Study on high efficient aromaticity degrading bacteria in oily sludge

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Abstract. The degradation of oily sludge is an important subject in the field of environmental remediation, especially the degradation of aromaticity in oily sludge, because its harm to human body is immeasurable, it is urgent to find an effective method to degrade oily sludge. Taking the oily sludge of Karamay oil field in Xinjiang as the research object, the chemical composition of the oily sludge was determined through the analysis of the chemical composition of the oily sludge. A strain was isolated from nearby contaminated soil, and was identified by ultraviolet spectroscopy, liquid chromatography, Gas chromatography-mass spectrometry, and other methods, it was found that the strain had a good degradation ability to aromaticity, with a degradation rate of 98.2%.

1. Introduction
Oily sludge is dangerous to humans, water and soil, especially aromaticity, which can cause genetic mutations in humans and other organisms[1]. These substances are considered to be extremely harmful pollutants because of their mutagenic, teratogenic and carcinogenic effects, their genetic damage and their continued accumulation in plants and animals through the food chain[2]. With the rapid development of petroleum industry, the output of oily sludge is increasing, and the disposal of oily sludge is imminent[3].

At present, the main treatment methods of oily sludge include incineration, pyrolysis, solvent extraction, solidification and biodegradation. In the above methods, the disadvantage of incineration method is that it will produce a large amount of gas pollutants[4]. The pyrolysis process requires a high reaction temperature and conditions, which requires a lot of energy consumption to degrade the pollutants[5], the solvent extraction method can not be widely used in industry due to the use of a large number of extraction liquid[6], the solidification treatment method is only temporary fixation of pollutants, pollution can not be eliminated fundamentally[7]. Biodegradation is the most environmentally friendly and cost-effective treatment method. Bioremediation technology is efficient and convenient to treat pollutants in oily sludge[8].

In this paper, We took the oil sludge from Karamay oil field in Xinjiang as the research object, screened out the oily sludge tolerant strain from the nearby soil and fermented it, then mixed it into the oily sludge as a loose agent, detected its characteristic peak by UV, and calculated its degradation ability. The strains with high efficient degradation were obtained by comparison.
2. Materials and methods

2.1. Materials

2.1.1. Main instruments.
The instruments mainly include constant temperature incubator, high pressure steam sterilization pot, thermostatic oscillator, ultraviolet (UV) spectrophotometer, Fourier transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR), high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), etc.

2.1.2. Main reagents.
Luria-Bertani (LB) medium: 1% Peptone, 0.5% yeast extract, 1% NaCl (add 1.5% agar in solid medium); Basic inorganic nutrient solution: 0.5% NaCl, 0.1% (NH₄)₂SO₄, 0.4% MgSO₄·7H₂O, 0.5% NaH₂PO₄, 1.0% Na₂HPO₄, 0.2% NaNO₃; Screening medium: add 2% oily sludge into the base inorganic nutrient solution. The above medium needs to be sterilized in high pressure steam sterilization pot at 121 ℃ for 20 minutes.

2.2. Preliminary screening of oil sludge tolerant strains
Add one gram of soil from the contaminated sludge into 50 ml screening medium. After overnight culture, dilute the bacterial solution to the appropriate concentration, then spread it on the solid LB medium, and incubate at 37 ℃ for 12 hours. Observe the colony morphology and collect the different forms of strains.

2.3. Second screening of degrading strains
The preliminary screened oily sludge tolerant strains were put into 100 ml LB medium for further cultivation. When they reached the logarithmic phase, they were added to the sterilized 10 g rice husk and stirred evenly. Then they were placed in a constant temperature incubator and cultured at 37 ℃ for 2 days. The mixed fermentation broth was used as a loose agent. A certain amount of loose agent was added into 100g oily sludge and fully stirred. At the same time, the sludge without the addition of loose agent was used as a natural control. The degradation of oil sludge was detected every three days, and the strains with strong degradation ability could be screened out.

2.4. Determination of degradation rate by UV Spectrophotometry
Take the oil sludge sample and the mixed oily sludge sample with a certain amount of loose agent, respectively add 20ml trichloromethane for extraction, take 3ml of the extracted supernatant into the colorimetric dish, scan the whole band with ultraviolet spectrophotometer, record the peak value of ultraviolet absorption peak, and make the standard curve by gradient dilution of the original oil sludge. The degradation rate was calculated by the change of UV absorption peak value.

2.5. Optimization of degradation conditions

2.5.1. Effect of culture time on degradation rate.
The UV absorption peak value of oily sludge was detected every three days. The degradation rate of different strains was calculated by standard curve and the degradation trend was observed.

2.5.2. Effect of the amount of loose agent on degradation rate.
5 g, 10 g, 15 g, 20 g and 25 g loose agent were separately added into 100 g oily sludge, and the degradation rate was calculated after seven days of incubation.

2.5.3. Effect of supplementary culture medium on degradation rate.
Three groups of experimental control were set up. Culture medium, water and nothing were added to each group every three days. The degradation rate was calculated after one month.
2.6. Stability test
After 1 month, 3 months and half a year, the UV absorption peaks of the samples were detected to verify the stability of the degradation ability of the selected strain.

2.7. Strain identification
The strain with the highest degradation rate was identified by 16S rRNA gene sequence analysis. Genomic DNA of bacteria was extracted, amplified by PCR and detected by electrophoresis. Blast software was used to compare the homology and analyze the phylogenetic relationship.

2.8. Composition analysis of oily sludge extract
The components of oily sludge extract were analyzed by means of FTIR, NMR, HPLC and GC-MS.

3. Results and discussion

3.1. Screening results of oil sludge tolerant strain
After overnight culture, the medium became turbid, which indicated that the oil sludge tolerant strain could grow with oil sludge as the sole carbon source. After diluting the bacterial solution to the appropriate concentration, it was evenly coated on the solid LB medium. After 12 h, nine different forms of bacteria were collected and numbered as A , B , C , D , E , F , G , H , I.

3.2. Standard curve
Added 20ml trichloromethane into 1g oily sludge for extraction. Then put 3ml of the extraction solution into the colorimetric dish. The UV spectrophotometer was used to scan the whole band. It was found that the absorption peak was strong at 290nm, and the absorbance was about 2.086. The concentration under this absorbance was recorded as 1μg/ml. Then, diluted the extracted samples into the concentrations of 0.8, 0.6, 0.4, 0.2, 0.1 (μg/ml) and determined the absorbance at 290nm. Make standard curve as shown in Figure 1.

Figure 1.Standard curve of absorbance and concentration.

3.3. Results of degradation rate of selected strains
The absorbance of nine strains at 290nm was determined after three days, the concentration was calculated by the formula of standard curve, and the degradation rate was calculated by formula (1). In this formula, C1 represents the initial concentration and C2 represents the concentration after degradation.

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\text{Degradation rate} (%) = \frac{(C_1 - C_2)}{C_1} \times 100\%
\]

Table 1 Degradation rate of different strains.

|     | A    | B    | C    | D    | E    | F    | G    | H    | I    | Blank |
|-----|------|------|------|------|------|------|------|------|------|-------|
| Absorbance | 0.842 | 1.577 | 1.368 | 1.598 | 1.425 | 1.776 | 1.489 | 1.204 | 1.115 | 2.055 |
| concentration   | 0.406 | 0.760 | 0.659 | 0.770 | 0.687 | 0.856 | 0.718 | 0.581 | 0.538 | 0.990 |
|-----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Degradation rate| 59.4% | 24.0% | 34.1% | 23.0% | 31.3% | 14.4% | 28.2% | 42.0% | 46.2% | 1.0%  |

It can be seen from Table 1 that strain A has better degradation effect than other strains, the degradation rate of strain A can reach 59.4% in three days, while the degradation rate of the blank control group is only 1%, which indicates that the degradation of this substance is extremely difficult and slow under natural conditions.

### 3.4. Results of optimization of degradation conditions

#### 3.4.1. Comparison of degradation rate under different culture time.

The degradation rate of each strain after 6 days, 12 days, 18 days, 24 days and 30 days is shown in Table 2.

|  | A   | B   | C   | D   | E   | F   | G   | H   | I   | Blank |
|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| 6d | 82.9% | 33.2% | 47.6% | 39.9% | 51.2% | 42.4% | 49.1% | 51.5% | 57.6% | 2.4%   |
| 12d| 93.7% | 51.9% | 62.1% | 50.0% | 62.4% | 57.8% | 66.3% | 59.2% | 64.2% | 4.5%   |
| 18d| 95.2% | 55.4% | 66.7% | 53.0% | 66.0% | 61.2% | 68.3% | 60.2% | 69.9% | 5.5%   |
| 24d| 97.7% | 55.7% | 68.8% | 55.9% | 66.3% | 64.2% | 69.4% | 61.5% | 72.3% | 6.8%   |
| 30d| 98.2% | 56.2% | 69.3% | 57.1% | 67.9% | 64.7% | 70.9% | 62.0% | 73.1% | 8.1%   |

It can be seen from Table 2 that the content of aromatocity decreases rapidly in the initial stage, and the degradation rate gradually tends to be stable in the later stage. After 30 days of continuous degradation, the degradation rate of strain A can reach 98.2%, which is far higher than other strains. Therefore, strain A was selected for follow-up experiment and identification.

#### 3.4.2. Comparison of degradation rate by adding different amount of loosening agent.

Adding 5g, 10g, 15g, 20g and 25g of loosening agent into 100g oil sludge, the degradation rate was measured after 7 days.

It can be seen from Figure 2 that the degradation rate of 15g loose agent is greatly improved compared with the former two, while the increase of more loos agent on the degradation rate is not significant. Considering the convenience and cost in practical application, Adding 15g loose agent was the best.

![Figure 2. Degradation rate with different amount of loosening agent.](image1)

![Figure 3. Degradation rate with different supplement.](image2)

#### 3.4.3. Comparison of degradation rate of supplementary culture medium.

The control group was set as a comparison. In one group, the culture medium was supplemented appropriately every 3 days to provide nutrition for bacterial growth. In the other group, only water was
added, and no substance was added in the blank group. The degradation rate was determined after 30 days.

It can be seen from Figure 3 that timely supplementation of nutrients and water during the degradation process can significantly improve the degradation effect.

3.5. Results of stability test
After half a year, the absorbance of the oil sludge samples degraded by Strain A was almost zero, and the degradation rate was still above 98%, indicating that the degraded aromaticity did not regenerate, and the degradation effect of the strain had excellent stability.

3.6. Results of strain identification
The strain A was identified and compared with blast, it was found that it had the closest genetic relationship with Bacillus horneckiae strain, and its matching degree was up to 99%. The phylogenetic tree is shown in Figure 4.

3.7. Results of composition of oily sludge extract
3.7.1. FTIR spectrum. It can be concluded from Figure 5 that the main component of the sample is chain alkanes.
3.7.2. NMR.
As shown in Figure 6, there are many peaks in the position from 0.8 to 1.5, which indicates that there are many kinds of methyl and methylene. There are a few peaks in NMR 7.0 to 8.0, indicating that there are a small amount of aromatic compounds. The triple peak at position 2.3 indicates that the sample contains a small amount of carboxylic acid and salt.

3.7.3. HPLC.
Using methanol water as separation system, the substances with absorption peak at 290nm were separated by UV detector.

![Figure 7. HPLC spectra of oily sludge extract before degradation.](image1)

![Figure 8. HPLC spectra of Oily sludge extract after degradation.](image2)

By the comparison of Figure 7 and Figure 8, it could be found that the content of aromatic compounds in the sample after degradation is close to zero. The outflow liquid was collected for GC-MS detection about 19 minutes.

3.7.4. GC-MS.
It could be found in Figure 9 and Figure 10, there are a large number of aromatic compounds in the samples before degradation, including toluene, ethylbenzene, xylene and other short chain aromatic compounds, as well as many long chain complex aromatic compounds. However, there are almost no such compounds in the samples after degradation, which can prove that the degradation is complete.
4. Conclusion and Prospect

By screening and acclimating the bacteria in the soil near the oily sludge, a strain capable of effectively degrading the aromaticity in the oily sludge was obtained. The results of 16s rRNA sequencing showed that the strain had the closest genetic relationship with Bacillus Hornicornis and the matching rate was close to 99%. UV Spectrophotometer was used to test the degradability of the strain. The results showed that the degradation rate was 98.2% after 30 days treatment. After a long time test, it was found that the degraded aromaticity could not regenerate, which indicated that the degradation ability of the strain was stable. At the same time, the degradation of aromaticity in the samples was confirmed by HPLC and GC-MS.

We expect the strain to play a more prominent role in the actual production, to achieve the degradation of a large number of oily sludge, and to contribute to environmental correction treatment.

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