Experiment study on the treatment effect of the hydrocarbon degradation microorganism to treat the oily wastewater

Zhiyong Han¹, Chengzhen Du and Ziming Shang

Lanzhou University of Technology

¹ Email address: hanzhy_009@sina.com

Abstract. In view of the large amount of oily wastewater, the complexity of pollutant composition and the difficulty of effective treatment, the idea to adopt the microbial technology to treat it is put forward and the influencing factors of oil-degrading microbial treatment of oily wastewater, optimal degradation conditions and degradation kinetics have been studied in depth. The degradation effect and the influencing factors of the hydrocarbon degradation microorganism to treat the oily wastewater were investigated in this study. Firstly, the hydrocarbon degradation microorganism which can degrade the oil pollution quickly and effectively are screened and identified and the physiological and biochemical characteristics are detaily researched. Secondly, the influencing factors and effects of oil-degrading bacteria treatment of oily wastewater and the optimal degradation conditions, which including pH value, rude oil mass concentration and the biological inoculum size are studied in depth and determined. Thirdly, the degradation kinetics characteristics of the petroleum hydrocarbon degrading stains are investigated. At last, the degradation effect of the oil pollution under the optimal condition is studied as the focus research is studied to verify the biodegradation effects of the strains in order to provide some technical reference for the practical application of this technology. The research results indicate that: (1) the Petroleum hydrocarbon degrading bacteria we screened all have good treatment effect on oily wastewater; (2) Analytical identification of morphological and physiological and biochemical characteristics indicated that both JA and JB are all Pseudomonas sp and their optimal growth times are 18-23h, and 20-21h respectively; (3) The degradation effect of the mixed strain on the pollutants is much better than that of the single one, Under the JA to JB mixed rate of 1:2, the average degradation rate of crude oil can be increased from 23.4% of the single bacteria to 49.6% of the mixed one; (4) The most important influencing factors affecting the degradation performance of petroleum-degrading bacteria are pH value, followed by crude oil mass concentration and biological inoculum. Among them, under the condition of pH 7.15, crude oil mass concentration of 3387mg/L and biological inoculum size of 75ml/L, the mixed bacteria had the highest degradation rate of crude oil, up to 55.76%.

1. Introduction

Petroleum is a very complex mixture of the carbon, hydrogen, nitrogen, sulfur, oxygen and hydrocarbons, containing hundreds of different components ranging from light-weight linear paraffins to heavy-weight polycyclic aromatic hydrocarbons (for example, PAHs)[1-2]. The diversity of petroleum components has increased its use in industry, agriculture, and social services. At present, it is not only an important chemical raw material for many products, but also an indispensable energy source for some industrial enterprises, which is of great significance to the development of the national economy. However, the pollution of surface water, groundwater, soil ecological and atmospheric
environment caused by the large amount of oily wastewater generated during petroleum exploration, mining and processing has become more serious. Therefore, how to effectively control and treat oily wastewater has become one of the most concerned global environmental problems. The purpose of this study is to improve the treatment rate of oily wastewater by cultivating low temperature petroleum degrading bacteria, in order to provide some references for the conditions, effects and influencing factors of the treatment of oily wastewater by degradation microorganisms, and it lays a theoretical foundation for the application of chemical microorganism technology in oily wastewater treatment. The oily wastewater mainly comes from industry, agriculture, transportation, social service industry, and the settlement of petroleum hydrocarbons that have escaped into the atmosphere [3]. Among them, industrial oily wastewater has the largest variety, mainly including oil production wastewater, refinery wastewater, tank cleaning wastewater and accidental oily wastewater [4]. In addition, the amount of oily wastewater generated by industry is large. The relevant data show that the average amount of oily wastewater generated by industrial crude oil refining enterprises is about 0.75t per 1t of crude oil. However, most of China's crude oil refining enterprises are mainly processing heavy oil, and the processing technology is more complicated. The average amount of oily wastewater from processing 1t crude oil is about 2.1t [5]. According to the latest statistics, China's crude oil processing level reached $4 \times 10^8$ t/a in 2013, from which the calculation of oily wastewater production is about $8.4 \times 10^8$ t/a.

At the same time, with the increasing oil exploration, mining and processing activities, the amount of oily wastewater is increasing year by year. The traditional oily wastewater treatment technology can no longer meet the existing environmental protection technology requirements. Therefore, research and development of efficient and simple oil-containing wastewater treatment process is extremely urgent and has been put on the research agenda.

At present, the environmental protection technology for oil pollution mainly includes physical, chemical and biological methods. There are three major categories, among which the biological method is the research hotpot, development trend and final attribution of petroleum pollution wastewater treatment technology.

## 2. Methods and materials

### 2.1. Determination of crude oil degradation rate

Under sterile conditions, the mixed bacteria were added to the inorganic salt medium, a certain amount of crude oil was added, and the degradation rate of the crude oil was measured after 5 days. The solution after degrading for 5 days was transferred to a separatory funnel, and 15 mL of dichloromethane, 1 g of NaCl, and 1 mL of sulfuric acid were sequentially added thereto, and the mixture was shaken for 2 minutes, and then allowed to stand for separation. The extract was passed through a sand core funnel containing anhydrous sodium sulfate. The mixture was filtered into a constant weight beaker, and the residual fluid was extracted twice again. [4], after the end of the extraction, the extract was placed in a fume hood and evaporated to dryness in a water bath at 54 °C, and evaporated to constant weight and weighed and measured [5]. The crude oil degradation rate is calculated according to Equation 1.

$$\eta = \frac{m_1 - m_2}{m_1} \times 100\%$$

Where: $\eta$ - is the degradation rate (%);
$m_1$—the mass of crude oil in the crude oil medium (g);
$m_2$—the mass of crude oil remaining in the crude oil medium after degradation for 5 days (g).
2.2. Crude oil degrading bacteria strain culture screening and domestication
Soil samples from Changqing Oilfield, which were polluted by petroleum hydrocarbons for a long time and polluted by 10-20cm, were used as the degradation strains, and the indigenous microorganisms were used to eliminate inferior strains and enrich the targets with high petroleum hydrocarbon degradation ability. The strain was enriched in a petroleum-degrading strain with stable community function under the condition of selecting petroleum hydrocarbon as the sole carbon source and inoculated into crude oil medium. The acclimation to the degradation rate tends to be constant, the degradation function of the flora is stable, and the physicochemical properties and growth characteristics of the strain and the degradation performance of petroleum hydrocarbons are analyzed.

2.3. Experimental factors affecting microbial degradation
The ratio of strain mixeding, crude oil mass concentration, carrier dosage and pH value are the main factors affecting the degradation rate of the crude oil. Based on the single factor experiment, determine the optimal ratio of mix ratio, crude oil mass concentration, carrier dosage and pH value to the degradation rate of crude oil, so as to determine the optimal conditions for degradation microorganisms to degrade petroleum pollutants. Provide some reference and reference for subsequent practical engineering applications.[6]

2.4. Effect of free degrading bacteria on oily wastewater treatment and its kinetic analysis
The dominant degrading bacteria JA and JB were selected as experimental bacteria groups, and the main factors affecting the degradation rate of crude oil, such as the optimal ratio of crude oil degrading bacteria, crude oil mass concentration, pH value and microbial inoculum, were studied in batches. The best strain ratio, crude oil quality concentration, pH value and microbial inoculum were studied. At the same time, the kinetics of petroleum hydrocarbon degrading bacteria degrading petroleum contaminants was studied to prepare the preliminary work for the subsequent treatment of oily wastewater by degradation strains.[7]

3. Results and discussion

3.1. Degrading strain culture screening and domestication
Six high effect oil degrading strains are screen purified and trained from the long -term heavily polluted soil of Changqing Oilfield, mong them JA and JB which have the highest degradation performance were selected as the experiment strains.

3.1.1. JA and JB strain identification and physiological and biochemical experiments
(1) Identification of strains
The oxidase, starch hydrolysis, oil hydrolysis, glucose oxidation fermentation, contact enzyme and nitrite experiments were performed on JA and JB, respectively. Comparing the experimental results with the Berger's Bacterial Identification Manual and the Common Bacterial System Identification Manual, both of them are Pseudomonas sp.
(2) Morphological characteristics
The colony of strain JA was yellow, the rounded edge was smooth and tidy, and the surface was moist and low. The colony of strain JB was light yellow, the rounded edge was smooth and tidy, and the surface was wet and flat, as shown in Figure 1 and Figure 2.

3.1.2. Identification results of crude oil degrading bacteria. According to the Gram staining experiment and the spore staining experiment, the strains JA and JB cells were short rod-shaped, Gram-negative, and no spores were produced. The microscopic results are shown in Figure 3-6.
3.1.3. Growth characteristics of crude oil degrading bacteria. The growth and reproduction of microorganisms can be divided into four stages, namely, stagnation, logarithm, stationary and fading. The stagnation period, also known as the lag phase, the adjustment period or the adaptation period, refers to a period in which the number of cells does not increase when a small amount of microorganisms are inoculated into a new culture medium. The exponential phase, also known as the logarithmic phase, refers to the period in which the cells in the growth phase are split at a geometric
base rate in the growth curve. The stationary phase is also called the constant period or the highest growth period. In the fading period, the rate of cell death exceeds the rate of reproduction, and the entire microbial population exhibits negative growth and diverse cell morphology. Some microorganisms are autolyzed by the increased activity of proteolytic enzymes, and some microorganisms produce or release secondary metabolites such as antibiotics at this time [6].

The OD value of the strain suspension during the growth of the strain was determined by ultraviolet spectrophotometry. The suspension of the strain with large concentration was appropriately diluted with the enrichment medium, and the OD value of the suspension was controlled within the range of 0-1, and the OD was recorded. The growth curve of crude oil degrading bacteria JA and JB were obtained by plotting the enrichment culture time of the strain as the abscissa and the OD value as the ordinate.

![Figure 7. Growth curves of JA and JB.](image)

![Figure 8. The influence of the JA and JB mixture ratio.](image)

It can be seen from Figure 7 that JA is in an adjustment period of 0-2 h, and the strain is in an adaptation stage to the medium. At the beginning of logarithmic growth of 2-8h, the metabolic activity of cells gradually increased, and the rate of synthesis of new cellular substances gradually increased. 8-16h is in the logarithmic growth phase, the metabolic capacity of microbial cells is in an optimal state, the growth rate of bacterial growth is accelerating, and the number of microbial cells is significantly increased. At the end of logarithmic growth, 16-18, the metabolic energy of the cells gradually stabilized. 18-23h is in a stable phase, the growth rate of bacteria is basically the same as the death rate, and the number of bacteria remains constant. After 23 hours, the growth of microbial cells entered a decline phase, and the death rate of the strain was significantly higher than that of the strain, and the number of microbial cells showed a sharp decline.

JB is in the adjustment phase at 0-2h, and the strain is in the adaptation phase to the medium. At the beginning of logarithmic growth of 2-6h, the metabolic activity of cells gradually increased, and the rate of synthesis of new cellular substances gradually increased. 6-20h in the logarithmic growth phase, the metabolic capacity of microbial cells is in an optimal state, the growth rate of bacterial growth is accelerating, and the number of microbial cells is significantly increased. 20-21h is in a stable phase, the growth rate of bacteria is basically the same as the death rate, and the number of cells reaches the maximum. After 21h, the growth of microbial cells entered a stage of decline. The death rate of the strain was significantly higher than the growth rate of the strain, and the number of microbial cells showed a sharp decline.
3.2. Influencing factors experiment
Through batch experiments, the effects of strain ratio, crude oil quality concentration, pH value and biological inoculum on the degradation of petroleum pollutants were studied and the optimal working conditions of the degrading bacteria were finally determined.

3.2.1. Optimal ratio of strains. In a 50 mL inorganic salt medium, 4.5 mL of a different ratio of the bacterial suspension was added, and the degradation rate of the crude oil was measured after degrading for 5 days under the conditions of pH=7.5, temperature 15 °C, and crude oil concentration 4000 mg/L. The results are shown in Figure 8.

It can be seen from Figure 8 that JA and JB are mixeded in a ratio of 1:2, and the degradation rate of crude oil is the highest, reaching 49.6%, and the degradation ability of mixed bacteria to crude oil is higher than that of single bacteria. This is because the two strains are mixed in proportion, which is beneficial to play the role of mutual assistance in the species and improve its ability to degrade crude oil [7]. Therefore, select the strain mixed ratio of 1:2 for subsequent experiments.

3.2.2. Crude oil mass concentration. The pH value was 7.5, the temperature was room temperature, and the microbial inoculum was 90 ml/L as the experimental constant condition. 50 mL of crude oil medium with different crude oil mass concentrations were prepared to study the effect of crude oil mass concentration on the degradation rate of crude oil. The results are shown in Figure 9.

3.2.3. pH value. The temperature was set to room temperature, concentration of the microbial inoculum was 90ml/L, and the crude oil mass concentration was 4000mg/L as the experimental constant condition. 50mL of crude oil medium with different pH values were prepared to investigate the effect of pH value on the degradation rate of the crude oil. The results are shown in the Figure 10.

It can be seen from Figure 9 that when the mass concentration of crude oil is lower than 4000 mg/L, the degradation rate of crude oil increases linearly with the increase of crude oil concentration. When the crude oil concentration continues to increase after reaching the peak value, the degradation rate of crude oil gradually decreases. This is because when the crude oil concentration is not high, the increase of the substrate concentration in the crude oil medium can promote the growth of the strain, thereby increasing the degradation rate of the crude oil. If the concentration of crude oil is too high, the molecular diffusion property of crude oil in solution will be lowered, and the growth and reproduction of the strain will be inhibited, resulting in poor removal of crude oil by the mixed crude
oil degrading bacteria. In summary, the crude oil mass concentration is selected as 4000mg/L as the center point of the BBD test.

It can be seen from Figure 10 that when the pH is lower than 7.5, the degradation rate of the crude oil increases with the increase of it, but when the pH is > 7.5, the degradation rate of the crude oil decreases with the increase of the pH value. It is because the too high or too low pH values can affect the stability of the cell enzyme and the dissociation state of the cell enzyme activity center and the dissociation state of the crude oil group, thereby reducing the cell metabolism ability [8]. Therefore, the pH was chosen to be 7.5 as the center point of the BBD test.

3.2.4. Microbial inoculum. With the pH value 7.5, room temperature, crude oil mass concentration 4000 mg/L as the experimental constant condition, and different amounts of microorganisms were inserted into each 50 mL crude oil medium to investigate the influence of the mixed bacteria inoculum size on the degradation rate of the crude oil, as shown in Figure 11.

It can be seen from Figure 11 that when the inoculum size of the mixed bacteria is <90 mL/L, the degradation rate of the crude oil increases with the inoculum increase, when the inoculation amount of the mixed bacteria is >90 mL/L, the dosage of the mixed bacteria is increased. largely, the lower the degradation rate of crude oil. This is because when the inoculum of the mixed bacteria is small, an excessively high crude oil concentration suppresses the activity of the microorganism. On the other hand, when the inoculum of the mixed bacteria is large, the microorganisms start to compete for the carbon source and other nutrients required for their own growth, resulting in inhibition and competition between the microorganisms, and finally reducing the ability of the mixed bacteria to degrade the crude oil, so the mixing is taken. The optimal inoculum size of the bacteria was 90 mL/L.

3.2.5. Kinetic experiment of degradation of crude oil by petroleum hydrocarbon degrading bacteria. Under the optimal degradation conditions obtained in the above experiments, the kinetics of crude oil degradation was carried out. The change of crude oil concentration with time in the process of free bacteria degradation is shown in Figure 12. The first and second-order kinetics fitting curves and fitting equation analysis were shown in Figures 13, 14 and Table 1.

![Figure 11. The effect of mixed bacteria dosage on the degradation rate of crude oil.](image1)

![Figure 12. The relationship of residual oil degradation change with time of free compound bacteria.](image2)
Table 1. Levels of biodegradation kinetics model.

| Reaction | Reaction rate equation | $K(h^{-1})$ | $R^2$ |
|----------|------------------------|-------------|--------|
| 0 grade  | $C=-198.59t+3094.74$   | -198.59     | 0.937  |
| I grade  | $\ln C=-0.08983t+8.07058$ | -0.08983   | 0.983  |
| II grade | $1/C=4.15069\times10^{-5}t+2.96446\times10^{-4}$ | 4.15069×10⁻⁵ | 0.9986 |

It can be seen from the results of Figure 13, 14 and Table 1 that under the optimal experimental conditions, the second-order kinetic model has a good fitting effect on the biodegradation process of mixed bacteria. The rate of biodegradation in the rate equation is proportional to the concentration of the substrate. Increasing the mass concentration of the crude oil within the acceptable concentration range of the microorganism can accelerate the rate of degradation of the crude oil by the fixed bacteria.

4. Conclusions
1) The oil-degrading bacteria JA and JB screened in this study have good degradation properties for petroleum pollutants.
2) Analysis of morphological characteristics, physiological and biochemical characteristics JA and JB are both Pseudomonas sp. The optimum growth time of JA and JB are 18-23h and 20-21h respectively.
3) After the 1:2 mixing of JA and JB, the average degradation rate of crude oil can be increased from 23.4% of single bacteria to 49.6% of mixed bacteria.
4) Influencing factors and the kinetics experiments show that pH value is the most significant influencing factor, followed by the crude oil mass concentration and biological inoculum here in the optimal conditions are pH 7.15, crude oil mass concentration 3387mg/L and the biological inoculum size of 75ml/Lunder which the mixed bacteria had the highest degradation rate of petroleum pollutants, up to 55.76%, and the second-order kinetic model has a good fitting effect with the biodegradation process of bacteria.

Acknowledgement
Sponsored by the Si chuan Provincial Key Laboratory of Chemical Synthesis and Pollution Control, Fenglin Tang (NO. CSPC201804).
References

[1] Zhao L, Fan F, Wang P, et al. 2013 Culture medium optimization of a new bacterial extracellular polysaccharide with excellent moisture retention activity[J] *Appl Microbiol Biotechnol* 97(7) 2841-2850

[2] Zhao L, Fan F, Wang P, et al. 2013 Culture medium optimization of a new bacterial extracellular polysaccharide with excellent moisture retention activity[J] *Appl Microbiol Biotechnol.* 97(7) 2841-2850

[3] Han S J, Park H, Lee S G, et al. 2011 Optimization of cold-active chitinase production from the antarctic bacterium, sanguibacter antarcticus KOPRI 21702[J] *Appl Microbiol Biotechnol.* 89(3) 613-621

[4] Liu C J, Liu J, Li J, et al. 2013 Removal of H2S by co-immobilized degradation bacteria and fungi biocatalysts in a bio-trickling filter[J] *Process Safety and Environmental Protection* 91(1-2) 145-152

[5] Iqbal M and Edyvean R G J 2004 Biosorption of lead, copper and zinc ions on loofa sponge immobilized degradation biomass of Phan-erochaete chrysosporium *Miner. Eng.* 17 217-223

[6] Katsivela E, Moore E R B, Kalogerakis N 2003 Biodegradation of aliphatic and aromatic hydrocarbons: specificity among bacteria isolated from refinery waste sludge [J] *Water, Air, and Soil Pollution: Focus* 3(3) 103-115

[7] Henderson S B, Grigson S J W, Johnson P 2006 Potential impact of production chemicals on the toxicity of produced water discharges from North Sea oil platforms [J] *Marine Pollution Bulletin* 53 260-265

[8] E M Adetutu, C Bird, K K Kadali, et al. 2014 Exploiting the intrinsic hydrocarbon-degrading microbial capacities in oil tank bottom sludge and waste soil for sludge bioremediation [J] *International Journal of Environmental Science and Technology* DOI; 10.1007/s13762-014-0534-y