Bioremediation of Diesel Oil Polluted Seawater by a Hydrocarbon-degrading Bacterial Consortium with Oleophilic Nutrients

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Abstract

The use of oleophilic nutrients that contained nitrogen and phosphorus is a valid strategy to enhance bioremediation rate in the open marine environments contaminated with hydrocarbons where the presence of nutrients is limited. The bioremediation effectiveness of the natural oleophilic nutrients (uric acid and soya bean lecithin) and an artificial oleophilic fertilizer prepared in this study were tested with an efficient bacterial consortium. The artificial oleophilic fertilizer was prepared using urea solution, soya bean lecithin, alcohol and oleic acid to form a water-in-oil type microemulsion system. The bioremediation potential of the oil-degrading bacterial consortium and these oleophilic nutrients were implemented by flask-shaking tests and laboratory mesocosm experiments. The diesel oil degradation ratios of the natural oleophilic nutrients and oleophilic fertilizer were upto 73% and 60%, respectively, at the optimal application rate during the shake flask trials. In the mesocosm experiments, the oil concentration of the oleophilic fertilizer + bacterial consortium group was decrease from about 8000 mg/L to 823 mg/L after six days of incubation, and the number of bacteria in the seawater increased from 3×10^4 to 1.8×10^{10} CFU/mL. The combination of these oleophilic nutrients and the consortium was an effective strategy to enhance bioremediation rate. This method could be exploited further for the development of an effective bioremediation technology for the marine oil pollution.

Introduction

The limited presence of nutrients, such as nitrogen and phosphorous, is a major factor affecting the effectiveness of bioremediation in the marine environments contaminated with hydrocarbons (Fragkou et al. 2021). In the open marine environments, it is difficult to supply nitrogen and phosphorus, because water-soluble nutrients would be diluted into the surrounding water rapidly (Azubuike et al. 2016). An efficient approach to supply nutrients is by developing oleophilic fertilizers that contained nitrogen and phosphorus. Since oleophilic fertilizers can target the oil at the oil-water interface where the oil degradation is occurring (Gertler et al. 2015). Inipol EAP 22 is the first well-known oleophilic fertilizer, an oil-in-water microemulsion containing urea, lauryl phosphate, 2-butoxy-1-ethanol, and oleic acid (Pritchard *et al*. 1995; Zhu et al. 2001). However, the emulsifier (2-butoxy-1-ethanol) could be harmful to the environment and humans, and Inipol EAP 22 is not effective in the open marine environments, because the emulsion would break rapidly when it contacts with seawater (Ron and Rosenberg 2010). Another oleophilic fertilizer S200 was used for treatment of heavy fuel oil spill at the Prestige. The difference between S200 and Inipol EAP22 is only the surfactant component (Gallego *et al*. 2007). Developing environmentally friendly oleophilic fertilizer which is effective in the open marine environments is a research hotspot in recent years (Okeke et al. 2022; Bertha *et al*. 2021).

Uric acid is a natural nitrogen source from the excrement of birds, insects and reptiles, and then it is often used for production of inexpensive guano fertilizer. Because uric acid is insoluble in water, it might be used as oleophilic nitrogen source for oil biodegradation. Due to low water solubility of hydrocarbons, the inadequate bioavailability is another limiting factor in biodegradation for microorganisms (Al-Hawash et al. 2018). Surfactants can reduce surface tension and increase the surface area of oil, which facilitate

bacteria to contact with the hydrocarbons, and then increase its bioavailability (Ferguson et al. 2017). Many reports proved the use of surfactants could enhance the biodegradation rate of hydrocarbons (Ganesan et al. 2022; Naughton et al. 2019). Because of the low cost and good dispersant property of soya bean lecithin, it would be a good oleophilic natural phosphorus source and surfactant for oil biodegradation.

Lack of different microorganisms to metabolize the different components of hydrocarbons limited the degradation rate and range (Upasani and Varjani 2016). The consortium had a greater capacity to degrade hydrocarbon than the individual strain (Varjani and Upasani 2016). From application perspective, using a consortium was more advantageous than an individual strain because the consortium had more metabolic diversity and robustness (Luo et al. 2021).

In this study, uric acid and soya bean lecithin were used as natural oleophilic nitrogen and phosphorus sources, and an oleophilic fertilizer was prepared using urea solution as a nitrogen source, soya bean lecithin as the phosphorous source and surfactant, alcohol as a co-surfactant and oleic acid to form a water-in-oil type microemulsion system (MES). An efficient bacterial consortium constructed in our previous study was used as the degrading microorganisms (Luo et al. 2021). The bioremediation potential of the oil-degrading bacterial consortium and these oleophilic nutrients for diesel oil polluted seawater were implemented by flask-shaking tests, laboratory mesocosm experiments.

Materials And Methods

Bacterium and media

Four strains (Table 1) screened from the oil-contaminated seawater were used to construct an efficient oil-degrading bacterial consortium in this study (Luo *et al*. 2021). Luria-Bertani (LB) medium contained (per liter) 10.0 g tryptone, 5.0 g yeast extract and 10.0 g NaCl (pH 7.0). The seawater was collected from Yangshan port, Shanghai City, China. Dichloromethane was high-performance liquid chromatographic (HPLC) grade (Tedia Company, USA). The chemicals, uric acid and soya bean lecithin were analytical grade (China National Pharmaceutical Group Corporation).

Table 1. Strains used in this study.

Strains	Species	Source
Y9	*Acinetobacter* sp.	Laboratory collection (Sun *et al*. 2012a)
W3	*Acinetobacter* sp.	Laboratory collection (Sun *et al*. 2012b)
F9	*Acinetobacter* sp.	Laboratory collection (Luo *et al*. 2015)
X1	*Gordonia* sp.	Laboratory collection (Luo *et al*. 2021)

Biodegradation assays for the natural oleophilic nutrients of uric acid and soya bean lecithin

Fresh cultures of every one of the four bacterial isolates (Y9, W3, F9, X1) used to construct the bacterial consortium were separately inoculated with a bacteriological loop into a flask containing 50 mL of LB medium, and incubated at 30 °C and 180 rpm for about 12 h in a shaker. Thereafter 1 mL of each culture were mixed and collected by centrifugation (4000 rpm, 5 min) and washed twice with the sterilized seawater. The wet bacteria were inoculated to 50 mL sterilized seawater supplemented with 0.5 mL (1%, v/v) diesel oil as the sole carbon source and corresponding oleophilic nutrients in 250-mL flasks. Uric acid and soya bean lecithin (with a purity of 55%) were used as oleophilic nutrients to provide nitrogen and phosphorus. In order to optimize the mass ratio of diesel oil to uric acid, the ratios were set at 100:6, 100:12, 100:18 and 100:24 (namely, about 2, 4, 6, 8 g N/100 g diesel oil) under constant mass ratio of soya bean lecithin to diesel oil of 2/100. When the ratio of diesel oil to soya bean lecithin was optimized, the mass ratios of diesel oil to soya bean lecithin were set at 100:6, 100:4, 100:2 and 100:1 under constant mass ratio of uric acid to diesel oil of 12/100. The cultures without inoculation of the bacterial consortium and oleophilic nutrients were used as blank controls. The cultures inoculated with bacterial inoculum and without oleophilic nutrients were used as negative controls. All flasks were incubated at 30 °C with shaking at 180 rpm until they were removed for sampling. The entire flask was used for the degradation ratio analysis. The analysis method used was similar to our previous study (Luo *et al*. 2021). All of seawater sample in the flask was extracted three times with 60 mL petroleum ether (60 90 °C). The organic phase was collected after extraction, and the absorbance was determined by UV spectrophotometer at the wavelength of 255 nm. The standard curve method was determined to assay the diesel oil concentration in the organic phase (petroleum ether). The remaining diesel oil concentration of seawater sample in the flask was converted from the oil concentration in the organic phase. The degradation ratio (η) was defined as Equation (1):

$$\eta = \frac{(C_0 - C_1)}{C_0} \times 100\% \qquad (1)$$

Where C_0 is the remaining diesel oil concentration in the blank control after incubation; C_1 is the remaining diesel oil concentration in the test sample after incubation. All biodegradation assays were performed in triplicate. The value of the degradation ratio was the average of three samples.

At the optimized nutrient conditions, the biodegradation ratios were detected each two days from the first day to the eleventh day. At the end of the experiments, the rest diesel oil was extracted from the liquid culture with dichloromethane three times to monitor the changes of composition by gas chromatography-mass spectrometry (Thermo Focus DSQ GC-MS, American). The GC-MS analysis method was the same with our previous study (Luo *et al*. 2021).

Preparations of oleophilic fertilizer

The preparations of oleophilic fertilizer were carried out at room temperature (about 20-25 °C). A pseudo-ternary phase diagram was used to characterize the water-in-oil type MES of saturated aqueous solution of urea /soya bean lecithin/alcohol/oleic acid. The mixtures of soya bean lecithin and alcohol were

prepared according to the mass ratio (Km) of 1:2, 1:1, 2:1, and 3:1. After being heated at 70 °C for 10 min, the mixtures were mixed for 2 min with a vortex mixer. For every Km value, these mixtures and oleic acid were mixed according to the mass ratio (K) of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 in penicillin bottles. Then, water solution of urea was added dropwise until the microemulsion became turbid. Depending on the content of each component, the composition of each component (oleic acid, urea water solution, and the mixture of soya bean lecithin and alcohol) could be determined. The type of MES was determined by the dilution method. The microemulsion were added dropwise into the water; if the droplets scattered on the water surface, the MES was oil-in-water type; however, if the droplets floated on the water surface, then it was water-in-oil type. On the basis of the mass ratio of urea water solution in MES, the formulations were optimized. The MES which has the maximal mass ratio of urea water solution was selected as the oleophilic fertilizer.

Characterizations of oleophilic fertilize*r*

The centrifugal stability of oleophilic fertilizer was examined by centrifugation at 5000 rpm for 10 min. The temperatures of phase separations were recorded for evaluation the temperature stability. Total nitrogen of the oleophilic fertilizer was determined by alkaline potassium persulfate digestion UV spectrophotometric method, and total phosphorus was determined by the ammonium molybdate spectrophotometric method according to the People' Republic of China National Standard Method (HJ636-2012). To determine the nutrient slow-release characteristics of oleophilic fertilizer in seawater, 2.5 mL oleophilic fertilizer, 4 mL diesel oil, and 400 mL seawater were kept in a 1000 mL glass beaker covered and incubated at room temperature. After 1, 5, 12, 24, 36, 48, and 72 h of incubation, 3 mL seawater samples in the beaker were picked out to determine the total nitrogen. The contacting patterns between oleophilic fertilizer and oil were observed through the light microscope.

Flask-shaking tests of the oleophilic fertilizer

To optimize the amount of the oleophilic fertilizer for diesel oil biodegradation, the oil-degrading bacterial consortium (Y9+W3+F9+X1) was used. The cells (Y9, F9, W3 and X1) were incubated in LB medium at 30 °C and 180 rpm for about 12 h (OD600 = 1.0, approximately 10^9 cells/mL), respectively. Thereafter, 1 ml of each resulting culture were mixed and collected by centrifugation (4000 rpm, 5 min). The wet bacteria were inoculated into 50 ml sterilized seawater inoculated with 0.5 mL (1%, v/v) diesel oil and different volumes (50, 100, 150, 200, 300 and 400 μL, respectively) of oleophilic fertilizer. The cultures without inoculation of the bacterial consortium and oleophilic nutrients were used as blank controls. The cultures inoculated with bacterial inoculum and without oleophilic nutrients were used as negative controls. After seven days of incubation at 30 °C with shaking at 180 rpm, the entire flask was used for degradation ratio analysis. The analysis method used was similar to our previous study (Luo *et al*. 2021). All flask-shaking tests were performed in triplicates.

Laboratory mesocosm experiments of the oleophilic fertilizer

Five tanks were designed for laboratory mesocosm experiments. The internal size of the tank was 0.5 m (L) × 0.3 m (W) × 0.4 m (H). The mesocosm experiments were performed in five groups: the blank control 1# of tank was filled with 8 L of sterile seawater and spiked with 10 mL of diesel oil; the blank control 2# of tank was filled with 8 L of sterile seawater and spiked with 10 mL of diesel oil and 3 mL of oleophilic fertilizer; the blank control 3# of tank was filled with 8 L of non-sterile seawater and spiked with 10 mL of diesel oil; the oleophilic fertilizer group of tank was filled with 8 L of non-sterile seawater and spiked with 10 mL of diesel oil, and inoculated with 3 mL of oleophilic fertilizer; and the oleophilic fertilizer + bacterial consortium group of tank filled with 8 L of non-sterile seawater and spiked with 10 mL of diesel oil, and inoculated with 3 mL of oleophilic fertilizer and 20 mL of the consortium fermentation broth (5 mL for each strain, OD_{600}=1.0). These tanks were placed on a shaker, and cultivated at 30 °C and 40 rpm. The shaker was stopped, and the oil on the surface of seawater was stirred well before sampling. 30 mL of surface seawater was sampled from five sampling points in the tanks every 48 h. the seawater samples were mixed and extracted three times with 150 mL petroleum ether (60 90 °C). The organic phase was collected and analyzed by UV spectrophotometer at the wavelength of 255 nm for concentration calculation. The number of bacteria in the seawater samples were estimated by the most probable number (MPN) method as described by Nikolopoulou *et al.* (2008).

Results

Biodegradation assays for the natural oleophilic nutrients of uric acid and soya bean lecithin

The forms of diesel oil in seawater before and after addition of soya bean lecithin and uric acid were shown in Fig.1. Because of the good dispersant property of soya bean lecithin and water insolubility of uric acid, the oil film was dispersed to small oil droplets and the uric acid crystals were adhering to the oil droplets. Soya bean lecithin increased the dispersion of the oil, and resulted in the increase of oil available surface area for microbial colonization. The oil droplets and uric acid crystals were floating on the surface of seawater. Microorganisms could attack oil at the oil-water interface using uric acid and soya bean lecithin as nitrogen and phosphorus sources.

The difference in nutrients level profoundly affected the biodegradation of diesel oil. The effects of the application rate of uric acid on the biodegradation ratio of diesel oil by the bacterial consortium were shown in Fig.2(a). The biodegradation ratio was significantly increased after the addition of uric acid and soya bean lecithin (P<0.01). The most appropriate mass ratio of diesel oil to uric acid was 100:12, and the diesel oil biodegradation ratio (62%) was significantly higher than other mass ratios (P<0.05). At a higher application rate of uric acid, the inhibition effect had occurred, but there was not sufficient nitrogen for diesel oil degradation at a lower application rate. As shown in Fig.2(b), at the mass ratio of diesel oil to soya bean lecithin of 100:1, the biodegradation ratio (67%) was significantly higher than other mass ratios (P<0.05). Soya bean lecithin was sufficient for dispersion of the oil film and used as phosphorus source at this ratio. The higher application rate of lecithin decreased the biodegradation ratio of diesel oil.

The relationship between the degradation ratio of diesel oil and incubation time was an important consideration when evaluating the efficacy of the oil-degrading process. As shown in Fig.2(c), the diesel oil degradation ratio increased along with the increase of incubation time. The degradation ratio increased linearly from the first day to the ninth day, and then remained relatively constant. On the eleventh day, the biodegradation ratio was up to 73%. The rest diesel oil in the blank control and the experimental culture after eleven days incubation were analyzed by GC-MS. As shown in Fig.2(d), most components of diesel oil were degraded by the consortium.

Preparations of oleophilic fertilizer

The oleophilic fertilizer was prepared using urea water solution as a nitrogen source, soya bean lecithin as a phosphorous source, alcohol and oleic acid to form a water-in-oil type MES. The pseudo-ternary phase diagrams of the MES were shown in Fig.3. In this system, the water solution of urea acted as the aqueous phase, soya bean lecithin acted as the surfactant, alcohol acted as co-surfactant, and oleic acid served as the oil phase. There were two distinct characteristics in the pseudo-ternary phase diagrams. On one hand, the maximum content of the water phase increased with increasing content of the oil phase in the lectin/alcohol-rich region, and decreased with increasing content of the oil phase in the lectin/alcohol-poor region. On the other hand, the mass ratio of water increased with the increasing of Km value, and the maximal mass ratio of water was achieved at the maximal Km value of 2/1. At a higher Km value, the maximal mass ratio of water appeared at a lower mass ratio of oil. The microemulsion which had the maximal mass ratio of urea water solution was selected as the oleophilic fertilizer. The mass composition in the oleophilic fertilizer of each component was determined to be that oleic acid of 14.4%, urea water solution of 27.8%, and the mixture of soya bean lecithin and alcohol of 57.8%.

Characterizations of oleophilic fertilizer

The oleophilic fertilizer was a stable microemulsion system. There was no phenomena of phase separation by centrifugation at 5000 rpm for 10 min. The physical and chemical properties of this oleophilic fertilizer were shown in Table 2. The nitrogen slow-release characteristics of the oleophilic fertilizer in seawater were shown in Fig.4(a). At first 12 h, the nitrogen was released slowly, and it remained unchanged at 140 mg/L from 48 h. There was 300 mg of nitrogen in 2.5 mL oleophilic fertilizer. If all the nitrogen were released into the 400 mL seawater, its concentration should be 750 mg/L. This result indicated that most of the oleophilic fertilizer floated on the surface of seawater and the microemulsion system did not break. The forms of diesel oil before and after addition of oleophilic fertilizer were shown in Fig.4(b). After the addition of oleophilic fertilizer, the oil film was dispersed to oil particles, and then the particles immediately crossed link and formed large flocs. The oleophilic fertilizer was adhering to the oil particles.

Table 2. Properties of oleophilic fertilizer.

Total nitrogen (g/mL)	Total phosphorus (mg/mL)	Density (g/L)	Temperatures of phase separations	Storage temperature	Solubility
0.12	0.045	0.87	<10℃, >75℃	25℃~35℃	in sea water insoluble, in hydrocarbons soluble

Effects of oleophilic fertilizer on the biodegradation of diesel oil

Too high concentration of nutrients of N and P fertilizer inhibited the biodegradation of oil, and inhibition has occasionally been reported at lower application rates (Huang *et al*. 2008). The optimization of application rate of oleophilic fertilizer was necessary to acquire the basic information. The biodegradation ratios of diesel oil were shown in Fig.5(a) at different amounts of oleophilic fertilizer after seven days of incubation. The biodegradation ratio was significantly increased after the addition of oleophilic fertilizer (P<0.01). The highest biodegradation ratio (53.99%) was achieved at the amount of 150 μL, which was significantly higher than that of other amounts (P<0.05). The optimal ratio of diesel oil to oleophilic fertilizer was 10:3 (v/v). At higher or lower application rates, the biodegradation of diesel oil was inhibited. To confirm the optimization amount of oleophilic fertilizer, the biodegradation ratios were detected each two days for ten days. The changes of biodegradation ratio of diesel oil with time were shown in Fig.5(b). In the first two days, the biodegradation ratio increased rapidly, and the rate tended to be slow in next four days. It remained relatively constant from the sixth day to the tenth day, and it reached up to 60% on the tenth day.

Mesocosm experiments

To investigate the practical application and the feasibility of this oleophilic fertilizer in contaminated seawater, the oleophilic fertilizer was further applied in our simulated marine environment. At the beginning of the experiments, diesel oil was properly sprayed on the surface of seawater in the tanks, and then the oleophilic fertilizer was sprayed. In the tanks supplied with oleophilic fertilizer, the oil film was dispersed to oil particles and immediately crossed link to form flocs. During the culture process, the water became turbid due to the cell growth in the oleophilic fertilizer + bacterial consortium group. In contrast, the oil in the controls without oleophilic fertilizer remained unchanged, and a thick layer of oil was floating on the seawater. 150 mL of seawater samples were taken from the surface of seawater by the five-point method. The residual oil concentrations of the samples were shown in Fig.6(a). In the blank control 1# and 2# group, the residual oil concentration decreased with incubation time due to volatilization. In the blank control 3# group, the residual oil concentration decreased with incubation time due to volatilization and biodegradation from indigenous bacteria. The residual oil concentration of the oleophilic fertilizer + bacterial consortium group decreased more rapidly than that of the other groups. After six days, the residual oil concentration was decrease from about 8000 mg/L to 5264 mg/L and 5068 mg/L in the blank control 1# and 2# group. The residual oil concentration was decrease from about

8000 mg/L to 3402mg/L, 2356 mg/L, 823 mg/L, repectively, in the blank control 3# group, oleophilic fertilizer group and oleophilic fertilizer + bacterial consortium group.

During the biological treatment in mesocosm experiments, changes of the number of bacteria in seawater were shown in Fig.6(b). The initial inoculation bacteria concentration was 3×10^4 CFU/mL in the oleophilic fertilizer + bacterial consortium group, and the initial bacteria concentration in the control tank was about 100 CFU/mL. The number of bacteria increased by 5 orders of magnitude in the experimental tank supplied with the oleophilic fertilizer and bacterial consortium after four days of incubation, and it increased up to 1.8×10^{10} CFU/ mL on the sixth day. The number of bacteria in the oleophilic fertilizer group increased from 100 to 10^5 CFU/ mL, because the indigenous microorganisms was stimulated by the oleophilic fertilizer. The number of bacteria increased from 100 to 10^4 CFU/ mL in the blank control group after six days of incubation because of the addition of diesel oil.

Discussions

In the oil pollution marine environments, there were enough carbon sources from hydrocarbons pollutants for microorganisms. However, the nutrients of nitrogen and phosphorous were poor (Sun et al. 2018). It was essential to add nitrogen and phosphorous for effective bioremediation (Mapelli et al. 2017). Water-soluble nutrients of nitrogen and phosphorus has been proven effective in improving oil biodegradation in many reports (Roy et al. 2018). However, water-soluble nitrogen and phosphorus additives were only could be applied in sheltered marine environments or low energy shorelines. Meanwhile, high concentrations of water-soluble N and P could cause waste and secondary pollution such as eutrophication (Nikolopoulou *et al*. 2010). For the open marine environments, the valid strategy was to design fertilizers which targeted the hydrocarbons pollutants and were not readily diluted into the surrounding seawater (Nikolopoulou et al. 2008). Using oleophilic nutrients is an alternative strategy to overcome the problem of quick dilution and wash out of water-soluble nutrients.

Uric acid and soya bean lecithin are natural oleophilic nitrogen and phosphorus sources which are nontoxic to the environment. Uric acid crystals could adhere to the oil droplets and be used by oil-degrading bacteria. Soya bean lecithin is also a natural surfactant, and it could disperse oil film into oil droplets. The results of this study showed that uric acid and soya bean lecithin were good sources of nitrogen and phosphorous in oleophilic form for diesel oil biodegradation. A high diesel oil degradation ratio could be achieved at the relatively low application rate of uric acid and soya bean lecithin. The diesel oil degradation ratio was up to 73% after eleven days incubation. Nikolopoulou and Kalogerakis (2008) examined the effectiveness of uric acid and lecithin in combination with rhamnolipids and molasses to enhance the biodegradation of oil by indigenous microorganisms. Their results showed that the use of biosurfactants increased degradation of petroleum hydrocarbons as well as in a reduction of the lag phase.

The oleophilic fertilizer prepared in this study was a water-in-oil microemulsion containing urea (nitrogen source) and soya bean lecithin (phosphorous source) which were environmentally friendly. The

microemulsion was a thermodynamically stable mixture of water, oil and surfactants. Stability was one of the most important indexes evaluating microemulsion quality. Through centrifugation at 5000 rpm for 10 min, the samples of this oleophilic fertilizer were uniform and transparent, and there were not stratified phenomenon. The break of the oleophilic fertilizer as soon as they came in contact with water was an important problem pressed for solution. The nitrogen slow-release characteristics of the oleophilic fertilizer indicated that most of the emulsion did not break when they came in contact with seawater. These results indicated the oleophilic fertilizer had a good stability. The oleophilic outer shell of the oleophilic fertilizer adhered to the hydrocarbon and it was less dense than seawater, therefore it would go to the surface of seawater where more oxygen was available and the conditions for biodegradati.on were better. The oleophilic fertilizer, meanwhile, dispersed the oil film to oil particles, and resulted in the increase of oil available surface area for microbial colonization. In the mesocosms experiments, the number of bacteria increased by six orders of magnitude in the experimental tank supplied with the oleophilic fertilizer and bacterial consortium, and the oil concentration was decrease from about 8000 mg/L to 823 mg/L after six days of incubation. On the other hand, the increasing of autochthonous bacteria was not significant in the control groups (without inoculation) compared to inoculated group. This was probable because the seawater used in these experiments was sampled from unpolluted sea area, and then contained too little indigenous hydrocarbon degrading bacteria. Although the limiting factors of nutrients were added, the biodegradation ratio and number of bacteria increased slowly. This result proved that biostimulation could not be applied to every case and the growth of indigenous hydrocarbon degraders was always slow. Bioaugmentation was essential for sites that did not have sufficient indigenous hydrocarbon degrading bacteria. The combination of bioaugmentation and biostimulation might be an effective strategy to enhance bioremediation rate (Luo et al. 2021). The volume ratio of oil to oleophilic fertilizer was 10:3 in flask-shaking tests. The actual amount needed for bioremediation is site-specific that depends on the type and amount of oil components, and also the background concentration of nutrients in the marine environments.

The natural oleophilic nutrients (uric acid and soya bean lecithin) and the oleophilic fertilizer prepared in this study were effective for bioremediation of diesel oil polluted seawater with the efficient bacterial consortium. These oleophilic nutrients not only overcame the problem of water-soluble nutrients being rapidly diluted, but also was friendly to the environment. The application strategies of oleophilic nutrients for bioremediation should be adjusted according to the environmental conditions. The results of this study would benefit to acquire the basic information and optimum condition for development of an effective bioremediation technology in the open marine environments.

Declarations

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Data Availability: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

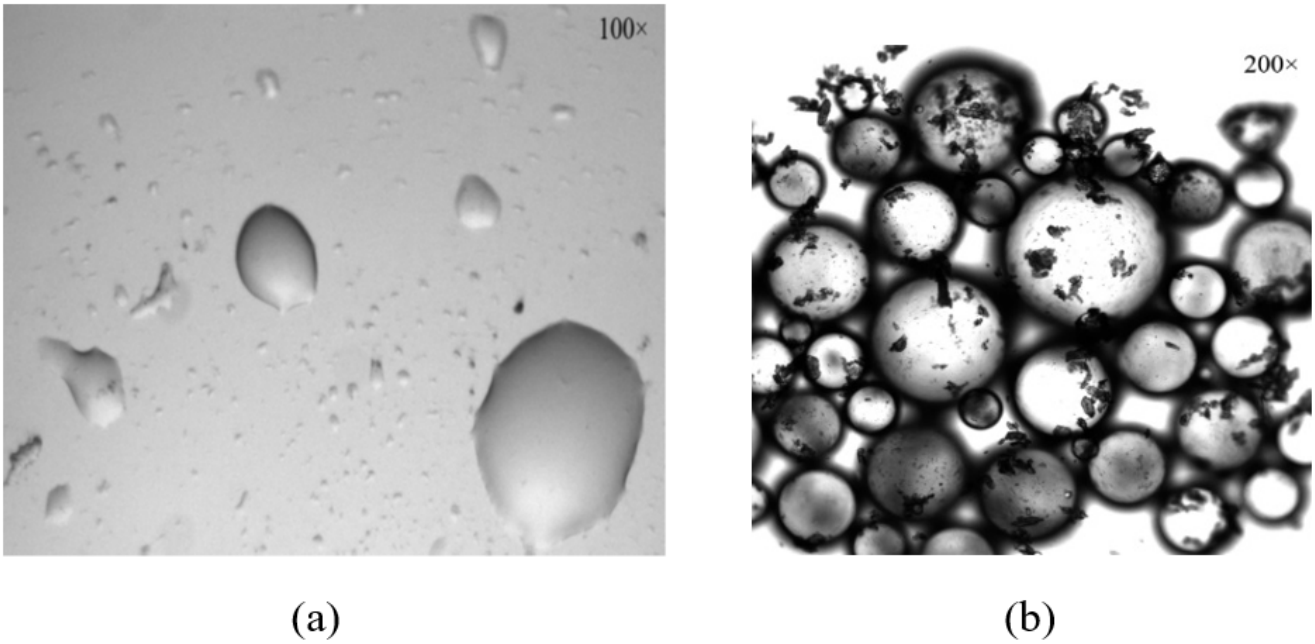

Figure 1

The forms of diesel oil in seawater before (a) and after (b) addition of soya bean lecithin and uric acid.

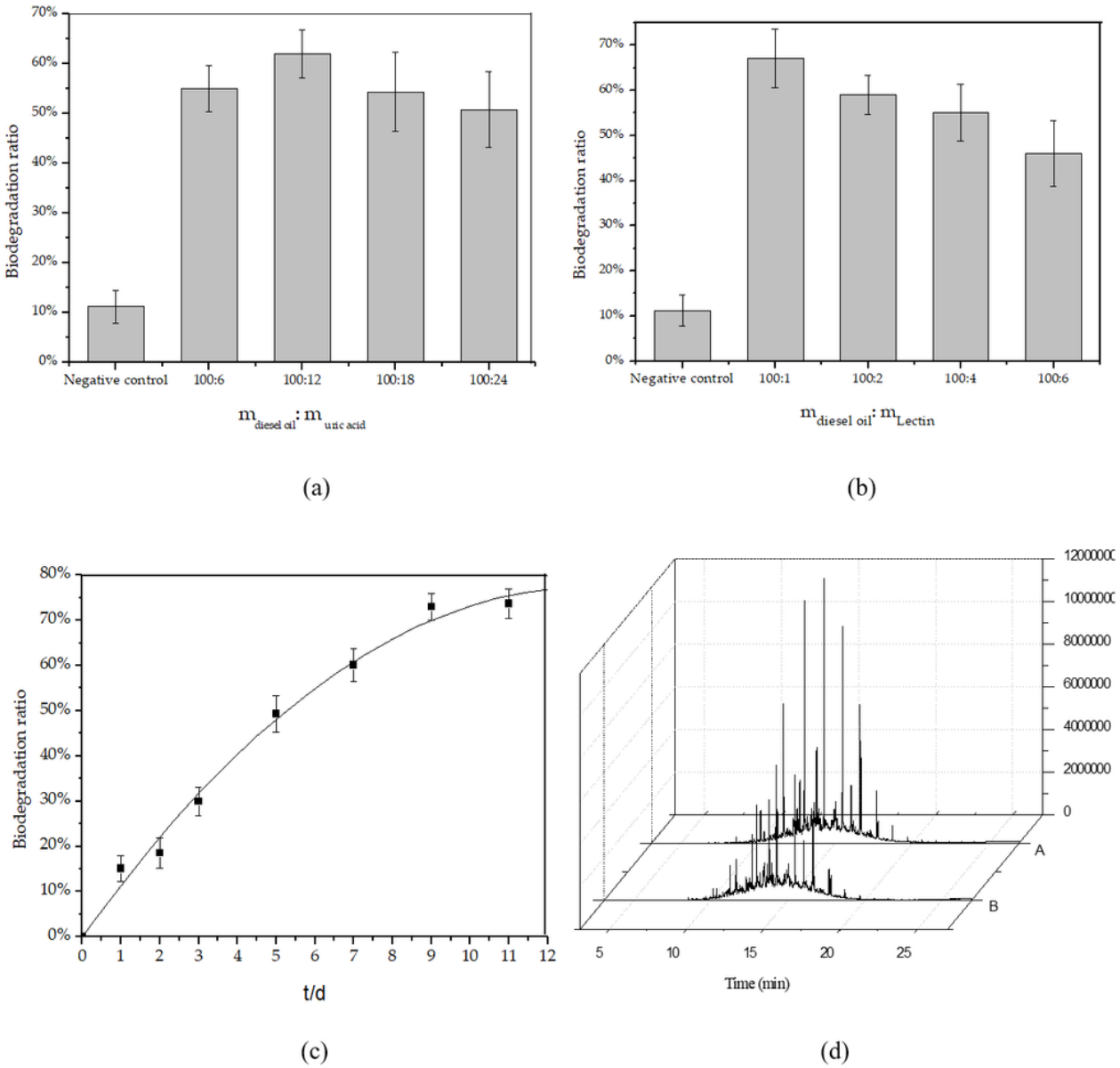

Figure 2

The biodegradation effects of diesel oil by the bacterial consortium with urea acid and soya bean lecithin. (a) Biodegradation ratios of diesel oil at different mass ratios of diesel oil to nitrogen. (b) Biodegradation ratios of diesel oil at different mass ratios of diesel oil to soya bean lecithin. (c) Changes of diesel oil biodegradation ratio with incubation time. (d) The chromatogram of rest diesel oil in experimental culture (B) and blank control (A) after eleven days incubation.

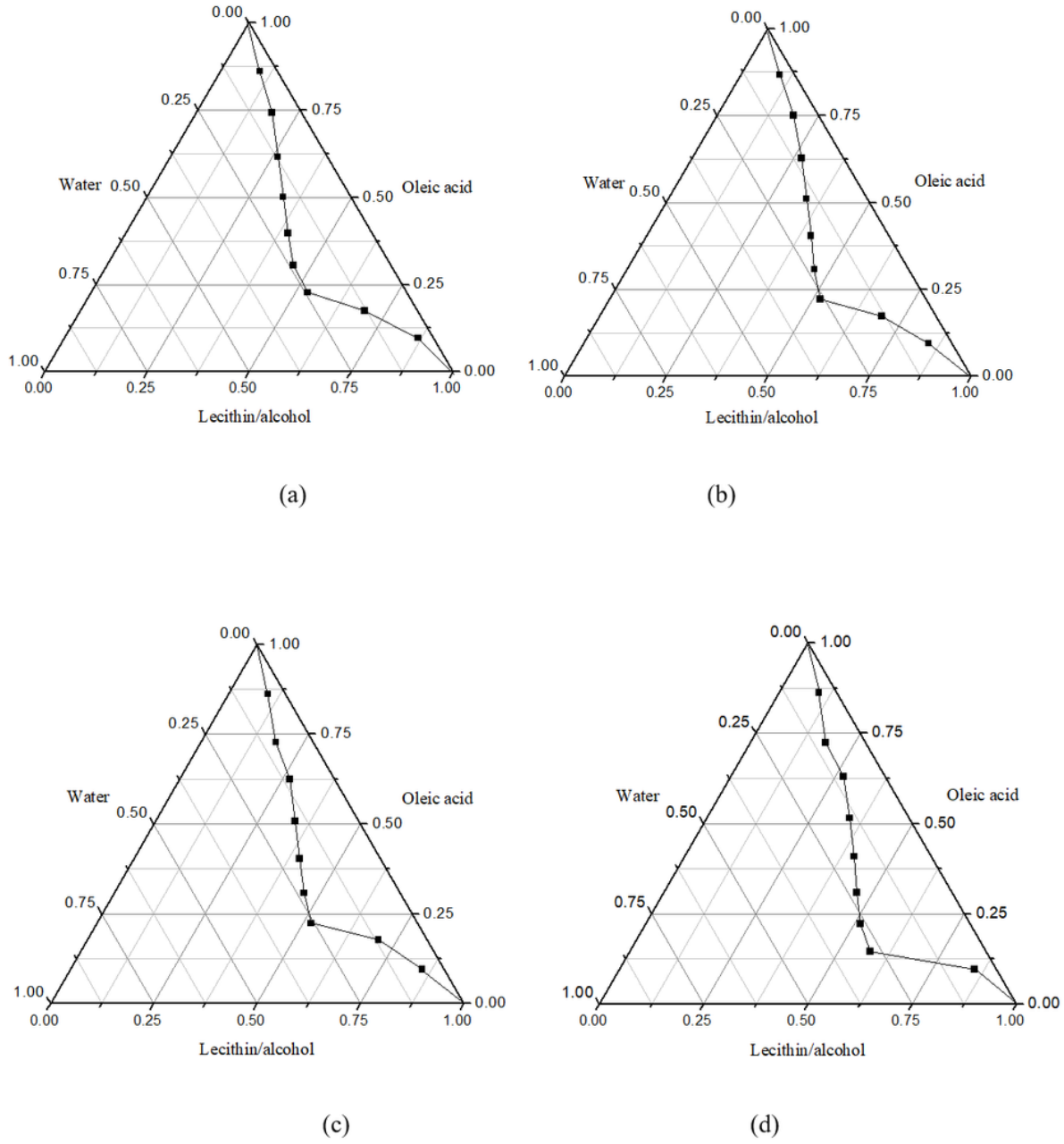

Figure 3

Pseudo-ternary phase diagrams of the W/O-type MES of water solution of urea/Soya bean lecithin/alcohol/oleic acid. (a)Km=1/3. (b) Km=1/2. (c) Km=1/1. (d) Km=2/1.

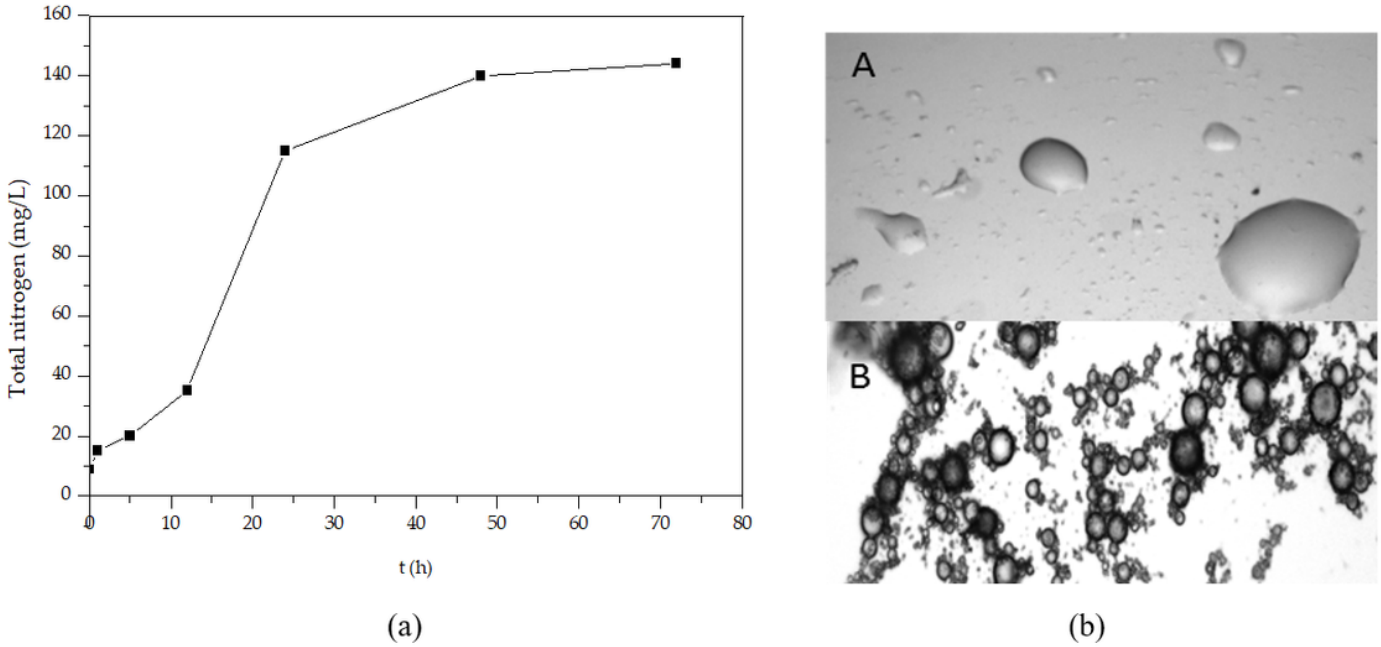

Figure 4

Characterizations of the oleophilic fertilizer. (a) Changes of total nitrogen concentration in seawater supplied with oleophilic fertilizer. (b) Forms of diesel oil in seawater before (A) and after (B) addition of oleophilic fertilizer, 100×.

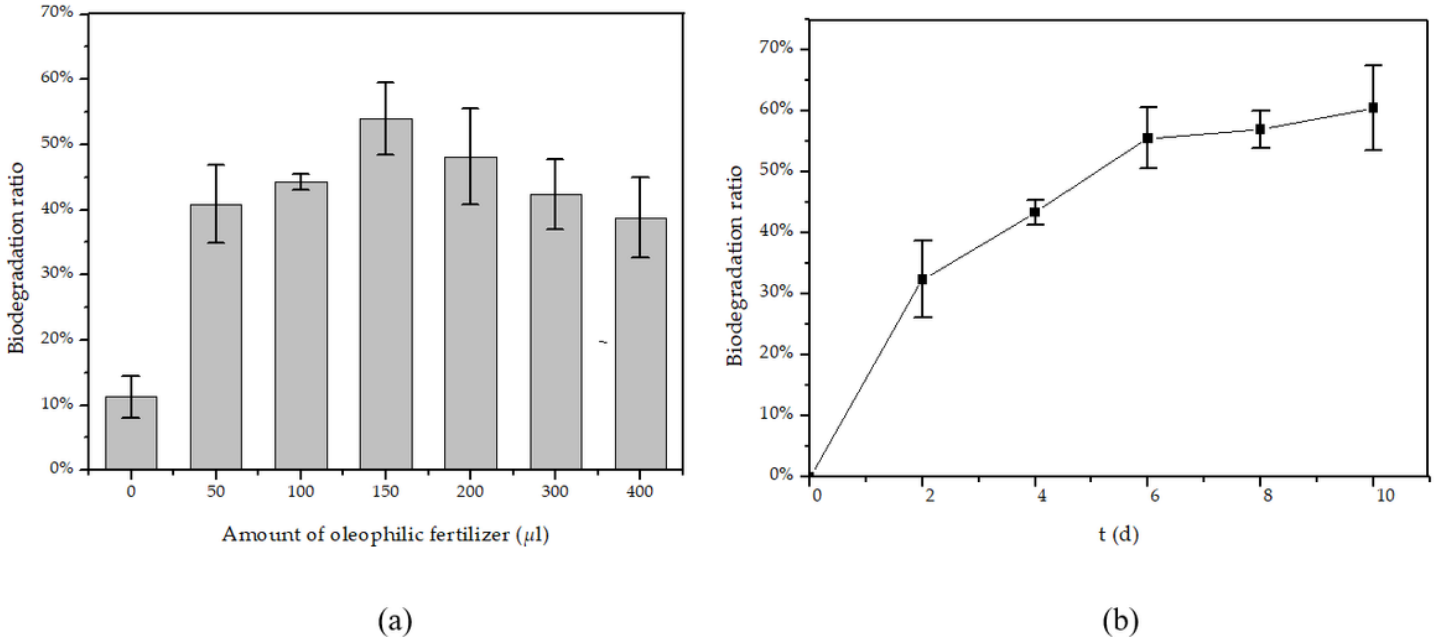

Figure 5

Biodegradation ratios of diesel oil. (a) Changes with the amounts of oleophilic fertilizer. (b)Changes with the incubation time at the optimization amount of oleophilic fertilizer.

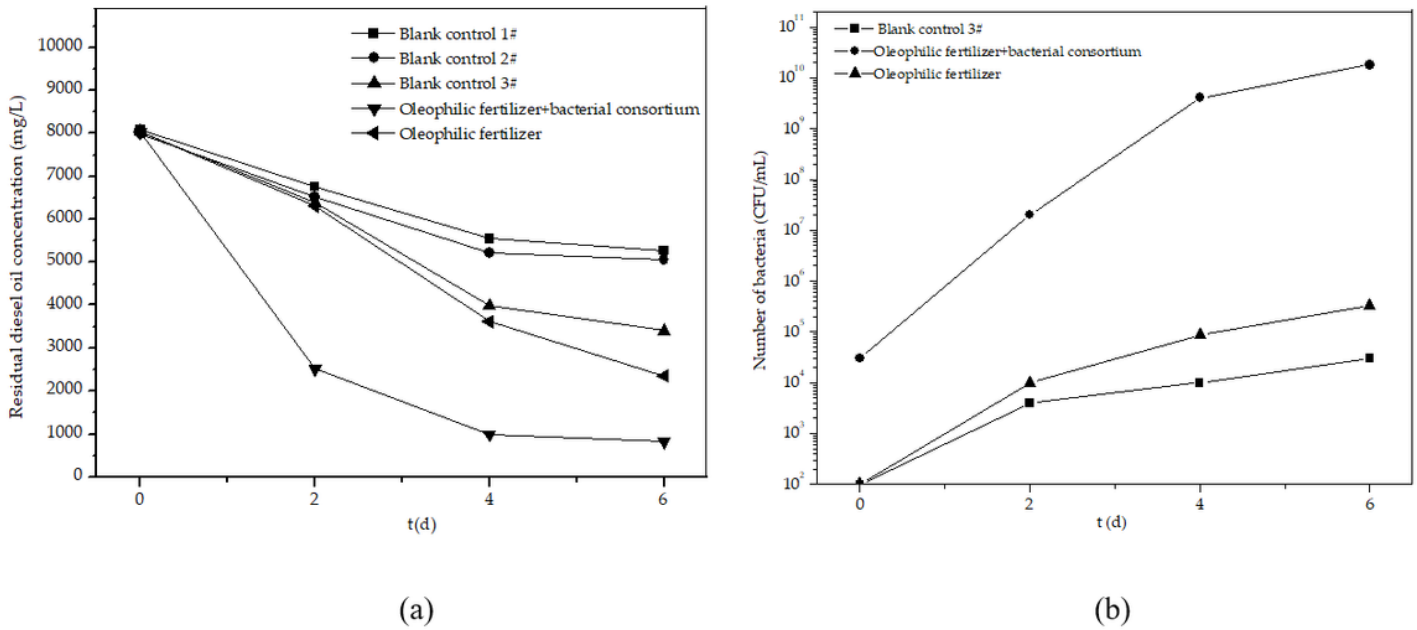

Figure 6

Changes of the residual oil concentration (a) and the bacteria number (b) with incubation time.