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Anoxic Bioremediation of Oil Contaminated Coastal Sediment

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In this paper, the adsorption and anoxic degradation of No. 0 diesel oil in Bohai coastal sediments were studied. The results showed that the adsorption of oil on the coastal sediments matched pseudo-second-order kinetics, which indicated that adsorption processes were essentially chemical reactions. Part of oil could be degraded in natural coastal sedimentary environment, and the additions of nutrients and proper electron acceptors could promote the degradation of oil effectively. The addition of nitrogen and phosphorus nutrients increased the removal ratio of oil from 54 % to 61 %. Different electron acceptors showed different effects on the degradation of oil in coastal sediment. Nitrate greatly promoted the degradation of oil, but sulfate had an adverse effect on that. Three oil-degrading strains named B1, B2, B3 were domesticated and isolated from Bohai coastal sediments. All the three strains could promote the degradation of oil, and the effect of B2 on the degradation of oil was the most significant, which increased the removal ratio of oil by nearly 30 %.

1. Introduction

Crude oil continues to be used as the principal source of global energy (Mirandola and Lorenzini, 2016; Xiao et al., 2016). The surging global oil demand has driven the transportation of large volume of oil through the busy sea lanes. During the past few decades, large volumes of oil have been released into the marine environment due to marine crude oil tanker collisions, and accidental spills. Because crude oil is more easily adsorbed by sediments than dissolving in water, sediment is the ultimate fate of the oil when it is released into the marine environment (Agarwal and Liu, 2015; Tyagi et al., 2011; Hii et al., 2009). The contamination of coastal sediments by crude oil has raised a global concern for the great threat to ecosystem and human health (Acosta-Gonzalez et al., 2015; Beolchini et al., 2010). Many techniques have been explored to remove the oil contaminants from the coastal sediment. Among these, bioremediation is gaining increasing prominence for its low environmental impact, low costs and high capability (Nikolopoulou et al., 2013; Wang et al., 2012; Dell'Anno et al., 2009; Tahhan and Abu-Ateih, 2009; Fuchedzhieva et al., 2008; Rahman et al., 2002).

The coastal sedimentary environment is always oxygen deficient because subsurface sediments normally contain a limited amount of oxygen, and the available molecular oxygen is consumed by indigenous microorganisms much faster than it can be replenished (Dou et al., 2009; Venosa and Zhu, 2003). Therefore, the anoxic degradation of crude oil plays a key role in the coastal sediment. Some studies have shown that anoxic microorganisms can degrade oil in the sediment or soil under anoxic conditions (Sherry et al., 2013; Chang et al., 2002; Grishchenkov et al., 1999). However, in anoxic coastal sedimentary environment, the rate and extent of hydrocarbon biodegradation are limited by the deficiency of terminal electron acceptors and nutrients (Dell'Anno et al., 2009; Hii et al., 2009; Tahhan and Abu-Ateih, 2009), especially nitrogen and phosphorus nutrients (Hii et al., 2009; Venosa and Zhu, 2003).

In this study, adsorption and anoxic bioremediation experiments were carried out on Bohai coastal sediment contaminated by No. 0 diesel oil, which were intensified with biostimulation. The biostimulation measures included the adding of nitrogen and phosphorous as nutrients, the adding of nitrate and sulfate as the terminal electron acceptors, and the adding of oil-degradaing strains. The aim of this study is to find beneficial measures that can effectively improve the anoxic biodegradation process of crude oil in coastal sediment and provide scientific basis for the artificial restoration of coastal sediment.

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2. Material and Methods

2.1 Chemicals

No. 0 diesel oil was obtained from China National Petroleum Corporation. Petroleum ether (analytical grade, boiling range: 30-60 ℃), n-hexane (purity ≥ 98 %), sodium sulfate (analytical grade), ammonium chloride (analytical grade), sodium nitrate (analytical grade), potassium dihydrogen phosphate (analytical grade) were supplied by Qinhuangdao Chemical Company.

2.2 Sampling

The samples of coastal water and sediments were collected at Qinhuangdao harbor in Hebei Province, P. R. China. The sediment samples were collected from a layer which was 10-20 cm depth under the surface sediment, and then were reserved at 4 ℃ in the refrigerator in 24 hours after collection. The pH values of the coastal water sample range from 7.4 to 8.2, and the salinity of the coastal water sample was 31 ‰. The contents of Total P and Total N in the sediment samples were 0.17 mg/g and 0.08 mg/g respectively.

2.3 Adsorption experiments

To get standard oil (SO), No. 0 diesel oil with a density of 0.84 kg/L and petroleum ether were added to a beaker in the volume ratio of 1:1, mixed on a shaker, and distilled at 90 ℃ in a water bath pot. The concentration of the standard oil stock solution (SOSS) was 5 mg/ml through diluting the SO with n-hexane. SOSS was diluted again with n-hexane to obtain oil standard solution (OSS) with a concentration of 200 μg/mL. 4ml OSS were added respectively to a series of 50 mL conical flasks, in which there were 4 g sterilized sediment and 20 ml deionized water. At the condition of 15 ℃, 175 r/min, the samples were shaken in thermostatic shaking bed, and were sampled and determined at 0, 10, 30, 60, 90, 180, 300, 420 minutes using ultraviolet spectrophotometry.

2.4 Biodegradation experiments

24 g fresh coastal sediments, each equivalent to 20 g of dry sediment, were placed into a series of 50 ml tapered bottle containing 8 ml sea water. 4 ml SOSS was added into the sediment to achieve the concentration of 1,000 mg/kg (oil/sediment). The bottles were then placed on an oscillator for 2 hours at 200 rpm, and then were incubated at 22±1 ℃ for 5 minutes after nitrogen gas injection and sealing. The residues of oil in the sediments were determined at different intervals.

To determine the proportion of biodegradation in the whole degradation, the comparison experiments were conducted using sterilized sediments (sterilized by autoclaving at 121 ℃ for 60 minutes) instead of fresh sediments. The sterilization series reflected the non-biological degradations (including hydrolysis and chemical oxidation-reduction reaction).

In order to explore the effects of nutrients and electron acceptors on the anoxic biodegradation of oil in coastal sediment, three groups of biostimulation experiments were conducted:

(1) Indigenous bacteria (IB): neither nutrients nor electron acceptor is added;

(2) Indigenous bacteria with the addition of nutrients (IB+N): 0.16 g ammonium chloride serving as the nitrogen source and 0.04 g potassium dihydrogen phosphate serving as phosphorus source are added;

(3) Indigenous bacteria with the addition of electron acceptors (IB+EA): one is 0.18 g sodium sulfate added with sulfate ion serving as the electron acceptor (IB+EAS), and the other was 0.15 g sodium nitrate added with nitrate ion serving as the electron acceptor (IB+EAN).

In order to explore the effects of oil- degrading strains on the anoxic biodegradation of oil, oil - degrading strains were domesticated by concentration gradient method, and isolated by streak plate method.

2.5 Determination of extractable residues

The diesel oil was extracted from the sediment using 20 ml of n-hexane in an ultrasonic bath. After partition, clean-up, and quantitative dilution, the residues dissolved in n-hexane were taken for ultraviolet spectrophotometry analysis (GB 17378.5-1998 The specification for marine monitoring: Sediment analysis).The optimum absorption wavelength of No. 0 diesel oil was 224 nm. Recoveries of oil residues in the sediment samples with the concentration of 200 mg/kg, 500 mg/kg, and 1,000 mg/kg range from 98.3 % to 102.1 %, with relative standard deviation (RDS) ranging from 1.33 % to 1.48 %.

3. Results and Discussion

3.1 Adsorption of diesel oil on Bohai coastal sediment

The adsorption behavior of No. 0 diesel oil on Bohai coastal sediment was shown in Figure1. From Figure 1, we could see that the adsorption of No. 0 diesel oil on the coastal sediment reached equilibrium at 180min, and the major adsorption happened in 90min. The experimental data were fitted with the pseudo-first-order and pseudo-second-order kinetic models respectively. The pseudo-first-order and pseudo-second-order equations can be expressed as:

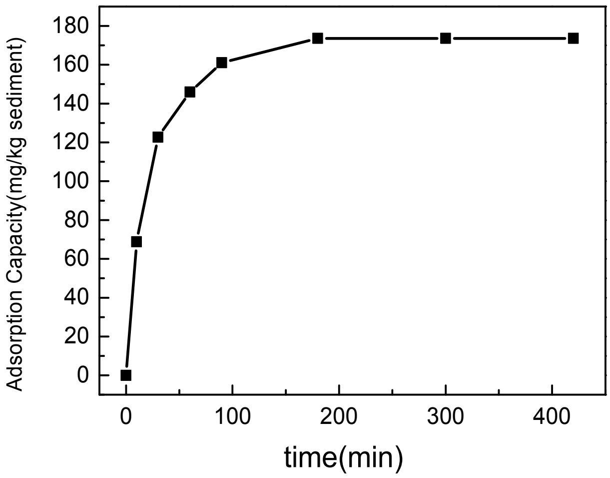

Figure 1: Adsorption curves of No. 0 diesel oil on Bohai coastal sediment

$$ln(Q_e-Q_t)=lnQ_e-k_1\times t \quad (1)$$

$$t/Q_t=1/(k_2\cdot Q_e^2)+t/Q_e \quad (2)$$

where Q_e and Q_t (mg/kg) are the equilibrium adsorption capacity and the adsorption capacity at time t, respectively, k_1(min^{-1}) and k_2(kg/(mg•min)) are the adsorption rate constants for the pseudo-first order and pseudo-second order kinetics, respectively. The fitting results were shown in Table 1.

Table 1: Adsorption kinetic parameters of the pseudo-first-order and pseudo-second-order models

Kinetic model	Q_e(mg/kg)	*Correlation coefficient*
pseudo-first-order model	54.2	0.7273
pseudo-second-order model	181.8	0.9995

As indicated by Table1, the linear fitting degree of the pseudo-second-order kinetics is higher, and the experimental equilibrium adsorption capacity (174.0 mg/kg) is more consistent to that of theory(181.8 mg/kg), which identifies that the adsorption of oil on Bohai coastal sediment follow the pseudo-second-order kinetics and the chemical adsorption plays a major role.

3.2 Effect of nutrients on No. 0 diesel oil degradation

To determine the influence of nutrients on the anoxic degradation of crude oil in coastal sediment, the experiments on the anoxic degradation of No. 0 diesel oil under different nutritional conditions were conducted. Results of the experiments were shown in Figure 2. According to Figure 2, 54 % of the oil in natural sediment was degraded in 112 days. In case of sterilized sediment, only 8 % of oil was removed from the sediment, that is to say, only 8 % was degraded by non-biological degradations (including hydrolysis and chemical oxidation-reduction reaction), so the main type of degradation of diesel oil in sediment is biodegradation. No obvious differences in degradation rate between the IB and IB+N groups were found in the first 28 days. However, the IB+N group had a higher diesel removal ratio than the IB group after 28 days, which indicated that nitrogen and phosphorus nutrient helped to enhance the anoxic degradation of diesel oil in coastal sediment. The promotion effect of nitrogen and phosphorus on degradation of oil happened mainly during the period of 28-70 days, which indicated that a large amount of nitrogen and phosphorus were needed during the period of 28-70 days, and the addition of the nutrients supplemented the shortage, thereby promoting the growth of microorganisms and enhanced the anoxic degradation of diesel oil. As the IB group, the degradation rate of IB+N group also presented a stable tendency during the period of 70-112 days. This might be due to the large consumption of electron acceptors, which were essential for degrading diesel oil in an anoxic environment. Therefore, the deficiency of electron acceptors limited the anoxic degradation of oil in the late period, although there were abundant nitrogen and phosphorus nutrients.

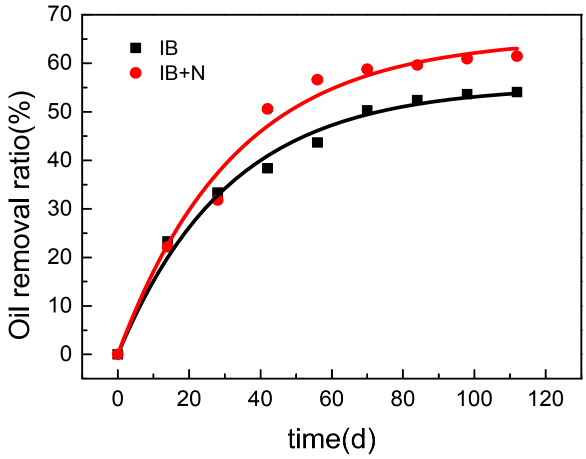

Figure 2: Effect of nutrients on the anoxic degradation of No.0 diesel oil in coastal sediment

3.3 Effect of electron acceptors on diesel oil degradation

Two kinds of electron acceptors, sulfate ion and nitrate ion, were added respectively to investigate their influence on the anoxic biodegradation of No. 0 diesel oil in Bohai coastal sediment and the results were shown in Figure 3. According to Figure 3, the degradation ratio of No. 0 diesel oil in IB+EAN group (with the addition of nitrate ion) achieved 73 % after 112 days, which was almost 20 % higher than that of the IB group. However, compared with the IB group, the removal ratio of IB+EAS group (with the addition of sulfate ion) decreased by 18 %. From the results, we concluded that the addition of nitrate promoted the anoxic biodegradation of oil in sediment significantly mainly in two ways. On the one hand, the nitrate could be the nitrogen source of oil-degrading bacteria; on the other hand, nitrate served as the electron acceptor in anoxic sediment. The nitrate-reducing bacteria in the coastal sediment could utilize nitrate ion as the electron acceptor to oxidize hydrocarbons in oil and reduce nitrate to NO_2^- or N_2. In contrast to the promotion effect of nitrate, the addition of sulfate restrained the anoxic biodegradation of diesel oil in sediment. The main reason might be that the indigenous bacterium contained sulfate-reducing bacteria, which could utilize sulfate ion as the electron acceptor to oxidize hydrocarbons in oil and reduce sulfate to sulfide like H_2S (Sherry et al., 2013). The sulfide suppressed microbial degradation activity because it was toxic to the microorganisms (Mahmood et al., 2007). In this experiment, the production of sulfide had been proved, which had a deadly effect on oil-degrading bacteria.

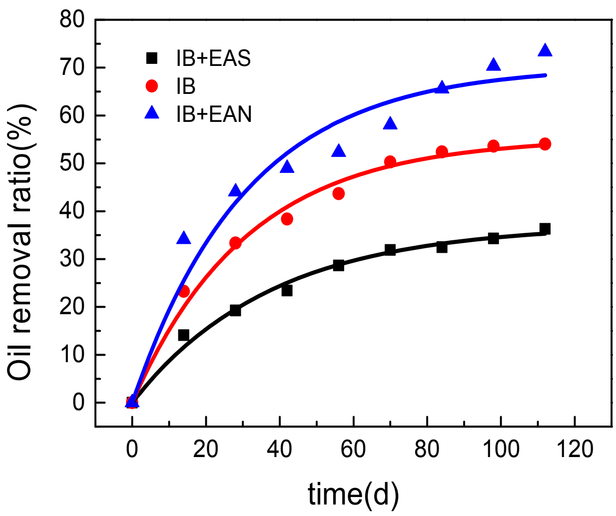

Figure 3: Effects of electron acceptors on the anoxic degradation of No. 0 diesel oil in coastal sediment

3.4 Effect of oil-degradaing strains on diesel oil degradation

Three oil-degrading strains named B1, B2 and B3 were domesticated and isolated from the coastal sediment. Under the anoxic condition, strains B1, B2 and B3 were added to the oil polluted sediments respectively. After 35 days of incubation, the degradation results of oil were shown in Figure 4.

Figure 4 showed that promoting effect of B2 was the most significant, the oil removal ratio increased by nearly 30 % compared to natural condition. The promoting effect of B1 was the second, the oil removal ratio increased by about 20 % compared to natural condition. B3 had only a slight promotion effect on oil degradation. Through physiological analysis, both of B1 and B2 have capsules, which can adsorb oil to the surface of bacteria and can also be used as carbon sources and energy sources when there is a lack of nutrition. According to the results of nitrate reduction test, B2 could reduce nitrate to nitrite, and then nitrite could continue to be reduced to ammonia or nitrogen, while B1, B3 could only reduce nitrate to nitrite, which indicated that the process of nitrate reduction by B2 required more electrons, thus promoted the anoxic degradation of oil significantly.

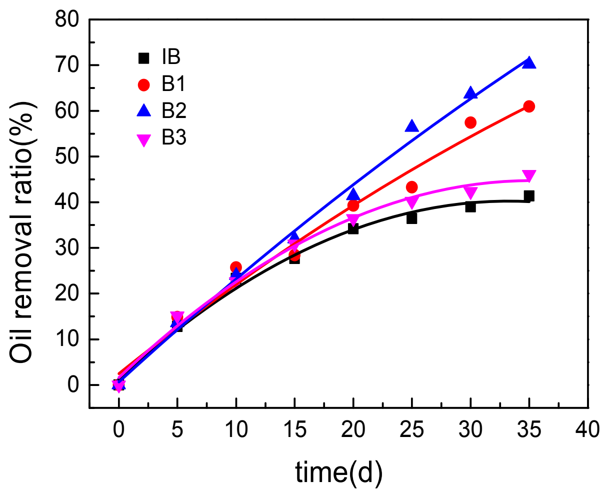

Figure 4: Effects of oil-degrading strains on the anoxic degradation of No. 0 diesel oil in coastal sediment

4. Conclusions

No. 0 diesel oil could be adsorbed onto the coastal sediment in a short period. The adsorption kinetics could be described by the pseudo-second-order kinetic model, which indicated that the adsorption process was essentially chemical reactions. The addition of nitrogen and phosphorus nutrients and adequate electron acceptors such as nitrate ion is helpful to promote the anoxic biodegradation of oil in coastal sediment. The removal ratio of oil in coastal sediment after 112 days grows from 54 % to 61 % because of the addition of nitrogen and phosphorus nutrients. The promoting effect of nitrate is more significant because the nitrate could be used both as nutrient and as electron acceptor. The removal ratio of oil with the addition of nitrate achieves 73 %. In addition, nitrate has the advantages of being easily soluble in water and is cheap. Therefore, it is very promising to add nitrate to promote the bioremediation of oil-contaminated coastal sedimentary environment. But the addition of sulfate as electron acceptor plays a negative role in the bio-degradation process of oil. All the three oil-degrading strains domesticated and isolated from the coastal sediment have the promoting effect on the degradation of oil. Strain B2 increased the removal ration of oil by nearly 30 % compared to natural condition. So the addition of oil-degraging bacteria is also an effective method to restore oil-contaminated coastal sedimentary environment.

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