Effects of Y3 Strain (*Pseudomonas Putida*) Physicochemical Properties of Sediment Polluted by Crude Oil

Jie He[1,2,*], Huan Liu[1,2], Xiaoru Fan[1,2], Yuan Liu[1,2] and Haifeng Wei[1,2]
[1] *College of Marine Technology and Environment, Dalian Ocean University, Dalian 116023, PR China;*
[2] *Key Laboratory of Nearshore Marine Environmental Science and Technology in Liaoning Province, Dalian Ocean University, Dalian 116023, PR China.*
Email: hejie@dlou.edu.cn

Keywords: Microorganisms, crude oil, physicochemical properties of soil

Abstract: There are a lot of microorganisms in crude oil polluted beaches, which have a great influence on the physicochemical properties of sediment. In this paper, Y3 (*Pseudomonas putida*) as the experimental object, the sediment contamination test with different crude oil concentrations (0, 4000, 8000, 12000, 16000, 20000 mg/kg), the sediment pH, total nitrogen, total phosphorus, total organic carbon (TOC) and sediment dehydrogenase activity were studied to investigate the effect of the microorganism on the physicochemical properties of the sediment before and after the addition of Y3 (*Pseudomonas putida*). The results showed that Y3 (*Pseudomonas putida*) had little effect on sediment pH; sediment TOC increased and dehydrogenase activity increased significantly; total nitrogen content did not change significantly, and total phosphorus content fluctuated with crude oil concentration. These showed that Y3 (*Pseudomonas putida*) have a certain influence on the physicochemical properties of sediment polluted by crude oil.

1 INTRODUCTION

According to statistics, the area of organic pollution in China is approximately 0.2 billion hm2, of which oil pollution accounts for a large proportion (Hui and Wang, 2018). In recent years, sediment crude oil pollution increased seriously. Frequent accidents such as oil spill on the sea have spread to coastal waters and beaches under the influence of waves, tides, and currents, causing certain damage to the ecosystem and the surrounding residents (Cheng et al., 2016). Physical and chemical methods are effective in removing oil. However, the cost is too high and most chemical methods generate secondary pollutants. Bioremediation in biological method refers to the use of indigenous microbial or exogenous microbial metabolic activities to improve the sediment physicochemical properties, so that microorganisms can accelerate the decomposition of oil hydrocarbons and other pollutants, thereby achieving the effect of repairing crude oil contaminated sediment (Finlay, 2008; Zheng et al., 2013; Roy et al., 2011; Ge et al., 2012), the advantages of low cost, convenient operation, no secondary pollution caused rapidly developed in recent years, and is also the most important and core component of bioremediation (Ren et al., 2004). The study of Qin Hua et al also pointed out that microbial reduction of oil also improves soil properties (Qin et al., 2005), especially in terms of increasing dehydrogenase activity.

In this paper, the sediment pH, sediment dehydrogenase activity, total organic carbon (TOC), total nitrogen and total phosphorus before and after the addition of Y3 (*Pseudomonas putida*) in the sediment contaminated by crude oil were tested to study the effect of Y3 (*Pseudomonas putida*) on sediment physicochemical properties. And provide reference for microorganism remediation of crude oil contaminated sediment.

He, J., Liu, H., Fan, X., Liu, Y. and Wei, H.
Effects of Y3 Strain (Pseudomonas Putida) Physicochemical Properties of Sediment Polluted by Crude Oil.
In *Proceedings of the International Workshop on Environment and Geoscience (IWEG 2018)*, pages 32-37
ISBN: 978-989-758-342-1

2 MATERIALS AND METHODS

2.1 Materials

The Y3 strain *(Pseudomonas putida)* was a laboratory-preserved strain isolated from Panjin beach of Liaoning province (Wang et al., 2012). The optimum growth temperature is 30°C, the optimum growth salinity is 10, and the optimum pH is 8.

Sediment: The sediment was collected from the coastal beach of Panjin, Liaoning Province

2.2 Methods

2.2.1 Sediment Treatment.

Dried in a drying box at 105°C, then crushed and sieved. Liquid crude oil was mixed into the sediment of each basin, so that the concentration of crude oil was 0, 4000, 8000, 12000, 16000, 20000mg/kg. Three parallel experimental groups were set for each concentration.

2.2.2 The Addition of Y3 (*Pseudomonas Putida*)

In each concentration of experimental group, 20 mL of selected Y3 (*Pseudomonas putida*) solution with a concentration of 1.0×10^{5}/mL was prepared, 2.0×10^{6} /pots (2.0×10^{6} bacteria per 200g of sediment, there were 10^{4} Y3 (*Pseudomonas putida*) in the soil). The fluidity of the bacteria liquid was used as much as possible to make it evenly distributed. The groups with the same concentration without Y3 (*Pseudomonas putida*) were used as control samples.

2.2.3 Test Methods

Determination of total bacteria in sediment by plate colony counting. Ultraviolet Spectrophotometry (GB17378.5—2007) Determination of crude oil content in sediment. Determination of pH by pH meter. Determination of Sediment Dehydrogenase Activity (DHA) with Triphenyltetrazolium Chloride (TTC) Colorimetry (TTC-UV Spectrophotometry). Total Organic Carbon Analyzer for Determination of Total Organic Carbon.

Kjeldahl titration (GB17378.5-2007) Determination of total N. Spectrophotometry (GB17378.5-2007) Determination of total P.

3 RESULTS AND ANALYSIS

3.1 Effect of Y3 Strain (Pseudomonas Putida) on pH in Sediment

As shown in Table 1, the pH was maintained between 6.68 and 7.89 during the entire experiment, which was basically neutral. The addition of Y3 (*Pseudomonas putida*) made the pH more neutral, the pH was between 6.68 and 7.85. However, it does not fluctuate. This shows that microorganisms have little effect on the pH of crude oil contaminated sediment.

3.2 Effects of Y3 Strain (*Pseudomonas putida*) on Dehydrogenase Content in Sediment

As shown in Figure 1, in this experiment, when sediment crude oil concentration was 0 mg/kg, sediment dehydrogenase was lower in the experimental group than in the control group， 4.0557 and 2.8865 respectively. When sediment crude oil concentration was 4000 mg/kg, the content of dehydrogenase in control group was 1.3397 and experimental group was 1.824. While 8000 mg/kg, the content were 2.8363 and 4.2254 respectively. Sediment dehydrogenase was higher in the experimental group than in the control group, indicating that the crude oil in the sediment can effectively promote the production of enzymes by microorganisms and reduce the damage to the environment. The concentration of sediment dehydrogenase in the experimental group with crude oil concentration higher than 12000mg/kg was basically the same as that in the control group, indicating that the promotion of sediment crude oil pollution on sediment microorganisms is limited, and if the concentration is too high, the stimulation effect will lost.

Table 1: The effect of pH on the Y3 (*Pseudomonas putida*) in the oil polluted sediment.

Crude oil concentration (mg/kg)	0		4000		8000		12000		16000		20000	
	CK[a]	+[b]	CK[a]	+[b]	CK[a]	+[b]	CK[a]	+[b]	CK[a]	+[b]	CK[a]	+[b]
30d	6.68	7.00	7.17	7.25	7.56	7.52	7.26	7.22	7.38	7.38	7.39	7.37
60d	7.08	6.93	7.51	7.04	7.44	6.95	7.27	6.99	7.42	7.12	7.39	7.19
90d	6.86	7.17	2.65	2.14	7.56	7.52	7.26	7.23	7.38	7.38	7.39	7.37
120d	7.11	6.93	1.69	1.57	7.74	6.79	7.27	6.99	7.42	7.12	7.39	7.19
150d	7.64	7.06	1.79	1.97	7.46	6.93	7.51	7.04	7.44	6.95	7.38	7.48
180d	6.68	7.00	7.17	7.25	7.56	7.52	7.26	7.23	7.38	7.38	7.39	7.37

[a] No bacteria, control sample
[b] Add bacteria, experimental sample

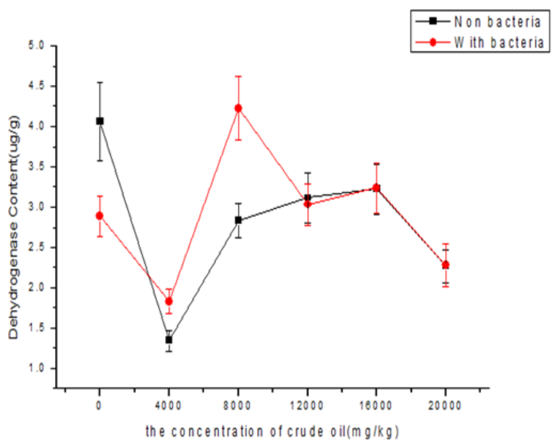

Figure 1: The effect of dehydrogenase content on theY3 (*Pseudomonas putida*) in the oil polluted sediment.

3.3 Effect of Y3 Strain (*Pseudomonas Putida*) on Total Organic Carbon(TOC) in Sediment

As can be seen from Figures 2, 3, as the concentration of crude oil increased, the sediment TOC content gradually increased, and the overall trend is consistent. As time goes on, the TOC in sediment first increased and then decreased. The sediment TOC increased to its maximum at 60 d, remained for 60 days. And sediment TOC was still high at 120 d, and then decreased similarly to the 30d. At 180 d, it slightly increased.

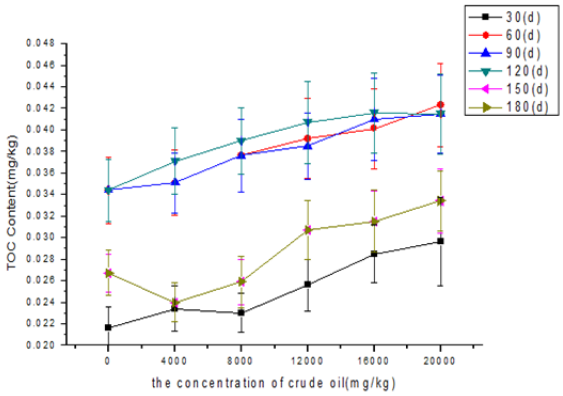

Figure 2: The effect of total organic carbon content on the Y3 (*Pseudomonas putida*) in the oil polluted sediment (Change over concentration).

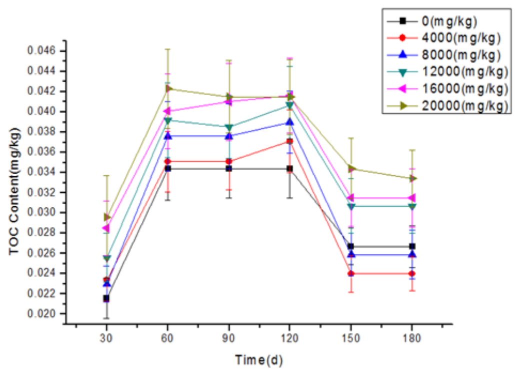

Figure 3: The effect of total organic carbon content on the Y3 (*Pseudomonas putida*) in the oil polluted sediment (Change over time).

3.4 Effect of Y3 Strain (*Pseudomonas Putida*) on Total Nitrogen(TN) in Sediment

From Figure 4, the total nitrogen content in the experimental group is similar to that in the control group, which is related to the concentration of crude oil in the sediment. When the concentration was 0mg/kg, the contents in the experimental group and the control group were 2.1504 and 2.4192. While 4000 mg/kg, the contents were 1.8816 and2.4192 , the sediment total nitrogen in the experimental group was lower than that in the control group. When the concentration was 8000 mg/kg, the experimental group and the control group were equal, all 2.1504. The contents were 2.4192 and 2.1504 in the concentration of 12000mg/kg and 20000mg/kg, experimental group total nitrogen was higher than that in the control group, while the crude oil concentration of 16000mg/kg, the experimental group was lower than the control group, the contents were 2.1502 and 2.4192. It shows that microorganisms have an effect on sediment total nitrogen content, but the effect is not significant.

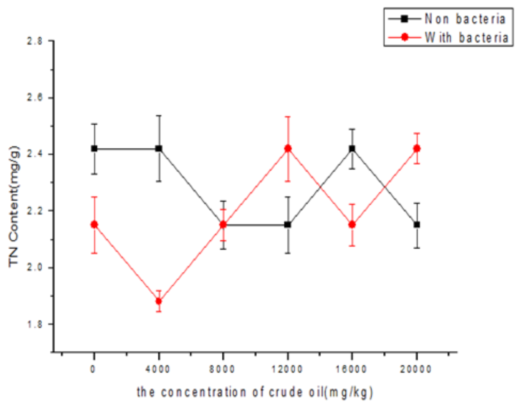

Figure 4: The effect of total nitrogen content on the Y3 (*Pseudomonas putida*) in the oil polluted sediment.

3.5 Effect of Y3 Strain (*Pseudomonas Putida*) on Total Phosphorus (TP) in Sediment

As shown in Figure 5, the total phosphorus content of the sediment increased first and then decreased as the crude oil concentration goes on, and the maximum was found when the crude oil concentration was 8000 mg/kg. Sediment total phosphorus contents in the experimental group at concentrations of 0mg/kg, 12000mg/kg, and 20000mg/kg were slightly higher than those in the control group, the contents were 1.6358, 1.2172, 2.8916, 2.4848 and 0.3918, 0.2108. Probably because of the metabolism of microorganisms in sediment may produce some soluble phosphate; When crude oil concentrations were 4000mg/kg and 80,000mg/kg, the total phosphorus contents in the experimental group was significantly lower than that in the control group, the contents were 1.9954、3.0036 and 3.1068、4.9993. Probably because of the existence and reproduction of microorganisms may use some of the phosphate in the sediment. The sediment total phosphorus content in the experimental group was 0.6571 in concentration of 16000 mg/kg was slightly lower than that 0.8104 in the control group. As a whole, in the low-concentration oil pollution samples, the microorganisms have an effect on the total phosphorus content in the sediment; when the concentration is high, the effect is not significant.

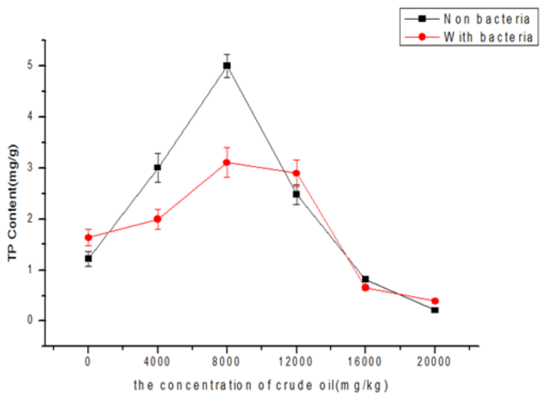

Figure 5: The effect of total phosphorus content on the Y3 (*Pseudomonas putida*) in the oil polluted sediment.

When sediment crude oil concentration is higher than 16000mg/kg, the contents of total phosphorus in sediment decreased significantly, indicating that crude oil pollution has a greater impact on sediment total phosphorus, leading to the loss of sediment phosphorus and impairing the sediment physicochemical properties.

4 RESULTS AND DISCUSSION

Y3 (*Pseudomonas putida*) only affect dehydrogenase and sediment carbon and nitrogen content. There are some studies have confirmed that the biological enrichment of inorganic nitrogen fertilizers by farmland microorganisms reduces the loss of nitrogen fertilizers (Shen et al., 1994). In controlling nutrient supply, sediment microbial biomass not only acts as a biocatalyst for many basic reactions, but also equivalent to the fast turnover library of sediment N and P elements (Burger and Jackson, 2003). It can be seen that the microbial activity and sediment physicochemical properties mutually restrict and promote each other: On the one hand, the metabolism of microorganisms improves the physicochemical properties of the sediment; on the other hand, the increase of the available organic carbon and nitrogen content of sediment greatly stimulates the activity of sediment microorganisms, making better promote the sediment physicochemical properties.

The suitable pH is favorable to the existence and distribution of sediment salts. From the experimental results, it can be known that the sediment pH was basically neutral

Dehydrogenase is one of the main enzymes in sediment, and its activity can be regarded as an important indicator of sediment microbial activity and functional diversity (Qin et al., 2005). Some studies have shown that there are certain differences in dehydrogenase activities in different sediments in different environments, and different agrochemical substances or pollutants may also affect the activity of sediment dehydrogenase (Guo et al., 2000). Chemical substances may also affect the microbial diversity of sediment. At present, the influence of organic compounds such as PAHs, organic pesticides and microbial diversity of crude oil sediment have been extensively studied at home and abroad (Kalayama et al., 2001; Aisllabie et al., 2004). The results showed that dehydrogenase reacts to intracellular enzymes in the sediment, microorganisms have an effect on the content of sediment dehydrogenase. The addition of Y3 (*Pseudomonas putida*) can significantly increase sediment dehydrogenase activity and accelerate sediment self-repair.

Sediment organic carbon content is one of the important indicators to measure sediment fertility. The carbon source is an essential nutrient for the growth of microorganisms. Organic carbon can be easily absorbed by microorganisms and utilized. The content of organic carbon in high-contamination samples is slightly higher, some of the crude oil contamination in sediment may be detected.It is also possible that as pollution concentration goes on, the Y3 (*Pseudomonas putida*) is seriously affected, the utilization of organic carbon in the sediment is getting lower and lower, so the content of the retained organic carbon is slightly higher. The organic carbon content in sediment with time first increased and then decreased may be due to the individual components of the crude oil in the sediment can be used as nutrients for the microorganisms, so the organic carbon content in the sediment first increased.

The total sediment nitrogen content is the sum of various forms of nitrogen in the sediment, including organic nitrogen and inorganic nitrogen, but does not include molecular nitrogen in the air. Sediment total nitrogen content is related to the amounts of microorganisms and dehydrogenase in the sediment environment. Dongyun M et al showed that the activity of soil dehydrogenase was significantly positively correlated with the nitrogen application. A timely and appropriate application of nitrogen could promote the development of the root systems, thereby increasing the amount of soybean roots, increasing the secretion of roots.The powerful root systems promotes the reproduction of sediment microorganisms (Ma et al., 2007).

From the comparison of the N and P assay results of each experimental group with the control group. The content of N changed little, while the content of P fluctuated with the concentration of crude oil, the concentration of P and Y3 (*Pseudomonas putida*) in 8000mg/kg and 12000mg/kg experimental group were higher. It shows that N and P are the main nutrient elements and are related to the amounts of bacteria. Congsheng Z et al pointed out that studying the distribution characteristics of main nutrient elements in soil is the important foundation for the study of Geochemistry in wetland ecosystem,which is helpful to the study of the plant rhizosphere ecosystem (Zeng et al., 2009). Hanfeng X et al have confirmed that there is a significant correlation between organic carbon and N content in soil layers, indicating that organic carbon and N have similar spatial distribution rules; soil organic carbon and P are related because P has small mobility and is mainly influenced by organic matter and parent material are similar to that of organic matter (Xiong et al., 2005). It shows that the physicochemical indicators do not exist independently. In the entire sediment ecosystem, the indicators are interdependent and mutually promote or restrict and maintain the sediment ecosystem. The results show that Y3 (*Pseudomonas putida*) has an impact on the TOC, total N, and total P contents of the sediment. On the one hand, microorganisms use carbon and nitrogen sources in the sediment for their survival. On the other hand, microbial metabolism produces easily decomposable substances that increase the TOC, total N, and total P which can be measured in sediment.

ACKNOWLEDGEMENTS

We acknowledge the Natural Science Foundation of Liaoning (No. 2015020616) the Wetland Degradation and Ecological Restoration Program of Panjin Pink Beach (PHL-XZ-2017013-002).

REFERENCES

Aisllabie J M, Balks M R and Foght J M 2004 Hydrocarbon spills on Antarche soils：Effects and management *Environmental Science and Technology* **38** 1265

Burger M and Jackson L E 2003 Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems *Soil Biol. Biochem* **35** 29

Cheng C C, Li Q Q and Wang J 2016 Advanced oxidation technology for remediation of petroleum-contaminated tidal flat *Journal of Environmental Engineering* **10** 2700

Finlay R 2008 Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium *Comparative Bio-chemistry & Physiology Part A Molecular & Integrative Physiology* **59** 220

Ge G F, Gao H J and Zheng B 2012 Current research situation and prospect of microorganism response to polycyclic aromatic hydrocarbons contaminated soil *Journal of Anhui Agricultural University* **39** 973

Guo M,Chen H J and Wang C L 2000 Effect on soil dehydrogenase activity of four pesitcides *Environmental Chemistry* **19** 524

Hui Y F and Wang H F 2018 Progress on bioremediation in oil-contaminated soil *Acta Agriculturae Boreali-occidentalis Sinica* 27 1

Kalayama A, Funasaka K and *Fujie* K 2001 Changes in respiratory quinine profile of a soil applied with pesticides Biology and Fertility of Soils. **33** 454

Ma D Y, Guo T C and Song X 2007 Effects of urea application rate on the quantity of microorganisms and activity of enzymes in wheat rhizosphere *Acta Ecologica Sinica* **27** 5222

Qin H, Lin X G and Chen R R 2005 Effects of DEHP on dehydrogenase activity and microbial functional diversity in soil *Acta Pedologica Sinica* **42** 829

Ren H F, Shan D C and Li S Q 2004 Study progress on microbial remedy technology for contaminated soil by petroleum *Journal of Northeast Agricultural University* **35** 373

Roy E, Naresh l and Simon S 2011 Surfactants and bacterial biore-mediation of polycyclic aromatic hydrocarbon contaminated soil unlocking the targets *Critical Reviews in Environ-mental Science & Technology* **41** 78

Shen Q R, Yu L and Liu Z P 1994 Effects of combining application of organic and inorganic nitrogen fertilizers on biomass nitrogen and nitrogen-supplying characteristics of coastal saline soil *Acta Pedologica Sinica* **31** 287

Wang B, Zhou Y F and Zhang X 2012 The growth and petroleum degradation characteristics of bacteria in coastal beach *Journal of Dalian Ocean University* 27 306

Xiong H F, Liao Q Z and Wu Q F 2005 Distribution on soil Nutrients and Correlation Analysis in Lake Liangzi Wetlands, Hubei Province *Journal of Lake Science* **17** 93

Zeng C S, Zhong C Q and Tong C 2009 Contents of Soil N,P and K Under Different Land Uses in Estuary Wetland of Min River *Journal of Soil and Water* Conservation **23** 87

Zheng C J, Shu Z and Ye Z B 2013 Research progress on oil sludge treatment technology *Applied Chemical Industry* **42** 332