

Degradation Performance of Petroleum Hydrocarbon Degrading Bacteria and Bioremediation of Oil Polluted Soil

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Abstract: Starting from strain Z-3, the factors affecting the degradation efficiency of strain Z-3 were explored in order to determine the optimum degradation conditions. The strain was used to simulate the remediation of oily soil in laboratory. The conclusions are as follows: (1) The degradation effect of the strain at higher or lower pH and temperature is not significant. The optimal degradation conditions are determined by experiments: temperature 30°C, pH 7.0, initial salt concentration 9 %, initial crude oil concentration 5mg/mL. (2) The optimal inoculum size of the strain for the simulated repair of oily soil is 106 cfu/g, and the optimum soil water content is 30%.

1. Introduction

Petroleum hydrocarbons are difficult to biodegrade and generally have a long degradation time. In the process of oil exploitation, it will inevitably cause spills and residues, causing pollution of soil and even groundwater. Soil polluted by oil will reduce its aeration and water permeability, which will weaken the absorption of nutrients by plant roots, causing decay and even death [1].

At present, most of the treatment methods for soil pollution focus on adding N, P and other nutrients to the soil to promote the growth of the original microorganisms, and to achieve its strengthening effect, but this method is often very slow. Using microbial remediation technology to treat petroleum hydrocarbon pollution has certain advantages, and has achieved some results. In this chapter, *Bacillus cereus* Z-3, which was successfully screened out before, was used to carry out a series of simulation experiments on the degradation of petroleum hydrocarbon components in soil in the laboratory in order to provide a new way for microbial remediation of oil-polluted soil [2,3].

2. Test material

2.1 Strain

The strain Z-3 is provided by the Microbiology Laboratory of Jilin Agricultural Science and Technology College.

2.2 Experimental soil

The tested soil was taken from oil-contaminated soil (0-20cm) near an oil well in Xinli Oil Production Plant of Jilin Oilfield. The impurities such as roots and stones were removed and screened through 20mm mesh for reserve.

3. Test method

3.1 Study on Degradation Conditions of Petroleum Hydrocarbon Degrading Bacteria

3.1.1 Effect of Crude Oil Concentration on Petroleum Degradation

The strain Z-3 was cultured for 3 days with shaking, and the bacterial suspension was prepared by centrifugation, OD₆₀₀=0.4, and 100 μL was taken to access different crude oil concentrations below 100 mL (1.0 mg/mL, 3.0 mg/mL, 5.0 mg/mL, 7.0 mg/mL). In the inorganic salt liquid medium of 9.0 mg/mL) and pH=7.0, the cells were cultured at 30°C and 180 r/min for 36 days at a

constant temperature. The crude oil degradation of strain Z-3 was explored and the test was carried out three times in parallel.

3.1.2 Effect of initial pH on petroleum degradation

The strain Z-3 was cultured for 3 days with shaking, and the bacterial suspension was prepared by centrifugation, OD₆₀₀=0.4, 100 µL was taken, and the different pH (5.0, 6.0, 7.0, 8.0, 9.0) and the optimal crude oil concentration of the inorganic salt liquid medium were added. Medium, 30°C, 180 r / min constant temperature shaking culture for 36 days. The crude oil degradation of strain Z-3 was explored and the test was carried out three times in parallel.

3.1.3 Effect of salt concentration on petroleum degradation

Strain Z-3 was cultured in oscillatory medium for 3 days. The suspension was prepared by centrifugation. OD₆₀₀=0.4. The suspension was transferred to different salt concentration below 100 mL (1%, 3%, 5%, 7%, 9%). The optimum concentration of crude oil was inorganic salt liquid medium. The suspension was cultured in constant temperature oscillatory medium at 30°C and 180 r/min for 36 days. Degradation of crude oil by strain Z-3 was investigated. The experiments were carried out three times in parallel.

3.1.4 Effect of Temperature on Petroleum Degradation

Strain Z-3 was cultured in a shaking medium for 3 days. The suspension was prepared by centrifugation. OD₆₀₀ = 0.4. 100 µL was removed and added to the inorganic salt liquid medium with the optimum crude oil concentration, pH value and salt concentration under 100 mL for 36 days. Degradation of crude oil by strain Z-3 was investigated. The experiments were carried out three times in parallel.

3.2 Bioremediation of Oily Soil

Preparation of bacterial suspension: The strain Z-3 was inoculated into liquid LB medium and cultured until its logarithmic growth stage. The culture medium was centrifuged and collected, then washed twice with phosphoric acid buffer to control the concentration of bacteria to the required value for reserve.

Soil preparation: each container contains 0.1 kg soil sample, 20 ml water, so that its water content reaches about 20%, sealed with film, and leaving a small amount of stomata, to maintain the water and ventilation in the repair process.

Take 5g of soil sample, dry it at 35°C for 24h, crush it, put it in a test tube with plug, add 5ml petroleum ether (60-90°C), shake for 30s, put it in ultrasonic cleaner for 10min, repeat 1 The above operation. The upper petroleum ether phase was taken out with a pipette; the remaining solid phase was further mixed with 10 mL of petroleum ether, and the extract was combined and centrifuged at 2800 r/min for 10 min. The supernatant was collected and the content of petroleum in the soil was determined by gravimetric method [4, 5].

4. Results and analysis

4.1 Effect of crude oil concentration on petroleum degradation

After 36 days of continuous culture, the degradation rate was measured as shown in FIG. The higher the concentration of crude oil, the lower the degradation rate. The degradation rates were close at 1, 3, and 5 mg/ml. The lower the concentration of crude oil, the easier it is to be utilized by the cells. Therefore, the higher the concentration of crude oil, the less the bacteria can use and the lower the degradation rate.

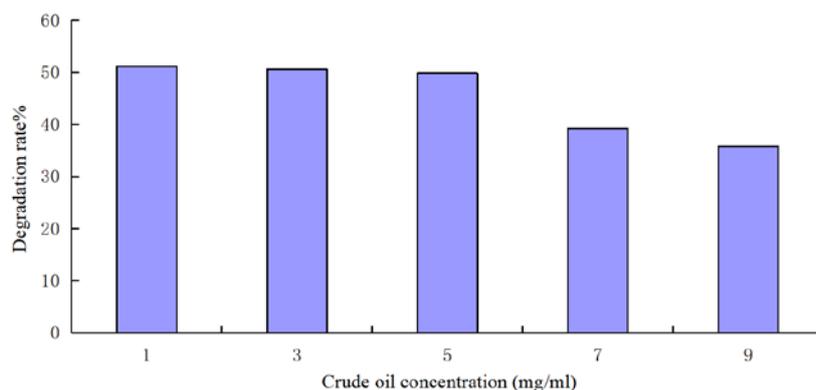


Fig.1. Effect of Different Crude Oil Concentration on Degradation Rate of Crude Oil

4.2 Effect of Initial pH Value on Petroleum Degradation

After 36 days of continuous culture, the degradation rate was determined as shown in Fig. 2. The degradation rate of bacteria Z-3 was 50.1% at pH=7. When pH=8, the degradation rate was 48.5%, which was close to each other. The degradation rate was 33.6% at pH=5, 39.7% at pH=6 and 36.4% at pH=9, which indicated that microbial degradation of crude oil would be slowed down when the pH was too high or too low.

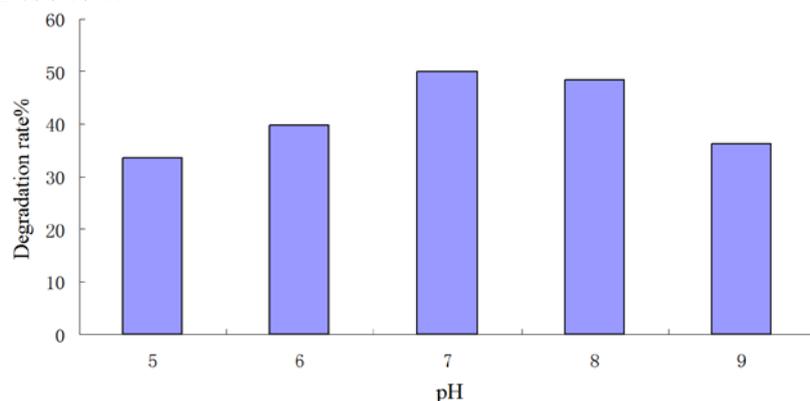


Fig.2. Effect of Different pH on Degradation Rate of Crude Oil

4.3 Effect of Different Salt Concentrations on Petroleum Degradation

After 36 days of continuous culture, the degradation rate was determined as shown in Figure 3. When NaCl concentration was 9%, the crude oil degradation ability of the strain was very strong, and the degradation rate was 61.2%. The degradation rates were 48.4%, 49.9%, 50.6% and 52.8% at 1, 3%, 5% and 7% salt concentration, respectively.

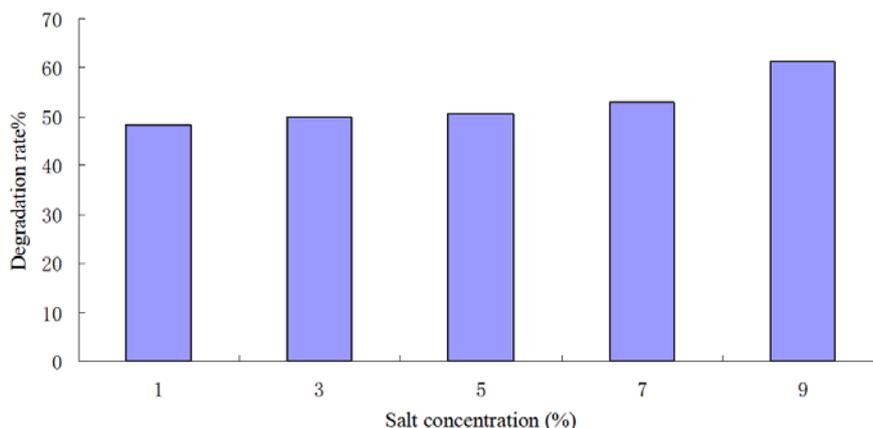


Fig.3. Effect of Different Salt Concentrations on Degradation Rate of Crude Oil

4.4 Effects of Different Temperatures on Microbial Growth and Petroleum Degradation

After 36 days of continuous culture, the degradation rate was measured as shown in FIG. As can be seen from the figure, the degradation rate at 35°C is 53.79%, reaching the highest level. The degradation rate was 33.23% at 15°C, and the degradation rate was 45.94% at 25°C. It can be seen that too low temperature will reduce the degradation rate of crude oil.

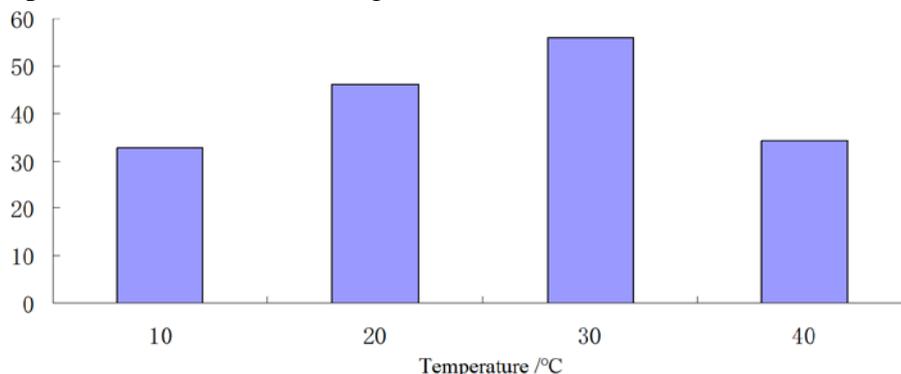


Fig.4. Effect of Different Temperatures on Degradation Rate of Crude Oil

4.5 Effect of original microorganisms on the degradation process of strain Z-3

In order to study the effect of the original microorganisms in the soil on the degradation of petroleum by foreign strains, the following experimental design was carried out. In three cases, each case was repeated three times, specifically the original microbial control group: the soil was not sterilized; the soil sterilization group: the degradation effect of the strain Z-3 was examined. Soil unsterilized group: The interaction between strain Z-3 and the original microorganism was examined. The soil sterilization temperature was 121°C for 2 hours. The inoculation amount was 106 cfu/g, cultured at 25°C, and the water content per day was kept at 25%. Sampling was performed every 6 days and cultured for 36 days. The results are shown in Fig. 5.

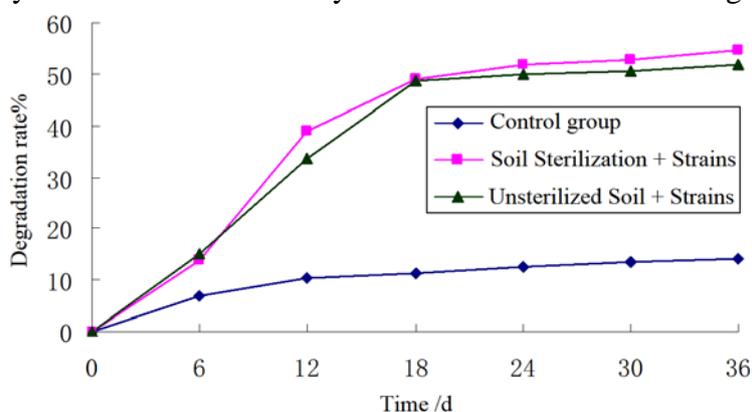


Fig.5. Oil degradation of strain Z-3 in sterilized and unsterilized soil

In the control group, the petroleum degradation process of the original microorganisms was relatively slow, and the oil degradation rate after 36 days was 13.1%. The oil degradation in the soil-sterilized and non-sterilized groups was concentrated in the first 18 days, after which the degradation process was slow. It is likely that the amount of microorganisms is large at the initial stage of inoculation, and the degradation is strong. As the degradation process progresses, the number of microorganisms decreases and the degradation rate decreases. In addition, as the degradation process progresses, the concentration of remaining petroleum hydrocarbons is too low, which also reduces the degradation rate. In the first 18 days, the degradation rate of soil sterilization group was slightly faster than that of non-sterilization group, probably because strain Z-3 was affected by the original soil microorganisms, which made the degradation rate of non-sterilization group decrease slightly. The purpose of this study is to explore the oil degradation of strain Z-3 in the field soil and provide a theoretical basis for its bioremediation. Therefore, the natural soil

without sterilization is used in the subsequent experiments.

4.6 Effect of inoculum size on petroleum degradation

The oily soil was separately connected to a bacterial suspension of 104, 105, 106, 107, and 108 cfu/g, and the culture temperature was 25°C, and each treatment was repeated 3 times. Daily water replenishment makes the soil moisture content 25%, sampled every 6d, the results are shown in Figure 6. It can be seen from the figure that the strain Z-3 can degrade the petroleum component of the soil well, and the larger the inoculum, the better the degradation effect. When the inoculum size is 106-108 cfu/g, the degradation rate of petroleum can reach 60% after 36 days of degradation, which is not much different. Therefore, in the process of in situ remediation of oily soil, the inoculum size can be selected to be 106 cfu/g.

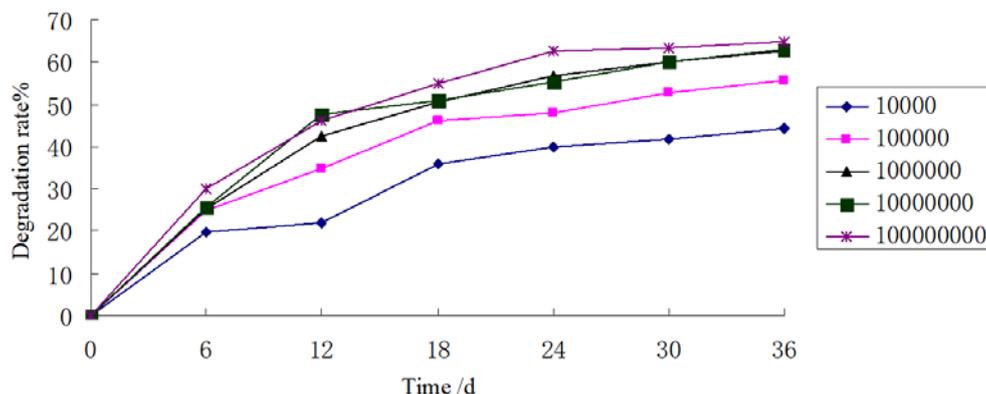


Fig.6. Effect of inoculum size on the degradation process of strain Z-3

4.7 Effect of Soil Water Content on Degradation of Petroleum by Strain Z-3

The water content of the soil was 5%, 10%, 15%, 20%, 25%, 30%, respectively, and degraded for 36 days, and each water content was repeated 3 times. To the test soil, 106 cfu/g of Z-3 was added, and the culture temperature was 35°C, pH = 7, and the sample was measured every 6 days. The results are shown in Figure 7.

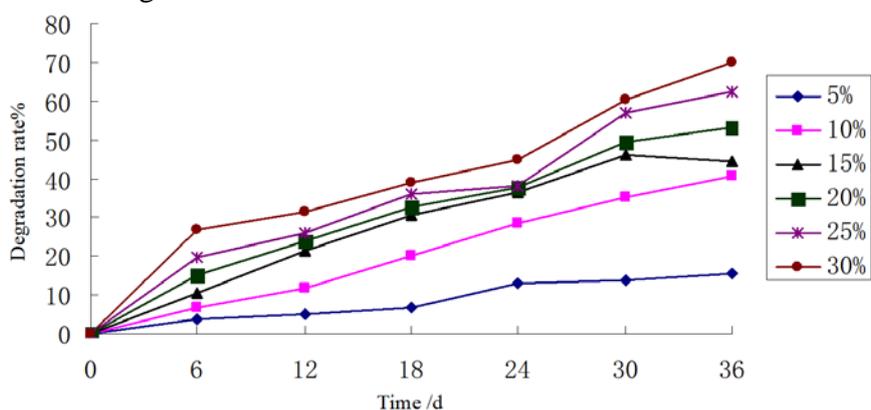


Fig.7. Effect of Soil Water Content on Petroleum Degradation by Strain Z-3

It can be seen from the graph that the degradation rate of petroleum hydrocarbons increases with the increase of soil water content. The water content is very low (5%), the degradation rate of petroleum hydrocarbons in soil is 15.4%; the water content is low (10%, 15%) and the corresponding degradation rates are 28.7% and 40.2%, respectively. When water content is 20%, 25% and 30%, the degradation rate of petroleum hydrocarbons is higher, corresponding degradation rates are 53.4%, 62.3% and 69.8%. This indicates that soil water content is an important factor affecting the degradation of petroleum hydrocarbons by strain Z-3. There may be two reasons: First, the microorganism itself needs a certain amount of water to maintain its own survival, and if the water content of the soil is too low, the activity of the microorganism is inhibited. Second, sufficient water can make the petroleum contaminants in the soil fully contact with water. The number of

microorganisms in the oil-water two-phase interface is more active, which is conducive to the degradation of petroleum pollutants.

5. Conclusion

Starting from strain Z-3, this paper explores the factors affecting its growth and degradation efficiency to determine its optimal growth conditions and degradation conditions. The strain was used to carry out laboratory simulation repair of oily soil. The results are as follows: (1) The degradation effect of the strain is not significant at higher or lower pH and temperature. The optimum degradation conditions are determined by experiments: temperature 30°C, pH 7.0, initial salt concentration 9%, initial crude oil concentration 5 mg/mL. (2) The optimum inoculation amount and soil water content of the strain were 106 cfu/g and 30% respectively.

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