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Study on Microbial Degradation Characteristics of Diesel Contaminated Soil

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Abstract. A novel and highly effective Acinetobacter (L15) were obtained from soil by morphological observation, physiological and biochemical tests and 16 SrDNA sequencing. The optimal reaction environment of L15 at different temperatures was determined by shaking test, and the catalytic activity was measured. It was found that the bacteria could withstand n-cetane value above 5000 mg/L when the initial culture pH was 7, the inoculation amount was 5%, the shaking speed was 150 r/min, and the culture temperature was 30℃. Under the optimal 7-day fermentation condition, the degradation rate of n-cetane compounds reached 94.09%, and the decomposition rate of 3000 mg/L diesel oil was basically complete. The results showed that the bacteria had good potential for bioreactor in diesel contaminated soil.

Keywords: N-Cetane; Dominant Bacteria; Microbial Degradation Characteristics; Diesel Pollution.

1. Introduction

Land is the basis of human survival and development, and it is also the main source of material and energy in nature. Pollution in the air and water will eventually enter the soil. Soil pollution is difficult to repair, only a small amount of unstable pollutants can be removed by natural volatilities or microbial decomposition, most of the pollutants will remain in the symbiosis with the human body, causing a great threat to human health, plant and animal growth and ecological balance. Using over 80% of hydrocarbons as the main component and utilizing degrading microorganisms to decompose residual hydrocarbons in the environment is an important method to solve the problem of crude oil pollution in soil. They mainly focus on the aromatic compounds that have good performance and are not easy to be degraded [1]. At present, there are few reports about alkane bacteria, and there are some deviations in the current research of alkane bacteria, such as short-chain alkane bacteria, pentadecane bacteria and hexafane bacteria, but because of their unstable characteristics, they are easy to be volatile and decomposed. Due to the lack of understanding of the hydrolysis process of mid-long chain alkanes, the degradation performance of the process is insufficient, and there are few systematic and comprehensive studies on the hydrolysis process of mid-long chain alkanes. The purpose of this paper is to study the screening, isolation and identification of high-efficiency long-chain alkane degrading bacteria, and their ability to degrade crude oil and various alkanes [2]. In this project, n-cetane compounds in diesel oil were taken as the main object, and a strain with strong catalytic activity was isolated from the petrochemical storage tank in China by microbial ecology method, in order to lay a foundation for the efficient degradation of n-cetane compounds in the petrochemical storage tank in China.

2. Materials and methods

2.1. Soil sources

Random sampling in the areas most affected by oil pollution. Remove branches, rocks and household garbage from the ground. From the affected soil sample 20 cm below the ground, a sample of 6.4 kg was collected and placed in a clean bag and stored indoors at 4℃. The soil was measured with a crude hydrocarbon concentration of 17.38 g/kg and a salinity of 25.7 g/kg.

2.2. Medium

LB's medium contained 10 grams of peptone, 5 grams of yeast extract, 10 grams of sodium chloride, and 1000 milliliters of deionized water. The inorganic salt medium consisted of 40.5g KH_2PO, 1g $Na_2HPO_4 \cdot 12H2O$, 1.1g NH_4Cl, 0.2g $MgSO_4 \cdot 7H_2O$, 2ml trace element solution, and 1000ml deionized water.

2.3. Strain screening

Ten times dilution method was used, saffranine method was used to stain, and light microscope was used. The main N-alkane-degrading bacteria obtained were cultured in vitro for 7 days with 5% inoculation rate, separated by 2ml and stored in centrifuge. The 16SrDNA sequence was determined [3]. MEGA7.0 analysis method was used to input the sequence data into NCBI official website, and the two subspecies with high similarity were screened out through Blast comparison and analysis, and the evolutionary tree was established through MEGA7.0.

2.4. Research on degradation performance

There are many influences on the biodegradation process of n-cetane. Using n-cetane as only one carbon source and energy, the effects of different temperatures, mass fraction of NaCl, initial pH, initial mass concentration of n-cetane, dissolved oxygen and inoculation amount on the removal effect of n-cetane were studied by single factor test. The initial pH of the medium was 7.0, the inoculum volume was 5%, and the culture was carried out at 30℃. The suspended bacterial solution of the selected dominant bacteria was inserted into the diesel medium, and after 7 days of vibration culture, the GC-MS analysis results of each component before and after the degradation of diesel oil with a mass concentration of 3000 mg/L were compared.

3. Results

3.1. Strain identification

3.1.1 Determination of morphological and physiological indexes of bacteria

Fifteen strains were selected from diesel contaminated soil samples to degrade n-cetane and were numbered L1~L15. Among them, L15 has the best degradability, and the degradation rate of n-cetane with initial mass concentration of 1000 mg/L is as high as 94.25% after 7 days, so it is selected as the dominant strain. The colony morphology and Gram Staining photos were shown in Figure 1 (the picture was cited in Simple Staining Procedure, Principle, Result). The shape of the colony is spherical, the volume is large, the color is opaque milky white; Product appearance smooth, moist, bright; The colony has a slight protrusion and forms a smooth strip around it. Gram staining, glucose, ethanol fermentation, starch hydrolysis, nitrate reduction, denitrification, contactase, methyl red, V-P, and nitrite reduction tests were negative.

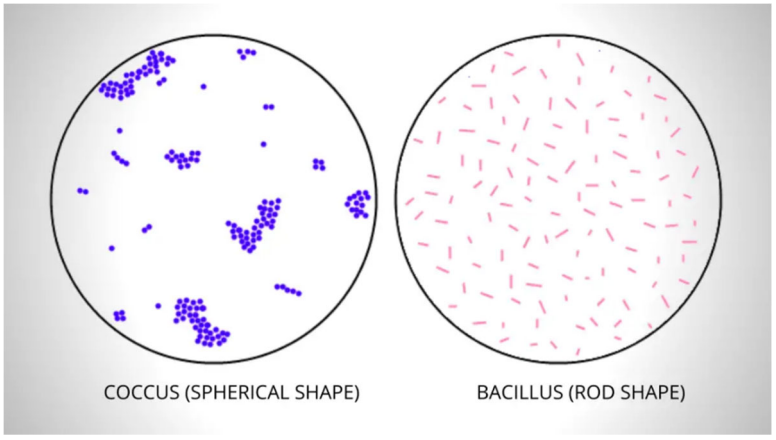

Fig. 1 L15 colony structure and Gram staining observation

3.1.2 16 SrDNA sequence analysis and establishment of molecular evolutionary tree

After 16 SrDNA sequencing of strain L15, a gene fragment with a length of 1439 bp could be obtained, which was input into NCBI, and Blast comparison was carried out. All the sequences with more than 98% homology with strain L15 were acinetobacter, from which some sequences with higher homology were selected for phylogenetic tree analysis. MEGA7.0 software was used to build the phylogenetic tree according to Blast comparison structure. It can be seen from Figure 2 that strain L15 belongs to the same clade as Acinetobacter baumannii strainDSM30007, and the 16 SrDNA homology of these two bacteria is as high as 99.99%, so strain L15 can be preliminarily classified into Acinetobacter.

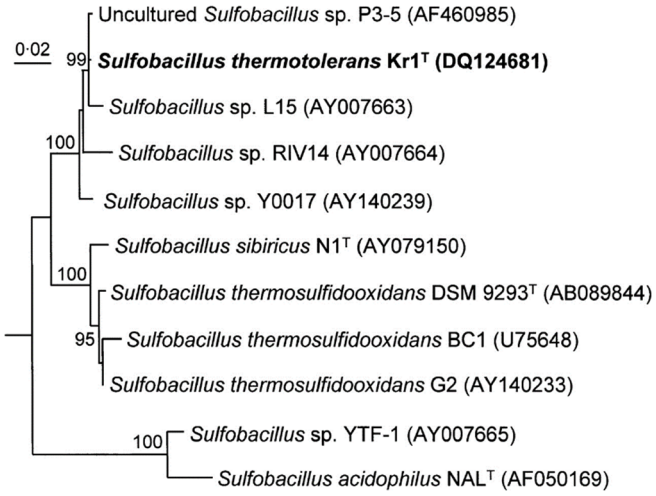

Fig. 2 Phylogenetic tree of strain L15

3.2. Optimization of degradation conditions

3.2.1 Influence of culture temperature

Figure 3 shows the culture temperature growth, reproduction and metabolic activity of bacteria [4]. As can be seen from Figure 3, the degradation rate of n-cetane increased step by step when the temperature increased from 15℃ to 30℃. This is because the microbial reaction and the general chemical reaction law is similar, the reaction rate will increase due to the increase of temperature, and in a certain temperature range, the higher the temperature, the microbial activity will also be strengthened. Under the condition of 30℃, the degradation rate of n-cetane compounds can reach 94.25%, when the bacteria activity is the strongest, the degradation rate of n-cetane compounds is the best. When the temperature continues to rise to 40℃, the degradation ability of n-cetane will decrease significantly, from 94.25% to 51.36%. This is because the process of microbial degradation of n-cetane depends on the catalysis of the relevant enzyme system in the body. If the temperature is too high, the activity of related enzymes will decrease. Objective strain L15 can degrade n-cetane at an optimum temperature of 30℃.

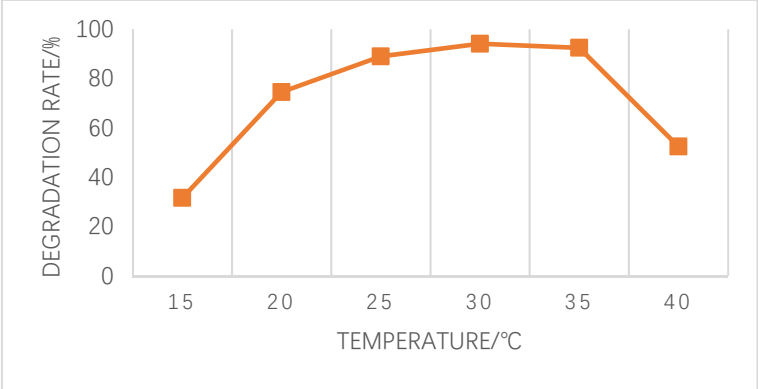

Fig. 3 Effect of temperature on degradation of strain L15

3.2.2 Influence of initial pH of medium

The pH on the degradation of strain L15 is shown in Figure 4. As shown in Figure 4, pH too high or too low is not conducive to bacterial growth and metabolism [5]. The bacterium has a wide pH adaptability and a high n-cetane removal rate under both neutral and weakly alkaline conditions. When the initial pH of the medium increased from 5 to 7, the n-cetane removal rate of the strain also increased correspondingly, and the n-cetane degradation rate of the strain with pH=7 reached the maximum 94.43%. This is because the increase of pH within a certain range can increase the flexibility of the bacterial cytoplasma membrane, making n-cetane easier to be used in the cell. At the same time, the moderate increase of pH can also strengthen the activity of related enzymes, accelerate the decomposition of n-cetane, and then improve the degradation rate of n-cetane by bacteria. When pH value is greater than 7. The results showed that the selected degrading bacteria had the best degradation rate at pH 7.

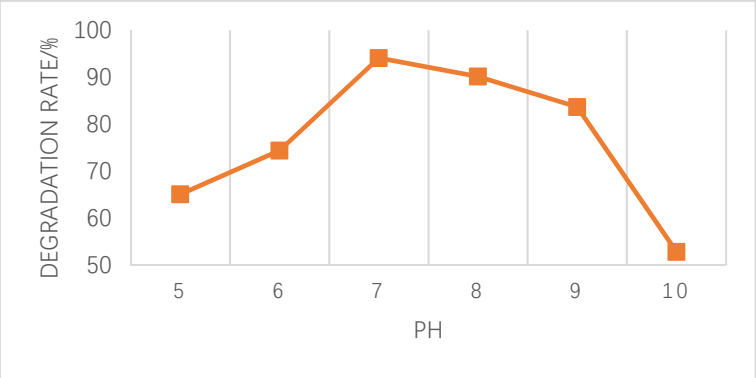

Fig. 4 Effect of initial pH on degradation of strain L15

3.2.3 Initial mass content of n-hexadecane compounds

Figure 5 shows the effect of positive cetane content on the degradation of L15. As can be seen from Figure 5, the selected dominant bacteria have strong degradation ability to substrates with initial n-cetane mass concentration between 1000 and 5000 mg/L, and the degradation rate is the highest when the substrate mass concentration is 1000 mg/L. Under 5 different concentrations of n-cetane, the growth rate of bacteria was slow due to insufficient n-cetane content in the fermentation process, and the degradation rate of bacteria to n-cetane was very small at 500 mg/L. When the content of n-cetane in the fermentation solution exceeds 1000 mg/L, the content of n-cetane in the fermentation process increases due to the abundance of matrix, which enhances the growth and metabolic activity of microorganisms in the fermentation process, and thus increases the degradability of fermentation products [6]. However, the degradation rate of n-cetane was almost unchanged when the mass concentration of n-cetane was continuously increased in the medium. Therefore, the dominant strain could tolerate higher mass concentration of n-cetane, and the mass concentration could reach 5000 mg/L.

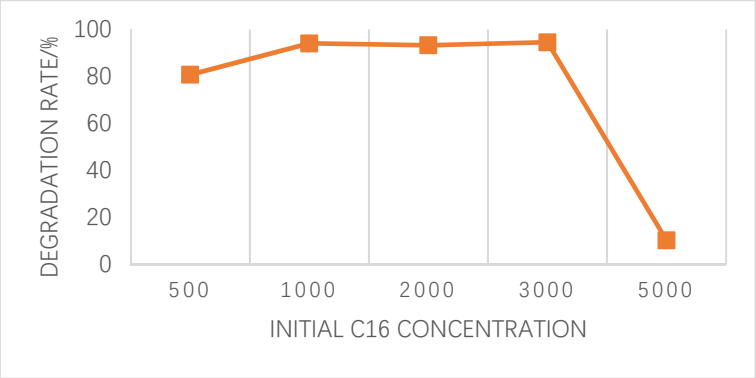

Fig. 5 Catalytic effects of different concentrations of n-hexadecane on L15 bacteria

3.2.4 Effect of salt content on water vapor evaporation

The effect of salt content on the degradability of bacteria L15 is shown in Figure 6. As can be seen from Figure 6, the degradation ability of the strain to n-cetane gradually decreased , the worse the degradation effect. This is because salt will have an impact on cell osmotic pressure [7]. When the salt mass fraction in the medium increases, the osmotic pressure inside the bacterial cell is smaller than that outside, and the water inside the cell flows out, resulting in dehydration. Therefore, the bacterial degradation reaction to n-cetane slows down and the degradation rate decreases. The results show that the degradation rate of n-cetane is about 50% when NaCl concentration is 3%. When the salt mass percentage reaches 9%, the bacterial growth is difficult, the bacterial concentration is low, and the removal rate is only 8.02%. Therefore, the optimal NaCl mass percentage of the bacteria is 0%~1%, and the salt mass percentage is not more than 3%.

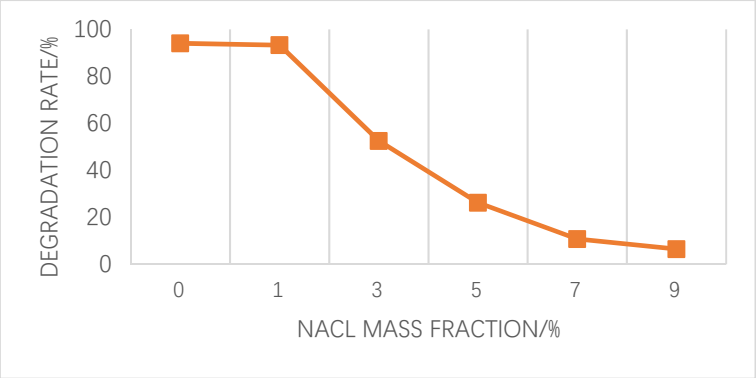

Fig. 6 Effect of salt on the degradability of L15 bacteria

3.2.5 Effects of dissolved oxygen

If other conditions remain unchanged, changing the capacity of the medium in the conical bottle is equivalent to changing the dissolved oxygen in the medium. Moreover, the influence of dissolved oxygen on the degradation performance of strain L15 is shown in Figure 7. As can be seen from Figure 7, as the capacity of the culture in the embryo bottle increases, the density of dissolved oxygen becomes smaller and vice versa. When the capacity of the medium was larger and larger, the degradation rate of the strain to n-cetane was also lower and lower. This is because under the same shaking speed, when the capacity of the culture liquid in the conical bottle was larger and larger, the dissolved oxygen in the medium would be smaller and smaller, and the strain could not get enough oxygen, its vitality would be slowed down, so the utilization rate of n-cetane would be slower and slower. The n-cetane degradation efficiency of this strain was above 90% when the culture medium capacity was 60 ml,80 ml and 100 ml, so 100 ml culture medium capacity was the best.

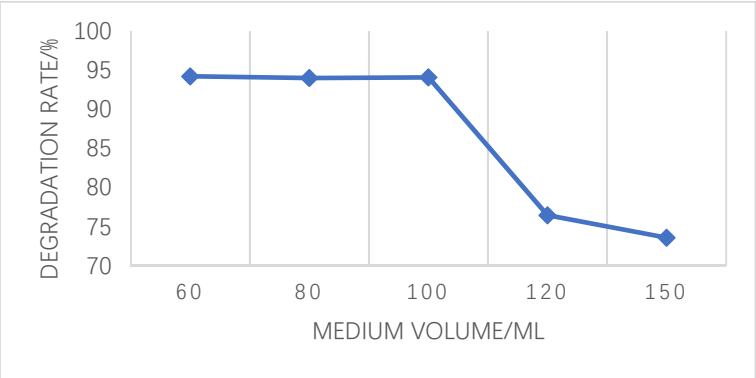

Fig. 7 Effect of dissolved oxygen on the biodegradability of bacteria L15

3.2.6 Influence of inoculation amount

Figure 8 shows the effect of the number of inoculations on the degradability of bacteria L15. As can be seen from Figure 8, the degradation rate of n-cetane to this strain was 90.33% when the inoculation

rate was 5%, and further increasing the inoculation rate could not significantly improve the removal rate of n-cetane at this time. In general, the effect of the amount of inoculation on the bacteria is most intuitively reflected in its extended time, and the more the amount of inoculation, the shorter the extended time. Under the same culture conditions, the larger the amount of inoculation, the earlier the logarithmic growth phase began, the earlier the rapid utilization of n-cetane would begin. However, when the amount of inoculation was increased after reaching 5%, the n-cetane degradation rate of the strain was not significantly improved, so 5% was considered as the optimal amount of inoculation.

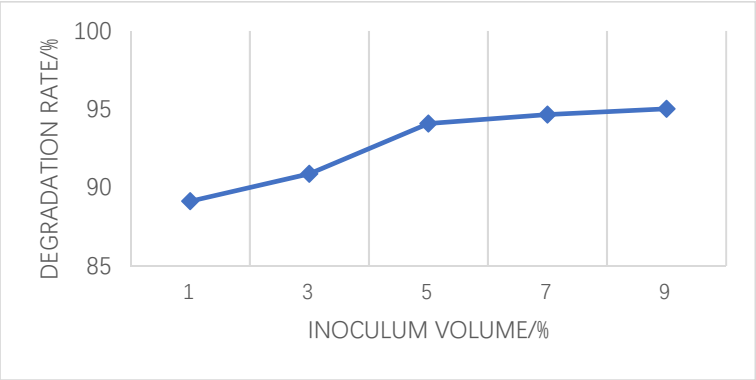

Fig. 8 Effect of inoculation dose on degradability of L15 bacteria

3.3. Degradation performance of diesel oil by the strain

Under the condition that the initial pH of the medium was 7.0, the inoculation amount was 5%, and 30℃, the suspensions of the dominant bacterium L15 were inserted into the diesel medium. After 7 days of oscillating culture, GC-MS analysis was performed on the components of the diesel oil with a mass concentration of 3000 mg/L before and after degradation, and the results were shown in FIG. 9 (a) and (b) respectively. The microorganism can effectively degrade various components in gasoline, especially the alkane components (Figure 10).

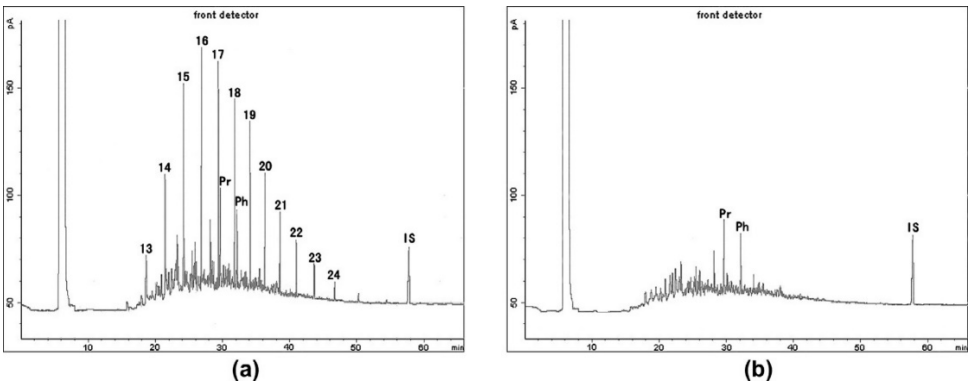

Fig. 9 GC-MS spectra before and after degradation

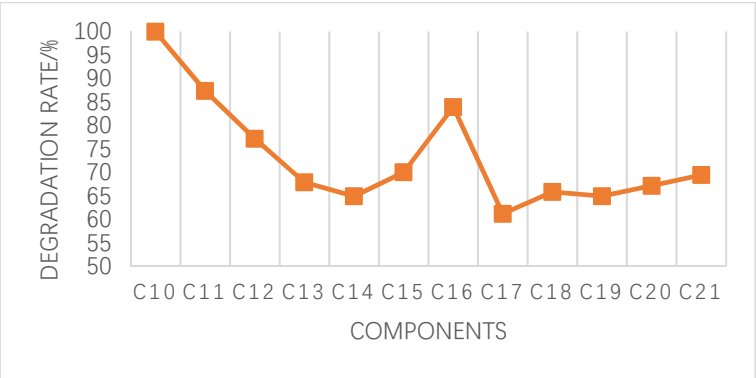

Fig. 10 Degradation rates of each alkane component in diesel oil samples

4. Conclusion

Through morphological observation, physiological and biochemical indexes and 16 SrDNA sequencing, L15 was preliminarily identified as acinetobacter. The results showed that L15 had the best degradation effect on n-cetane compounds under the culture temperature of 30℃, 5% inoculum volume and 100 ml medium volume. Its maximum tolerance can reach 5000 mg/L. The L15 strain has higher sulfur content, sulfur content and sulfur content, and can complete the advanced treatment of 3000 mg/L diesel oil in 7 days. It shows a strong application prospect.

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