Degradation of Crude Oil in Contaminated Sediment by *Pseudomonas Putida* Y3 Strain

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Abstract: In this study, *Pseudomonas putida* Y3 strain was used as the test object, and the sediment contamination test was conducted at different crude oil concentrations (0, 4000, 8000, 12000, 16000 and 20000 mg/kg) in order to study the remediation effect of microorganisms on crude oil-contaminated sediments. The degradation rate of crude oil in sediment after the addition of Y3 strain was determined, and the degradation of crude oil content in contaminated sediment by Y3 strain was analyzed. The results showed that the growth of Y3 strain was affected by crude oil pollution, and the exogenous bacteria had an adaptive positive process to the sediment environment. After the microorganisms adjusted themselves, they were able to adapt to the new environment. The Y3 strain had an effect on the degradation of sediment crude oil. With the increase of sediment crude oil concentration, the degradation rate constant gradually increased. When the crude oil concentration was 12000 mg/kg, the rate constant of the experimental group was the highest (10.584). Studies have shown that Y3 strain has the remedial function for crude oil-contaminated sediments.

1 INTRODUCTION

In recent years, a large amount of petroleum-contaminated sediment has been produced due to leakage, spillage, submergence and other causes in the development and production activities such as oil exploration, transportation, smelting and in the treatment process of oilfield wastewater. These oil pollutants spread in the environment through various means such as volatilization, infiltration into groundwater, and plant absorption, thereby causing serious soil environmental pollution problems (Wang et al., 2017; Zhang et al., 2018; Daniel and Philip, 2014). The microbial remediation of crude oil-contaminated sediment mainly relies on microbial metabolism to remove pollutants. It has the advantages of low cost, convenient operation and no secondary pollution, which has been rapidly developed in recent years (Xu, 2016; Crisafi et al., 2016). The study by Yanfei et al. showed that arbuscular mycorrhizal fungi (AMF) can stimulate soil microbial activity, improve soil structure and play an active role in petroleum-contaminated soil remediation (Lu and Lu, 2015). The study by Mehdi et al. showed that the paraquat bacteria in Boko Island had a certain effect on petroleum degradation, which can reach 95% (Mehdi et al., 2014).

In this paper, *Pseudomonas putida* Y3 strain (Wang et al., 2012) isolated from Panjin beach in Liaoning province was used as the test object. The Y3 strain in crude oil-contaminated sediment and the degradation efficiency of sediment contaminated by different concentration of crude oil were measured to evaluate the remediation of oil contaminated sediment by Y3 strain.

2 MATERIALS AND METHODS

2.1 Sediment

The sediment was collected from the coastal beach of Panjin, Liaoning Province, dried in a drying oven at 105°C for 1 hour, crushed, sieved and weighed in 200 g per portion, and placed in a pot for use.

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2.2 Bacterial Strain Used in This Study

The *Pseudomonas putida* Y3 strain was a laboratory -preserved strain isolated from Panjin beach of Liao ning province and cultured in inorganic salt medium with diesel as the sole carbon source (Wang et al., 2 012).

2.3 Experimental Setup

The crude oil was quantitatively stirred in each sedi ment pot, crushed and sieved so that the crude oil co ncentration was 0 (CK), 4000, 8000, 12000, 16000 a nd 20000 mg/kg. To make the crude oil mix well in t he sediment, 20 g of crude oil was mixed into 980 g sediment to prepare an initial sediment sample with a crude oil concentration of 20000 mg/kg. Then a tot al of 200 g of sediment was filled in each pot, ie the soil ratio was as shown in Table 1. The sediment we re poured into pot and mixed evenly. The devices w ere equilibrated for one month and three concentrati ons were set for each concentration. In each concent ration experiment group, 20 mL of *Pseudomonas pu tida* Y3 strain with a concentration of 1.0×10^5 cells/ mL were added and distributed homogeneously. The same concentration group without adding bacteria was used as a control sample. After adding quantitati ve Y3 strain to the sediments of crude oil concentrati on gradient as described above, the contents of Y3 st rain in sediments were counted using a plate with di esel as the sole carbon source, and the crude oil cont ent in sediments were determined by Ultraviolet spe ctrophotometry (Wang and Hu, 2010) at 30 d, 60 d, 90 d, 120 d, 150 d and 180 d. The rate of oil degrada tion was calculated. Three parallel experiments were set up for each experimental group.

Table 1: The ratio of experiment sediment.

Crude oil concentration (mg/kg)	0	4000	8000	12000	16000	20000
Initial sediment sample(g)	0	40	80	120	160	200
Pure sediment(g)	200	160	120	80	40	0

2.4 Measurement Methods

The *Pseudomonas putida* Y3 strain was counted usi ng a plate with diesel as the sole carbon source; The crude oil content in sediment were determined by Ul traviolet spectrophotometry (GB17378.5-2007) (Wa ng and Hu, 2010) .

2.5 Calculation Method

Number of *Pseudomonas putida* Y3 strains: The ave rage number of colonies on the three plates at the sa me dilution was calculated according to the followin g formula.

Colony forming units (cfu) per milliliter = avera ge number of colonies with three replicates of the sa me dilution × dilution factor × 5

The rate of oil degradation was calculated to:

$\% = (C_0 - C_i) / C_0 \times 100\%$

C_0-------initial petroleum concentration (mg/kg);

C_i-------residual diesel concentration (mg/kg)

Table 2: The quantity of *Pseudomonas putida* Y3 strain in different concentrations of oil polluted sediment (cfu/g).

Oil content(mg/kg)		30d	60d	90d	120d	150d	180d
0	CK($\times10^2$)	6.50^{Aa}	3.50^{Ba}	3.00^{Ca}	4.90^{Da}	3.90^{Ea}	2.00^{Fa}
	+($\times10^6$)	21.0^{Ab}	7.00^{Bb}	1.70^{Cb}	3.00^{Db}	1.70^{Cb}	2.60^{Eb}
4000	CK($\times10^2$)	9.00^{Aa}	2.75^{Ba}	3.00^{Ca}	4.10^{Da}	3.10^{Ea}	2.60^{Fa}
	+($\times10^6$)	19.0^{Ab}	3.75^{Bb}	2.40^{Cb}	2.40^{Cb}	2.50^{Db}	2.50^{Db}
8000	CK($\times10^2$)	8.00^{Aa}	2.50^{Ba}	3.10^{Ca}	3.50^{Da}	2.50^{Ba}	2.50^{Ba}
	+($\times10^6$)	19.5^{Ab}	4.00^{Bb}	1.50^{Cb}	1.70^{Db}	2.30^{Eb}	2.50^{Fa}
12000	CK($\times10^2$)	5.50^{Aa}	2.25^{Ba}	2.10^{Ca}	3.30^{Da}	2.30^{Ea}	1.90^{Fa}
	+($\times10^6$)	17.0^{Ab}	5.00^{Bb}	2.40^{Cb}	1.40^{Db}	1.10^{Eb}	2.20^{Fb}
16000	CK($\times10^2$)	10.0^{Aa}	2.50^{Ba}	1.70^{Ca}	2.70^{Da}	1.70^{Ca}	2.40^{Ea}
	+($\times10^6$)	13.5^{Ab}	5.75^{Bb}	2.50^{Cb}	1.60^{Db}	1.50^{Eb}	2.40^{Fa}
20000	CK($\times10^2$)	12.0^{Aa}	1.75^{Ba}	1.40^{Ca}	2.40^{Da}	2.00^{Ea}	1.90^{Fa}
	+($\times10^6$)	12.0^{Ab}	4.25^{Bb}	1.12^{Cb}	1.30^{Db}	1.20^{Ab}	2.30^{Eb}

CK : no bacteria, control sample; + : add bacteria, experimental sample.

the different capital (column)/lowercase (line) letters indicated significant differences between groups ($p < 0.05$); the same capital (column)/lowercase (line) indicated no significant differences between groups ($p > 0.05$).

3 RESULTS

3.1 Influence of Crude Oil Pollution on the Number of *Pseudomonas Putida* Y3 Strain in the Sediment

From Table 2, it can be seen that the cell number of Y3 strain in the experimental group was higher than that of the control group during the whole experiment, and the number was basically stable. This showed that the exogenous bacteria have been stabilized and balanced in the sediment. This may be due to the fact that the exogenous bacteria have a certain ability to adapt to changes in the environment, or due to the time of September, so the conditions of various aspects of sediment are suitable for bacterial growth. At the end of 180 d, the cell number of Y3 strain in experimental group at the crude oil concentration of 0 mg/kg, 4000 mg/kg and 8000 mg/kg reached the highest value.

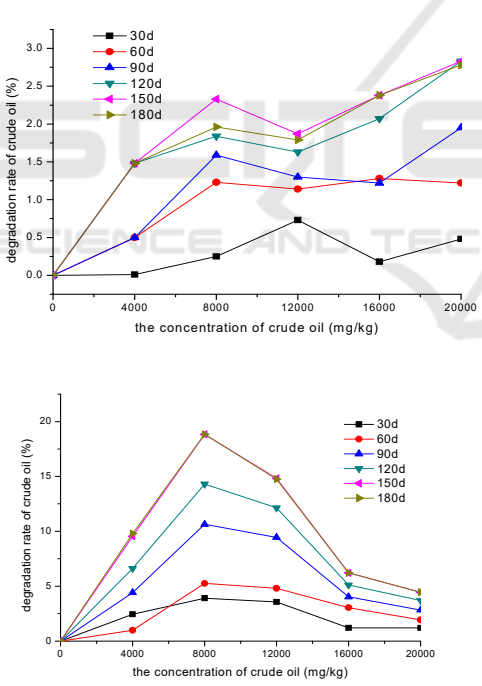

Figure 1: The degradation rate of crude oil changes with the concentration in the polluted sediment (Left: control group; right: experimental group).

3.2 Degradation of Crude Oil Contaminated by *Pseudomonas Putida* Y3 Strain

3.2.1 Effect of *Pseudomonas Putida* Y3 Strain on the Degradation Rate of Oil in Sediment

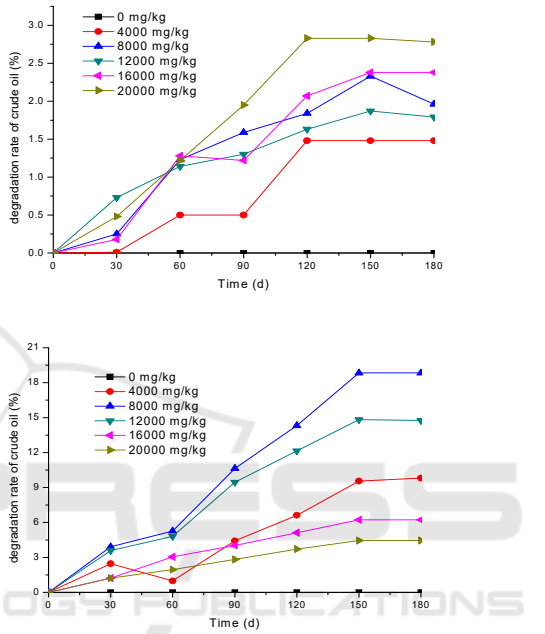

Figure 2: The degradation rate of crude oil changes with the time in the polluted sediment (Left: control group; right: experimental group).

Figure 1 and 2 showed the degradation rate of crude oil in sediment with concentration and time, respectively, compared with the control group, from the concentration point of view, with the increase of crude oil concentration, the degradation rate of crude oil increased first and then decreased. At 150 d, the concentration of crude oil in sediment increased from 0 mg/kg to 8000 mg/kg, and the crude oil degradation rate increased to 17% - 20%. As the concentration continued to increase, the degradation rate of crude oil decreased. When the crude oil concentration was 12000 mg/kg, the degradation rate of crude oil slightly decreased. At a concentration of 16000 mg/kg, the crude oil degradation rate rapidly decreased to about 6%. This showed that when the crude oil in sediment concentration was 12000 - 6000 mg/kg, it had the greatest impact on the Y3 Strain. When the concentration is 20000 mg/kg, the degradation rate of crude oil

was reduced to about 4%. It showed that the contamination of high-concentration crude oil in sediment has a great impact on Y3 strain, resulting in damage to the crude oil pollution resistance and elimination function. From a time perspective, the degradation rate of crude oil in each concentration group increased with time, and the speed was even. The degradation rate of the 8000 mg/kg group was always the highest, indicating that the Y3 strain had the strongest adaptability to this concentration of crude oil and the degradation was most effective. The crude oil degradation rate of the experimental group was greater than that of the control group.

3.2.2 Effect of *Pseudomonas Putida* Y3 Strain on Crude Oil Content in Sediment

Figure 3 showed the effect of Pseudomonas putida Y3 Strain on crude oil concentration in sediment. As can be seen from the figure, during the experiment, the crude oil content in sediment of each experimental group gradually decreased. The degradation rate, reaction order, reaction rate constant and kinetic equation of crude oil in sediment were listed in Table 3. The greater the reaction rate constant, the higher the reaction rate, indicating that the Y3 strain has greater influence on the degradation of crude oil in sediment. From Table 3, the reaction rate of the experimental group at the concentration of 12000 mg/kg was the highest, which was 10.584, indicating that the reaction rate was the fastest.

4 DISCUSSION

4.1 Effect of Crude Oil Concentration on the Number of *Pseudomonas Putida* Y3 Strain in Sediment

There are a large number of microorganisms that depend on organic substances for their existence in sediment, such as bacteria and fungi, which have the ability to oxidize and decompose organic matters. After being contaminated by oil, some microorganisms produce enzyme systems that decompose pollutants under the induction of pollutants, which can degrade pollutants and convert them. Microbial remediation refers to the use of microorganisms to degrade toxic and harmful crude oil contaminants present in the sediment into carbon dioxide and water or to convert them into non-hazardous substances. It is an extension of traditional biological treatment method (Kong, 2017; Li and Li, 2017; Ren and Huang, 2001).

When cultivating petroleum hydrocarbon degrading bacteria, it is generally believed that the richer the energy, the greater the number of bacteria. However, in this study, the number of *Pseudomonas putida Y3 Strain* was the highest in the 4000 mg/kg experimental group. After analysis, we mainly considered two factors: on the one hand, dissolved oxygen. The greater the amount of oil, the more difficult it was for oxygen to enter, thereby affecting the oxygen supply to the Y3 strain and inhibiting the growth of the Y3 strain. On the other hand, there was an imbalance in the nutritional ratio. The large amount of oil in the sediment resulted in a disproportionate ratio of N and P in the soil, thereby inhibiting the growth of the Y3 strain. Qingxin et al. (Liu and Yi, 2006) also pointed out in the research on the growth factors of petroleum hydrocarbon decomposing bacteria that the amount of degrading bacteria in the experimental group with the oil refueling amount of 1% was higher than that in the experimental group with the oil concentration of 2%, verifying that the amount of oil-degrading bacteria did not necessarily increase with the increase of oil concentration.

Table 3: Degradation rate constant and kinetic equation of different concentration of crude oil.

concentration(mg/kg)	reaction progression	dynamics equation	reaction velocity constant *K* /mg/kg·d
4000	0	y =-2.3425x + 4017.5 R^2 = 0.9196	2.3425
8000	0	y = -9.083x + 7997.1 R^2 =0.9727	9.083
12000	0	y = -10.584x + 11931 R^2 = 0.9637	10.584
16000	0	y = -5.8664x + 15936 R^2 =0.9613	5.8664
20000	0	y =-5.1315x + 19930 R^2 =0.9662	5.1315

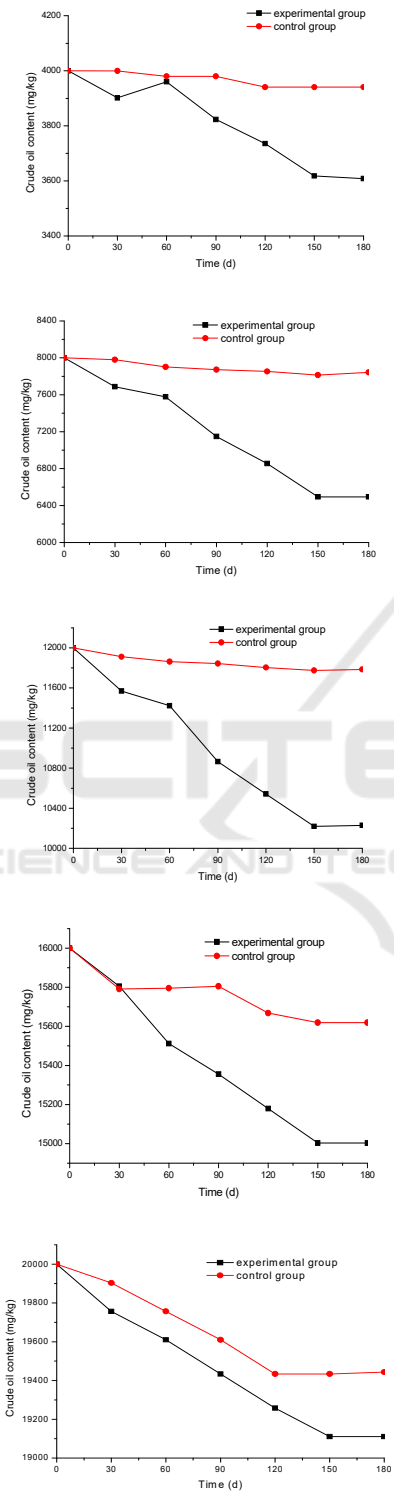

Figure 3: The effect of crude oil content in sediment on the Y3 strain.

4.2 Degradation of *Pseudomonas Putida* Y3 Strain on Different Concentrations of Contaminated Crude Oil

The physiological processes of microbial metabolism of crude oil pollutants are generally accomplished through intracellular metabolism of absorbents that contact and adsorb crude oil, secrete extracellular enzymes and generate crude oil contaminants (Ren and Huang, 2001). The key to the degradation of crude oil is the oxidation of crude oil by oxidase (Liu et al., 2009). Fungi and bacteria complete the oxidative metabolism of crude oil contaminants through the action of extracellular enzymes and intracellular enzymes. The microbial metabolic pathways of crude oil hydrocarbon compounds are crucial for elucidating the mechanism by which microorganisms degrade organic pollutants. Studies have shown that the key step in the degradation of crude hydrocarbons by bacteria and fungi is the oxidation of substrates by oxidases. In addition, microbial uptake of crude oil hydrocarbons and transport process is also an important part of the study of microbial degradation mechanism. Gas chromatography analysis shows that Pseudomonas bacteria selectively transports crude oil hydrocarbons from the extracellular to the intracellular and has a certain selectivity in composition (Liu et al., 2008). In the natural environment, the process of microorganisms degrading hydrocarbon organic pollutants in crude oil is very complicated, involving the interactions between microorganisms, substrates, and microorganisms and substrates.

From the comparison of the results of the experiments with and without the addition of bacteria, the degradation rate of crude oil is sorted by size: experimental group with bacteria > control group. Although bacterial contamination in sediment may have some influence on the experiment, *Pseudomonas putida Y3 Strain* still played a leading role in the degradation of crude oil. The activity of Y3 strain improved the physicochemical properties of sediment, thereby promoting the degradation of crude oil in sediment, which is similar to the study of Qiang et al. (Ma, 2008). The petroleum degradation rate was inversely proportional to the sediment crude oil concentration, which was consistent with the reports of Routani et al. (Routani, 1985). In other words, high oil concentration would inhibit the growth of oil-degrading bacteria, and even lead to a large number of deaths, resulting in a significant drop in oil removal rate.

5 CONCLUSIONS

The growth of *Pseudomonas putida Y3 Strain* was affected by crude oil pollution. With the increase of crude oil concentration, the number of Y3 strain gradually decreased. Exogenous bacteria can adapt to the sediment environment. When the sediment environment changed, the number of exogenous bacteria would be affected. However, after the adjustment of the microbes themselves, they were generally able to adapt to the new environment.

The Y3 strain had an effect on the crude oil degradation of sediments. With the increase of crude oil concentration, the reaction rate constant increased gradually. When the concentration was 12000 mg/kg, the rate constant of the experimental group was the highest (10.584).

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