Research of Isolation and Degradation Conditions of Petroleum Degrading Marine

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Abstract. A novel petroleum-degrading microbial strain was isolated from sediment samples in estuary of Bohai Sea estuary beaches. The strain was primarily identified as Alcanivorax sp. and named Alcanivorax sp. H34. Effect of PH values, temperature, nitrogen and phosphorus concentrations on degradation of H34 were investigated. The paraffinic components average degradation rate of H34 ungrowth cells under optimized conditions was studied. The results showed that the optimal growth conditions of H34 are were temperature of 30°C, initial PH of 7.0, nitrogen concentration of 3g/L, phosphorus concentration of 3g/L, and paraffinic components average degradation rates of H34 ungrowth cells was 41.6%, while total degradation rate was 45.5%.

1. Introduction

With the improvement of the petroleum extraction technology and development of petroleum industry, the maritime transport increased year by year. At the same time, the marine petroleum spills happens every year which results in large amounts of petroleum leaked into the sea and great harm to human life. Compared with chemical and physical methods, biological methods is an important way to completely eliminate the spilled petroleum by the microbial degradation. It can not only help us to understand the self-healing capacity of the environment, but also provide bioremediation with strain resources. Due to the hydrophobicity of the petroleum hydrocarbon, the microbial degradation of petroleum hydrocarbons is low. Thus, it is significant to isolate and identify degrading strains from the polluted seawater, and improve the microbial degradation by optimize their growth conditions.

There has been a growing environmental and research concern toward petroleum spills due to their wide distribution, persistence and toxicity to humanbeings and other biota. To effectively solve this problem, many methods are proposed. The result is demonstrate clearly that the bacterial consortium MPD-M was effective for treating hydrocarbons in water with salinity varying from 0 to 180g/L [1]. The efficiency of Bacillus subtilis DM-04 and Pseudomonas aeruginosa M and NM strains isolated from a petroleum contaminated soil sample from North-East India was compared for the biodegradation of crude petroleum-oil hydrocarbons in soil and shake flask study[2]. Two types of Indian crude oil (Bombay High and Gujarat) were tested for their biodegradability by Acinetubacter cakoaceticus and Alcnligenes odornns [3]. A mixed consortium was prepared with 15 bacteria isolated by enrichment technique from the sample collected from an oil contaminated site, and the degradation efficiency of the isolates in consortium was checked with 2% crude oil by shake flask transformation in mineral salt medium, at 37°C for 24 days [4]. The phenanthrene degrading novel bacterium strain USTB-RU was isolated from petroleum contami-nated soil in Dagan oilfield, southeast of Tianjin,

northeast China [5]. Isolation and identification of biosurfactant producing bacteria were assessed, and the potential application of these bacteria in petroleum industry was investigated [6].

In this paper, we name the petroleum-degrading microbial strain which is isolated tidal flat sediment in estuary of Bohai as Alcanivorax sp. H34, since it is primarily identified as Alcanivorax sp. Then, the effect of PH values, temperature, nitrogen and phosphorus concentrations on degradation of Alcanivorax sp. H34 is investigated. In addition, we also study the content and degradation rate of Alcanivorax sp. H34 non-growing cells alkane degradation

2. Materials And Methods

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2.1. Sampling

Sediment samples were collected from the Bohai Sea estuary beaches, and the oil samples were taken from the Dagang Oilfield.

2.2. Medium

2.2.1. Enrichment medium. Adjust pH of the medium (composition: NH_4NO_3 50g, KH_2PO_4 30g, K_2HPO_4 9g, $MgSO_4$-$7H_20$ 0.4g) to 7.0. Sterilize by autoclaving at 121°C for 15 minutes. Add diesel 5mL. Sterilize by autoclaving at 105°C for 15 minutes.

2.2.2. Isolation. Add the following to 300mL H_2O; Tryptone 0.5g; Yeast extract 0.5g; Beef extract 2g; Sodium acetate 0.2g; Agar 15g. Adjust pH to 7.0. Sterilize by autoclaving at 121°C for 15 minutes. Add 700mL seawater, and Sterilize by microporous membrane with 14um.

2.2.3. Re-screening medium. Sterilize 50mL seawater by microporous membrane with 14um. Add NH_4NO_3 50g, KH_2PO_4 30g, K_2HPO_4 9g ,$MgSO_4$-$7H_20$ 0.4g. Sterilize by autoclaving at 121°C for 15 minutes. Add diesel 5mL. Sterilize by autoclaving at 105°C for 15 minutes.

2.3. Enrichment, Isolation and Selection of Crude-oil Degrading Bacteria

The 30/300mL Enrichment medium was added to Erlenmeyer flasks and the flasks were incubated for 7 days at 30°C on rotary shaker with 120 rpm. Then the medium was diluted with 1 times, 100 times, 1,000 times with saline, and separated by the separation medium with streak plate method. After that, the solution was cultured by inverted until the colonies outgrow. At last, take the single colony, and isolated repeatedly using the streak plate method. Inoculated into 30/300mL re-screeningm medium. Incubated for 7 days at 30°C on rotary shaker with 120 rpm.

2.4. Alcanivorax sp.
H34 Non-growing Cells Degrade Petroleum Hydrocarbons

2.4.1. Strains cultured. The strain Alcanivorax sp. H34 was inoculated to tubes containing 5mL FM medium. Activated for 24 hours under the condition of 30°C and 120rpm. Two active strains were inoculated to two 2216E mediums (30mL and 300mL) with 5% inoculum. Incubated for 48 hours at 30°C and 120 rpm.

2.4.2. Collected cells, inoculated, degradation. Take 30mL H34 broth, centrifugate at 14000 rpm and 4°C for 15min, and discard the supernatant. Transfer the centrifugal thallus to degradation of petroleum hydrocarbon using 3mL sterilization natural seawater. Degradate 7 days at 3°C and 20 rpm.

2.4.3. Diesel group for Comparison. Degradation of petroleum hydrocarbon was sterilized by microporous membrane. Add to the flask, and degraded for 7-8 days at 30°C, 120 rpm.

3. Experimental Results and Analysis

To research the optimal growth conditions of H34, temperature, initial PH value, nitrogen and phosphorus concentrations were studied. Put H34 to 5ml FM medium under condition of 30°C, 120 rprn, activated for 24 hours. Then, put 5% inoculation amount to hydrocarbon-degrading matrix, 5mL tubes, 120 rpm and measured OD_{600} every 24 hours.

3.1. Influence of Temperature on Alcanivorax sp. H34

To research the influence of temperature on Alcanivorax sp. H34, set the temperature 15°C and 30°C, the initial PH value 7.0, the NH_4NO_3 concentration 3 g/L, K_2HPO_4 3 g/L, KH_2PO_4 1 g/L. the result is shown in Fig.1.

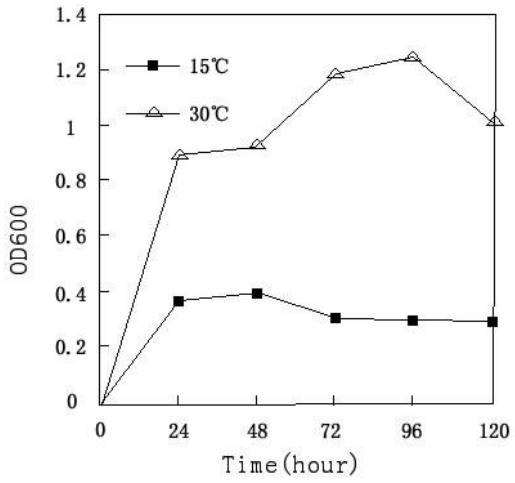

Figure 1. Influence of temperature on Alcanivorax sp. H34 consumption of alkanes

From Fig.1 we can see that during the growth of Alcanivorax sp. H34, the strain can grow at two different temperatures, the growth reaches the maximum at 96 h, and the optimum growth temperature of Alcanivorax sp. H34 is 30 ℃.

3.2. Influence of Initial PH on Alcanivorax sp. H34

The optimal initial PH value of Alcanivorax sp. H34 consumption of alkanes is researched under the condition of temperature 30°C, the NH_4NO_3 concentration 3g/L, K_2HPO_4 3g/L, KH_2PO_4 1 g/L. The initial PH values were set 6, 7, 8 and 9. Influence of pH on Alcanivorax sp. H34 consumption of alkanes is shown in Fig. 2.

Fig. 2 shows that during the growth of Alcanivorax sp. H34, the strain can grow at four different initial PH values, the growth reaches the maximum at 96 h, and the optimum growth initial PH value of Alcanivorax sp. H34 is 7.0. PH value affects the decomposition of petroleum hydrocarbons, and also inhibits the growth and metabolism of strains.

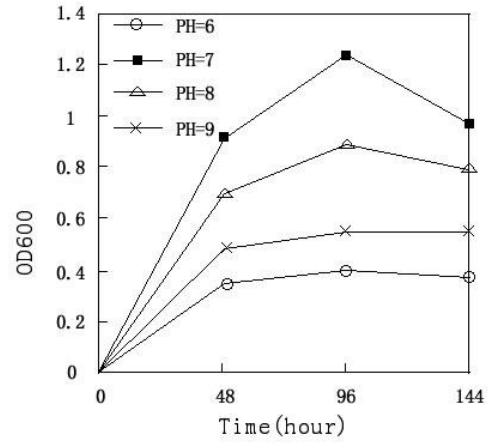

Figure 2. Influence of pH on Alcanivorax sp. H34 consumption of alkanes

3.3. Influence of Nitrogen Concentration on Alcanivorax sp. H34

The optimal nitrogen concentration of Alcanivorax sp. H34 consumption of alkanes is researched under the condition of temperature 30°C, initial PH value 7.0 , K_2HPO_4 3g/L, KH_2PO_4 1g/L. The nitrogen concentrations are set 1g/L, 2g/L, 3g/L, 4g/L and 5g/L. Influence of nitrogen concentrations on Alcanivorax sp. H34 consumption of alkanes is shown in Fig. 3.

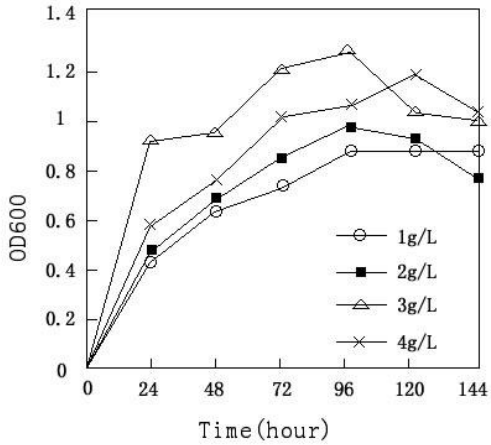

Figure 3. Influence of nitrogen concentrations on Alcanivorax sp. H34 consumption of alkanes

From Fig.3 we can see that during the growth of Alcanivorax sp. H34, the strain can grow at four different nitrogen concentrations. When the nitrogen concentration of Alcanivorax sp. H34 is 3g/L, the strains grow best and the growth reaches the maximum when 96h.

3.4. Influence of Phosphorus Concentration on Alcanivorax sp. H34

The optimal phosphorus concentration of Alcanivorax sp. H34 consumption of alkanes is researched under the condition of temperature 30°C, initial PH value 7.0, the nitrogen concentration set 3g/L.

Phosphorus concentrations are 1g/L, 2g/L, 3g/L, 4g/L and 5g/L. Influence of phosphorus concentrations on Alcanivorax sp. H34 consumption of alkanes is shown in Fig. 4.

Fig.4 shows that during the growth of Alcanivorax sp. H34, the strain can grow at four different phosphorus concentrations. When the phosphorus concentration of Alcanivorax sp. H34 is 3g/L, the strains grow best and when 144h, the growth reaches the maximum.

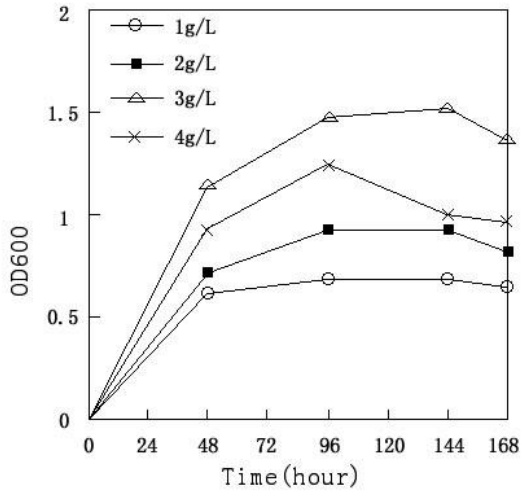

Figure 4. Influence of phosphorus concentrations on Alcanivorax sp. H34 consumption of alkanes

3.5. Alcanivorax sp. H34 Non-growing Cells Alkane Degradation

To research the Alcanivorax sp. H34 non-growing cells alkane degradation, experiments are carried by the method shown in section 2.4 and the results are shown in Fig. 5.

From Fig.5 we can see that Alcanivorax sp. H34 non-growing cells can degrade moderate carbon chain (CIQ - C24). The paraffinic components average degradation rates of H34 ungrowth cells is 41.6%, total degradation rate is 45.5%. The degradation rate slightly varies for different components. With the increasing of carbon number, the degradation rate decreases. The degradation rate of C10-C18 is high, 55.7% the highest and 21.6% the lowest.

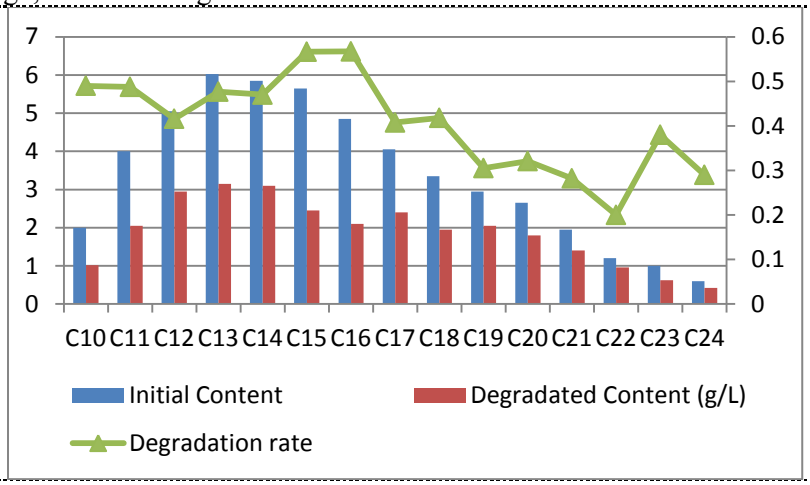

Figure 5. Content and degradation rate of Alcanivorax sp. H34 non-growing cells alkane degradation

4. CONCLUSIONS

During the growth of Alcanivorax sp. H34, the optimum growth temperature is 30 °C, the optimum growth initial PH value is 7.0 and the growth reaches the maximum at 96 h. When the nitrogen concentration of Alcanivorax sp. H34 is 3g/L, the strains grow best and when 96h, the growth reaches the maximum. When the phosphorus concentration of Alcanivorax sp. H34 is 3g/L, the strains grow best and when 144h, the growth reaches the maximum.

Alcanivorax sp. H34 non-growing cells can degrade moderate carbon chain (CIQ - C24). The paraffinic components average degradation rates of H34 ungrowth cells is 41.6%, total degradation rate is 45.5%. The degradation rate slightly varies for different components. With the increasing of carbon number, the degradation rate decreases. The degradation rate of C10-C18 is high, 55.7% the highest and 21.6% the lowest.

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