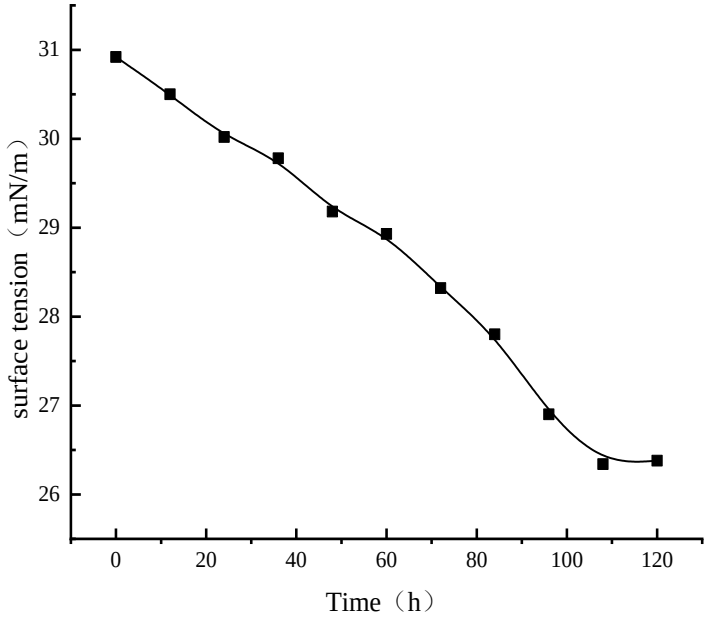

Fig. 9 Surface tension of microflora in different time periods after compounding dispersant

ISSN: 2664-9640

It can be seen from Fig. 9 that at the initial stage of microflora growth, due to the addition of oil eliminator, the surface tension of the culture medium began to decrease to 30.92 $mN•m^{-1}$. With the growth of the strain, biosurfactant was slowly produced, and the surface tension continued to decrease. Until 108 h, the surface tension reached the lowest value of 26.34 $mN•m^{-1}$. With the continuous growth of time, the surface tension increased slightly.

This is mainly because the biosurfactant produced by the microflora and the surfactant in the oil remover work together to reduce the surface tension of the culture medium. Due to the stable performance of the surfactant in the dispersant, the surface tension of the culture medium is significantly reduced at the beginning. The initial surface tension of the culture medium without dispersant is about 52.89 $mN•m^{-1}$. The dispersant can continue to reduce the surface tension of the culture medium to 30.92 $mN•m^{-1}$. With the increase of fermentation time, the microflora begins to produce biosurfactants, so that the surface tension of the culture medium continues to decrease to the lowest value of 26.34 $mN•m^{-1}$, After 108 h, the surface tension of the medium increased, which was the same as that in the microflora medium, indicating that the production of biosurfactants did not have a higher yield with the increase of culture time.

It can be seen from Fig. 7 ~ 9 that the surface tension of dispersant solution can be reduced to 28.42 $mN•m^{-1}$, the surface tension of flora culture medium can be reduced to 44.72 $mN•m^{-1}$, and the surface tension after the combination of dispersant and microflora can be reduced to 26.34 $mN•m^{-1}$. Therefore, the surfactant in dispersant and biosurfactant produced by microflora can jointly reduce the surface tension of the culture medium, and the compound effect is better than dispersant or microflora.

3.3. Dispersant technology and performance index

According to the standard GB18188.1-2000, the performance indexes of dispersantare determined, and the results are shown in Table 1.

Table 1 Specific performance indexes of dispersant

Indicator name		Sample	National standard
Appearance		Yellowish brown, Transparent liquid	Clear, Transparent, No stratification
Viscosity, $mm^2•s^{-2}$		28.39	<50
Ignition point, ℃		>70	>70
Emulsification rate, %	30 s	69.28	>60
	10 min	35.83	>20
Acute toxicity of fish, h		124	>24
Bio-degradability, %		46.54	>30

According to the concentration of 3000 mg / L specified in the national standard, the half lethal time at the concentration of the dispersant is measured. After 24 h, the test fish activity is normal, and half of the test fish die after 124 h, which is far more than the standard of 24 h or 96 h specified in the national standard. At the same time, BOD_5 and COD of the dispersant were measured at the concentration of 300 mg / L specified in the national standard, and the ratio of the two is far greater than 30%, which meets the requirements of the national standard.

3.4. Effect of dispersant on oil degrading microflora

In order to investigate the relatively suitable compound ratio of dispersant and oil degrading microflora, with the help of *Bioscreen C* automatic growth curve analyzer to monitor the growth of flora under different compound ratios. The growth of microflora is shown in Fig. 10.

ISSN: 2664-9640

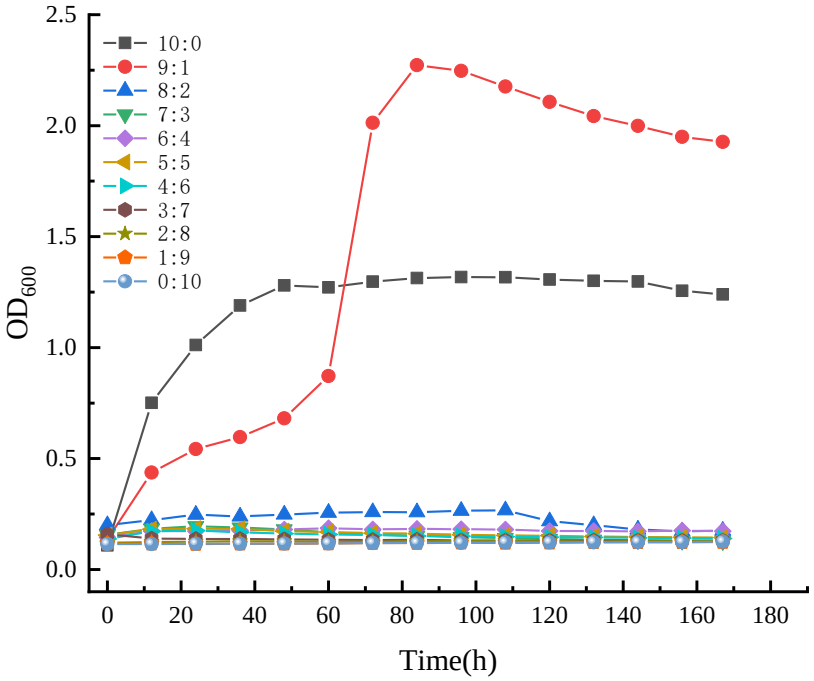

Fig. 10 Microbial growth curve under different microflora / dispersant compound ratio

It can be seen from Fig. 10 that the oil degrading microflora grow rapidly within 10 h before growth, enter the logarithmic period, and the constant geometric progression of the number of bacteria increases greatly. During this period, the bacteria have good adaptability and the strongest activity. Therefore, the best inoculation time is between 12 h ~ 24 h, and the number of bacteria reaches the maximum when it is about 40 h. Then the growth enters the stable period, in which the growth and decline of the microflora reach a balance, and a large number of microbial metabolites begin to accumulate in the cell. The metabolites have certain toxic and side effects on the strain, which will affect the continuous growth of the microflora. After the continuous accumulation of microbial metabolites, the growth of the microflora enters the decline period. Biosurfactants are one of the main products of microbial metabolism. Due to the production of biosurfactants, microorganisms can degrade crude oil.

When the volume ratio of bacterial solution to dispersant is 9:1, the retardation period of the flora will increase, and its growth rate will be lower than that of the simple flora. It will officially enter the logarithmic period after about 60 h, and enter the stable period after about 80 h, and the time of the stable period will be short, so that the decline period will come in advance. It shows that dispersant still has a certain inhibitory effect on the growth of flora. However, the number of bacteria in this proportion is greater than the total number of pure bacteria, which shows that the bacteria can use the effective carbon source components in the oil remover for growth and reproduction.

It can be seen from Fig. 7 that the microflora can effectively use the components in the oil remover as the carbon source in the culture medium for growth and reproduction, and its growth process will go through adjustment period, logarithmic growth period and stable period. However, compared with the enrichment medium without dispersant, the growth retardation period of oil degrading miaroflora with dispersant is longer, and with the increase of the proportion of dispersant, The retardation period increased and the growth rate decreased. With the increase of the proportion of dispersant, the total number of bacteria decreases. When the volume ratio of bacterial solution to dispersant exceeds 9:1, the absorbance value of microflora is similar, indicating that the inhibitory effect of dispersant on the growth of microflora is basically unchanged within this proportion range. Therefore, when dispersant is used as carbon source for strain growth, the growth of microflora is inhibited in varying degrees.

ISSN: 2664-9640

3.5. Comparison of petroleum hydrocarbon degradation rates under different conditions

In the previous experiments, the oil degrading microflora was immobilized. In order to verify that the biotype foam dispersing agent had a higher degrading effect on crude oil, the effects of free microflora, immobilized microflora, biological foam dispersant (free bacterial group dispersing agent) and immobilized microflora complex dispersing agent on the crude oil degradation were tested respectively. The test results are shown in Fig. 11.

The petroleum ether sample dissolved in petroleum hydrocarbon is scanned in the full wavelength range, and there is an obvious absorption peak at the wavelength of 255 nm. 255 nm is selected as the test wavelength. Taking the sample concentration as the x-axis and the corresponding absorbance as the y-axis, the standard curve is fitted. The fitting equation is: $y = 0.00912x + 0.10131$, the linear correlation coefficient R^2 is 0.99978, and the fitting degree is high. It is suitable for analyzing and testing the concentration of petroleum hydrocarbons.

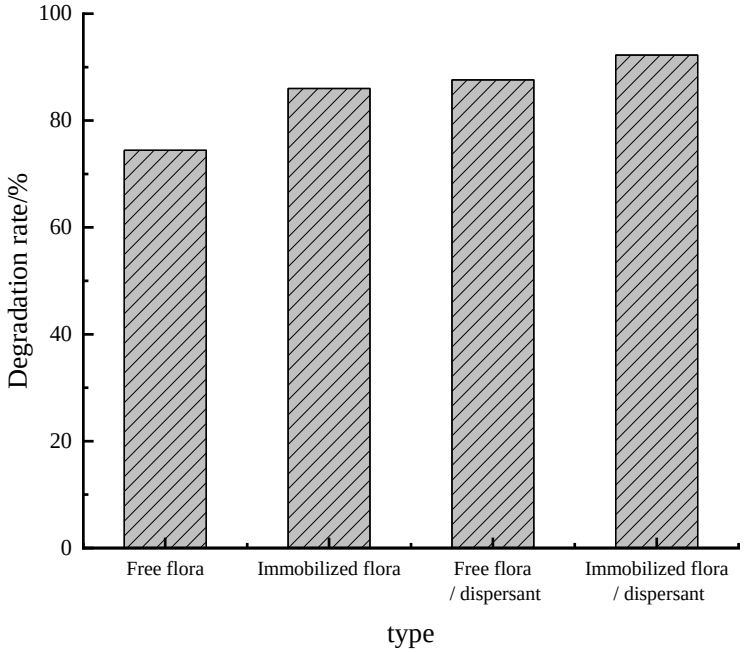

Fig. 11 7 d degradation rate of oil degrading microflora and dispersant

It can be seen from Fig. 11 that when the free oil degrading microflora has only 74.43% of the 7 d degradation rate of crude oil, the 7 d degradation rate of crude oil has reached 87.62% and the degradation rate has increased by 13.19% after the combination of free microflora and dispersant; After the immobilization of free microflora, the 7 d degradation rate of crude oil reached 86%. After the combination of fixed bacteria and dispersant, the 7 d degradation rate of crude oil reached 92.25%, which greatly improved the degradation efficiency. This may be because after fixing the oil degrading microflora, the ability of the bacteria to resist the external environment is increased; After compounding with dispersant, the carbon source of flora is increased. Surfactants in dispersants can provide effective carbon sources and other nutrients for the growth of flora. Secondly, the dispersant can disperse crude oil molecules and improve the contact area and oxygen flux between crude oil molecules and flora. Therefore, the 7 d degradation rate of crude oil by oil degrading microflora is significantly improved.

4. Conclusion

(1) A green oil spill dispersant was prepared based on biosurfactant rhamnolipid. Its service conditions were determined. When DOR was 25%, its 30 s emulsification rate of crude oil

reached 69.28%. The emulsifying effect is better when the pH value is 8, the temperature is 30℃ and the salinity is 3%.

(2) The viscosity, ignition point and other performance indexes of dispersant were measured, as well as the biodegradability and acute toxicity of dispersant to the fish. The results exceeded the national standards.

(3) The dispersant and bacteria groups were mixed to obtain bio-foam oil dispersant. The effect of dispersant on the growth of bacteria was determined, and the best compound ratio was 1:9. Through the change of the surface tension, it is verified that there is a synergistic relationship between them. The final biodegradation of the crude oil by bio-foam dispersant is 7, and the degradation rate of the crude oil is 87.62%, which has been improved significantly compared to the simple biodegradation.

Acknowledgements

Supported by the Graduate's Science Research Fundation of Shandong Jiaotong University(2020YK01); "Composite microbial missile" for degradation of the oil spill and residual oil at sea by National Natural Science Foundation of China (51609131); Joint training of graduate students integrating industry and education in Shandong Province - demonstration base for graduate students of ship and ocean engineering (2021001).

References

[1] Gong Yunfei, Zhao Pengfei, LAN Dongdong, et al. Characteristics and trend analysis of marine oil spill accidents in China[J]. Marine development and management, Vol. 35 (2018) No. 11, p. 42-45.

[2] Duan junxuan, Wu Wenfeng, Zhen Changwen, et al. Cause analysis and Countermeasures of offshore oil spill accident[J]. Water transportation management, Vol. 40 (2018) No. 10, p. 23-24.

[3] Li Dayan, Huang shenfa, Ye Chunmei, et al. The impact of oil pollution on marine ecosystem: A review[J]. Shanghai Environmental Science, Vol. 39 (2020) No. 4, p. 149-156.

[4] Chen Xinguang. Impact of marine oil pollution on marine ecological environment[J]. Environment and development, Vol. 169 (2020) No. 8, p. 205-207.

[5] Guo Feng. Review of pollution control technology[J]. Chemical management, 2021 (19): 51-53. Vol. 19 (2021), p. 51-53.

[6] Wu Kaijun. Analysis of hazards and treatment methods of ship oil spill accidents[J]. Chemical management, Vol. 24 (2020), p. 90-91.

[7] Molinier, Valerie, Le, et al. Optimization of chemical dispersants effectiveness in case of subsurface oil spill[J]. Colloids and Surfaces, A. Physicochemical and Engineering Aspects, Vol. 35 (2018) No. 11, p. 43-51.

[8] Jia Yi, Wang Jun, Jing Jing. Research progresses on toxic effects of Oil Spill Dispersants and dispersed oil droplets on marine organisms[J]. Bulletin of marine Limnology, Vol. 43 (2021) No. 4, p. 108-113.

[9] Stroski K M, Tomy G, Palace V. The current state of knowledge for toxicity of corexit EC9500A dispersant: a review[J]. Critical Reviews in Environmental Science and Technology, 2019: 1-23. (2019), p. 1-23.

[10] Afshar-Mohajer N, Fox M A, Koehler K. The human health risk estimation of inhaled oil spill emissions with and without adding dispersant[J]. Science of the Total Environment, Vol. 654 (2019) No. 1, p. 924-932.

[11] Nyankson E, Decuir M J, Gupta R B. Soybean Lecithin as a Dispersant for Crude Oil Spills[J]. ACS Sustainable Chemistry & Engineering, Vol. 3 (2015) No. 5, p. 920-931.

ISSN: 2664-9640

[12] Arp A, Dong M. Environmental risk of maritime territory subjected to accidental phenomena: Correlation of oil spill and ship grounding in the Exxon Valdez's case[J]. Results in Engineering, Vol. 49 (2019) No. 2, p. 81-103.

[13] Wei li'e, Yin Xin, Liu Jutao, et al. Review of bio-surfactant in oil spill pollution treatment[J]. Jiangxi water conservancy science and technology, Vol. 43 (2017) No. 4, p. 263-266.

[14] Yongrui Pi, Bao M, Liu Y, et al. The contribution of chemical dispersants and biosurfactants on crude oil biodegradation by Pseudomonas sp. LSH-7′[J]. Journal of Cleaner Production, Vol. 153 (2017) No. 1, p. 74-82.

[15] Hajibagheri F, Hashemi A, Lashkarbolooki M, et al. Investigating the synergic effects of chemical surfactant (SDBS) and biosurfactant produced by bacterium (Enterobacter cloacae) on IFT reduction and wettability alteration during MEOR process[J]. Journal of Molecular Liquids, Vol. 25 (2018) No. 26, p. 277-285.

[16] Shah M, Moniruzzaman M, Sivapragasam M, et al. A binary mixture of a biosurfactant and an ionic liquid surfactant as a green dispersant for oil spill remediation[J]. Journal of Molecular Liquids, Vol. 280 (2019), p. 111-119.

[17] Annex: national standard of the people's Republic of China - oil spill dispersant technical conditions GB 18188.1-2000[J]. Traffic standardization, Vol. 6 (2001), p. 19-26.