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Degradation Characteristics of Oil Degrading *Candida tropicalis*

Ya-Bin Zhan[1], Fang-Min Li[2], Wei-Chu Yu[2], Tao Jiang[3*], Li-An Ma[1#]

[1]College of Life Science, Yangtze University, Jingzhou, China
[2]College of Chemical and Environmental Engineering, Yangtze University, Jingzhou, China
[3]College of Animal Science, Yangtze University, Jingzhou, China
Email: 1766784675@qq.com,*jiangtao@yangtzeu.edu.cn, #malian@yangtzeu.edu.cn

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Abstract

Objective: The aim of study is to research the degradation characteristics of oil degrading *Candida tropicalis* (G-94). **Methods:** Effects of temperature, pH, oil concentration, salt concentration, inoculation amount, N and P source on the oil removal rate of G-94 were studied by single factor test. Meanwhile, the effects of temperature, pH, oil concentration and salt concentration on the oil removal rate of G-94 were optimized by orthogonal design. **Result:** The orthogonal design showed that the oil removal rate of G-94 could reach 25.83%, 28.56% and 30.90% at 10 d, 20 d and 40 d under the optimal conditions which was 25˚C, initial pH 8.0, oil concentration 1.0%, salt concentration 0.4%, inoculum amount 4%, the optimal N and P were $(NH_4)_2SO_4$ and K_2HPO_4. **Conclusion:** This experiment studied the degradation characteristics of G-94, which paved the way for the remediation of petroleum contaminated soil.

Subject Areas

Environmental Sciences

Keywords

Degrading Yeast, *Candida tropicalis*, Oil Degrading

1. Introduction

Oil contains many highly concentrated toxic materials, and oil contamination can negatively influence soil microbes and plants, as well as contaminate groundwater, which may be used for drinking or agriculture [1]. To eliminate these pollution compounds, processes have been developed based on physico-chemical techniques, including the vacuum extraction of hydrocarbons, soil

washing, electrokinetic incineration and recovery using solvents [2]. However, these methods produce toxic remnants that need to be decontaminated, which involve a high economic cost that is an obstacle to implementation [3] [4]. Fortunately, bioremediation is the microbial degradation of organic pollutants such as petroleum in soil and groundwater. This technique has the benefits of high treatment efficiency, low cost, relatively quick action, in site and ex site application, and compatibility with other techniques [5] [6] [7] [8].

Thus, in order to get insight of the bioremediation process of hydrocarbon in Qianjiang Guanghua Oilfield, the present study focused on degradation characteristics of indigenous hydrocarbon-degrading *Candida tropicalis* (G-94) with regards to the conditions for optimizing their activities and the efficient cleanup of the hydrocarbon pollutants.

2. Materials and Methods

2.1. Source of Isolation

The G-94 was isolated from Qianjiang Guanghua Oilfield on June 2015, stored in the laboratory of College of Life Science, Yangtze University [9].

2.2. Growth Study

Experiments were conducted in the oil medium described above with 0.5% oil (w/v) as sole carbon and energy source. Growth was monitored by measuring optical density at 420 nm with a TU-1900 spectrophotometer [10].

2.3. Determination of Oil Removal Rate

The oil removal rate was determined by gravimetric method [10]. A total of 80 ml methylene chloride was added to the oil triangle bottle to extract the oil. The water in the oil was dried at room temperature until the anhydrous sodium sulfate column was removed, until the organic solvent completely evaporated. Place the oil in the vacuum drying box at 40˚C and keep the vacuum at 0.04 Mpa for 30 min. Then remove it and leave it in the dryer for 30 min and to weigh. The oil removal rate is calculated according to the formula (1):

$$D = \frac{(C_0 - CS)}{C_0} \times 100\% \quad (1)$$

D: The oil removal rate, %; C_0: blank hydrocarbon concentration, mg/L; *CS*: concentration of petroleum hydrocarbon in the culture fluid, mg/L [10].

2.4. Study on Degradation Characteristics

2.4.1. Single Factor Test

The yeast was inoculated into potato sucrose liquid medium. The yeast were inoculated in logarithmic phase, centrifuged, removed supernatant, washed with sterile normal saline, and adjusted to OD_{420} = 9.185. In the basic culture medium at the same time, by changing the growth conditions in different temperature, pH, oil concentration, salt concentration, inoculation amount, N source, P

source on a horizontal shaker (150 rpm) at 35˚C in 100 ml oil medium for 5 d. Learning the suitable growth conditions of G-94 by the determination of biomass (OD_{420}).

2.4.2. Orthogonal Test

Four factors and 3 levels orthogonal test (Table 1) was carried out with temperature, pH, oil concentration and salt concentration. With 4% inoculation on a horizontal shaker (150 rpm) in 100 ml oil medium for 5 d. Learning the optimum growth condition of G-94 by the determination of biomass (OD_{420}).

2.4.3. Verification Test

Under orthogonal optimum conditions, the oil removal rate of G-94 was measured at 10, 20 and 40 day, respectively.

2.4.4. Statistic Analysis

SAS software was used to test the significance difference ($P < 0.05$) and extremely significant difference ($P < 0.01$).

2.5. Determination of Growth Curve

Under the optimum conditions, the strain of G-94 was injected into the oil medium with 4% inoculum, drawing the growth curve by measuring its OD_{420}.

3. Results and Analysis

3.1. Physiology

3.1.1. Single Factor Test

1) Temperature

The biomass of G-94 was effected by temperature as shown in Figure 1. The biomass decreased with the increase of temperature. There were larger biomass of G-94, when the temperature were 25˚C (OD_{420} = 1.622) and 30˚C (OD_{420} = 1.537). Compared with other treatments, there were significant differences.

Extremely significant difference ($P < 0.01$); Significance difference ($P < 0.05$). Same as below.

2) pH

The biomass of G-94 was effected by pH as shown in Figure 2. The biomass increased firstly and decreased lastly with the increase of pH. There was larger biomass of G-94, when the pH was 7.5 (OD_{420} = 0.960), 8.0 (OD_{420} = 1.183) and 9.0 (OD_{420} = 0.991). Compared with other treatments, there were extremely significant differences.

Table 1. Factor levels table.

	A (˚C)	B	C (%)	D (%)
1	25	7.8	0.1	0.3
2	28	8.0	0.2	0.4
3	30	8.2	0.3	0.5

A: Temperature; B: pH; C: oil concentration; D: salt concentration.

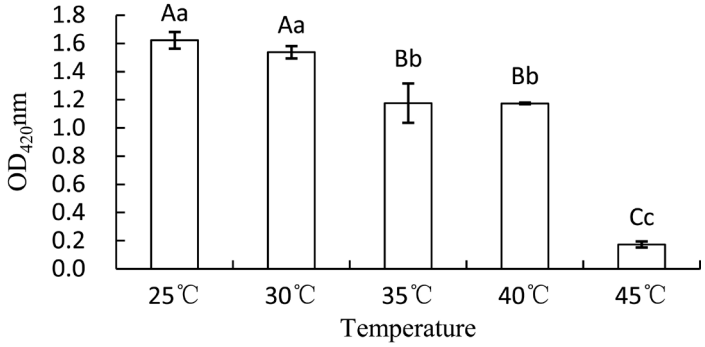

Figure 1. Biomass of G-94 under different temperature.

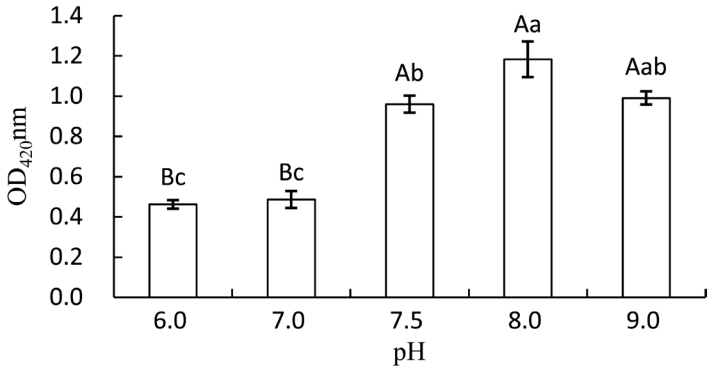

Figure 2. Biomass of G-94 under different pH.

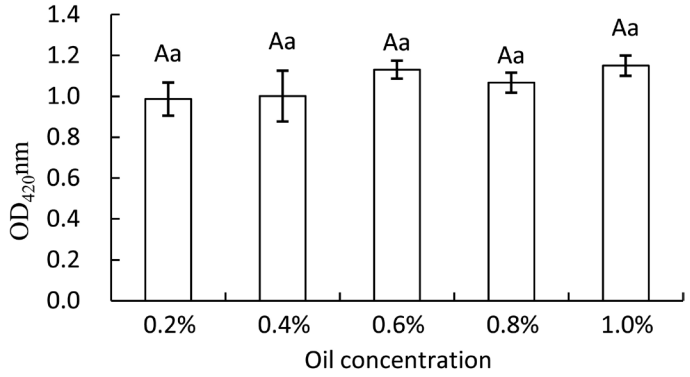

Figure 3. Biomass of G-94 under different oil concentration.

3) Oil concentration

The biomass of G-94 was effected by oil concentration as shown in **Figure 3**. There were larger biomass of G-94, when the oil concentration were 1.0% (OD_{420} = 1.150). Compared with the biomass of oil concentrate 0.2%, 0.4%, 0.6% and 0.8%, there were no significant differences.

4) Salinity

The biomass of G-94 was effected by salt concentration as shown in **Figure 4**. The biomass increased firstly and decreased lastly with the increase of salt concentration. There were larger biomass of G-94, when the salt concentration was 0.4% (OD_{420} = 1.722). Compared with other treatments, there were extremely significant differences.

5) Inoculation amount

The biomass of G-94 was effected by inoculation amount as shown in Figure 5. The biomass increased with the increase of inoculation amount. There was larger biomass of G-94, when the inoculation amount were 4% (OD_{420} = 1.739). Compared with other treatments, there were no extremely significant differences.

6) Nitrogen

The biomass of G-94 was effected by nitrogen source as shown in Figure 6. There were larger biomass of G-94, when the nitrogen source were $(NH_4)_2SO_4$ (OD_{420} = 0.593), NH_4NO_3 (OD_{420} = 1.314) and NH_4NO_3 (OD_{420} = 1.175). Compared with the biomass of NH_4Cl and KNO_3, there were extremely significant differences.

7) Phosphorus

The biomass of G-94 was effected by phosphorus source as shown in Figure 7. There were larger biomass of G-94, when the phosphorus source were K_2HPO_4 (OD_{420} = 1.356). Compared with the biomass of K_2HPO_4, Na_2HPO_4 and NaH_2PO_4, there were extremely significant differences.

3.1.2. Orthogonal Test

Different conditions had obvious influence on the oil removal rate of G-94. The maximum range of temperature is the key factor affecting the oil removal rate of

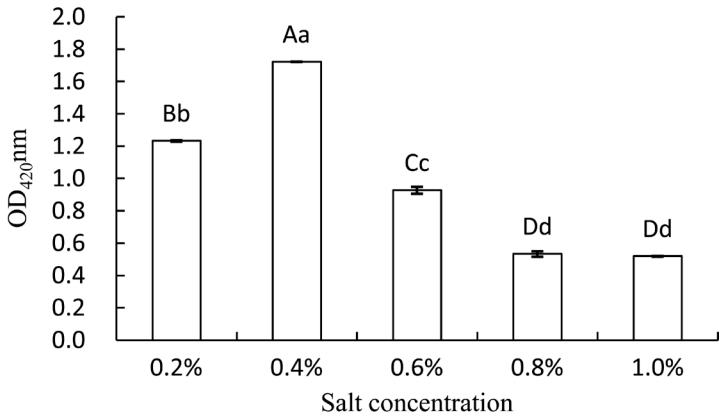

Figure 4. Biomass of G-94 under different salt concentration.

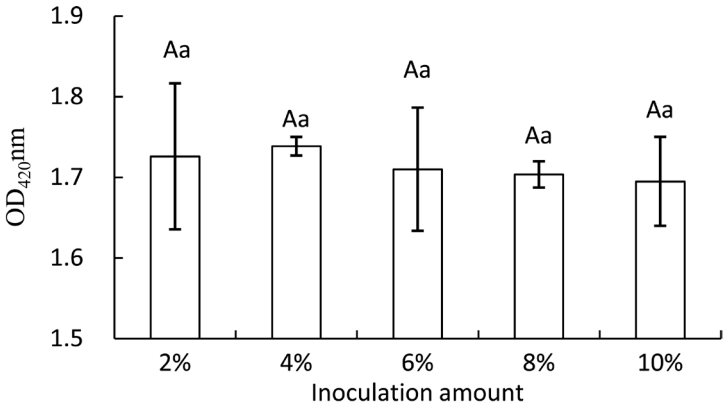

Figure 5. Biomass of G-94 under different inoculation amount.

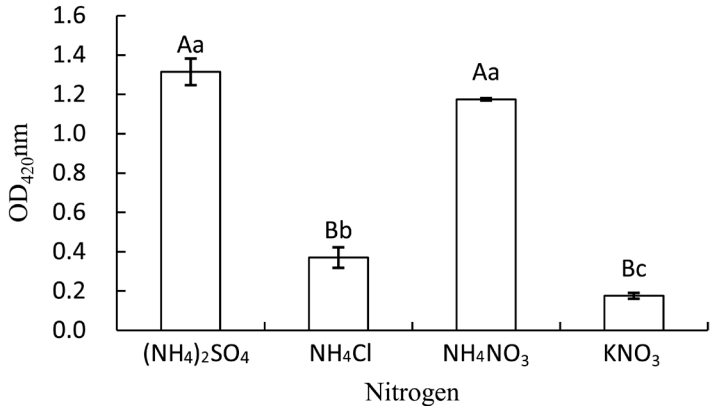

Figure 6. Biomass of G-94 under different nitrogen source.

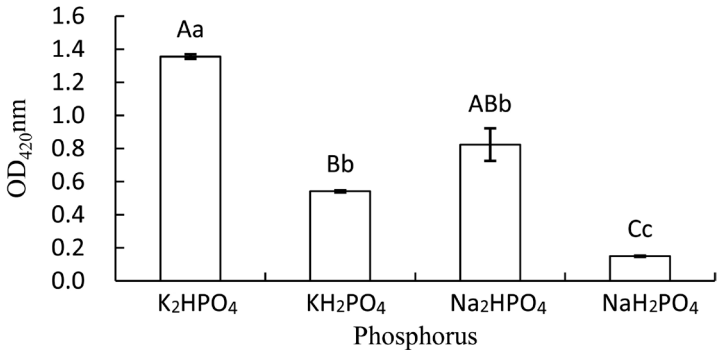

Figure 7. Biomass of G-94 under different phosphorus source.

Table 2. The orthogonal design and analysis of G-94.

Treatment	A (˚C)	B	C (%)	D (%)	Result
1	25	7.8	0.1	0.3	1.197
2	25	8.0	0.2	0.4	1.183
3	25	8.2	0.3	0.5	1.544
4	28	7.8	0.2	0.5	1.468
5	28	8.0	0.3	0.3	1.589
6	28	8.2	0.1	0.4	1.025
7	30	7.8	0.3	0.4	1.537
8	30	8.0	0.1	0.5	1.066
9	30	8.2	0.2	0.3	1.264
K1	1.308	1.401	1.096	1.350	
K2	1.361	1.279	1.305	1.248	
K3	1.289	1.278	1.557	1.359	
R	0.072	0.123	0.467	0.111	

A: Temperature; B: pH; C: oil concentration; D: salt concentration.

G-94, followed by pH and oil concentration, while salt concentration has the least influence. Therefore, the suitable condition of oil removal rate of G-94 was $A_2B_1C_3D_3$ which was 28˚C, pH 7.8, oil concentration 0.3%, salt concentration 0.5% (Table 2).

3.1.3. Verification Test

The results of Verification test were shown in **Figure 8**. Under orthogonal optimum conditions, the oil removal rate of G-94 reached to 25.83%, 28.56% and 30.90% at 10, 20 and 40 day, respectively.

3.2. Determination of Growth Curve

The result of determination of growth curve was shown in **Figure 9**. The 0 - 0.5 day was the lag phase of G-94; In this period, the reason why no biomass increase is that bacteria were first introduced into a fresh media. The 0.5 - 4 day was the exponential of G-94; In this period, the strain rapidly propagated and gradually turned the oil medium to muddy. The 4.5 - 13 day was the stationary phase of G-94; In this period, death rate = rate of reproduction of G-94 and continues for a long time.

4. Discussion and Conclusion

4.1. Discussion

The optimal oil removal condition of G-94 was determined through biomass of petroleum degradation microorganism, not the oil removal rate. The reason why we use the biomass is that biomass is positively correlated with degradation rate and it is more convenient than oil removal rate [10].

Candida tropicalis used to repair potato starch wastewater [11], produce xylitol [12] and Long Chain Dicarboxylic Acid [13]. There were few reports about *Candida tropicalis* degrading oil.

A strain of G-94 with petroleum as the sole carbon source was isolated from

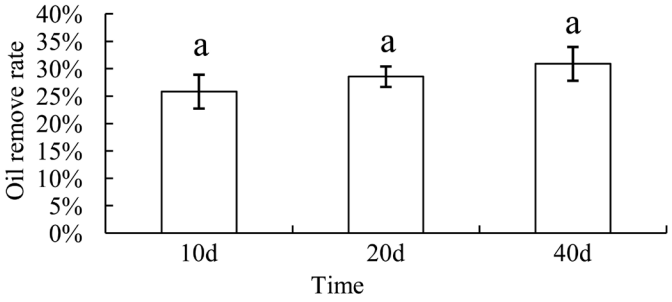

Figure 8. Oil degradation rate of strain G-94 on different time.

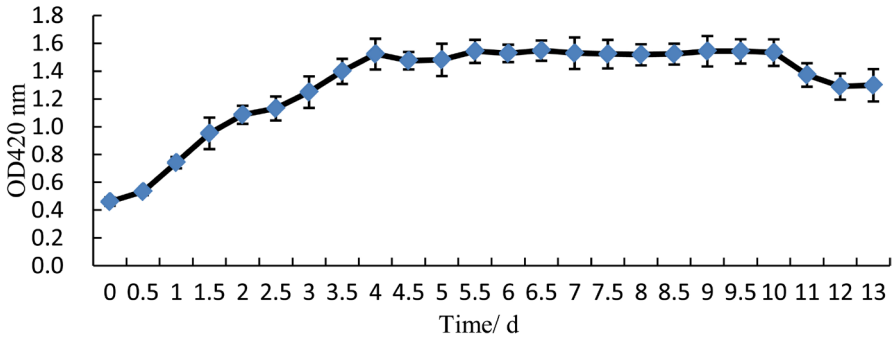

Figure 9. The growth curve of G-94 under the optimal conditions.

the soil contaminated by petroleum for a long time. The effects of temperature, pH, oil concentration, salt concentration, inoculum amount, N and P source on the degradation of oil were studied. Different conditions had great influences on the oil removal rate of G-94. The G-94 has larger biomass when the inoculation amount was 4% (OD_{420} = 1.739); Finally, the inoculation amount 4% was used to do the orthogonal experiment. The optimum N and P source was $(NH_4)_2SO_4$ and K_2HPO_4, which is basically consistent with the oil medium. Therefore, the temperature, pH and oil concentration and salt concentration were selected to do orthogonal test to further study the optimum conditions for degrading oil.

The oil removal rate of G-94 reached to 25.83% on 10th day. The oil removal rate of G-94 reached 28.56% on 20th day, increased by 10.57% compared with 10th day. The oil removal rate of G-94 reached 30.90% on 40th day, increased by 8.19% compared with 20th day. Maybe, the nutrition in the bottle had been consumed with the passage of time, so as to the oil removal rate of increase was not obvious. In order to improve the oil removal rate, nutrition can be added to the bottle; meanwhile, fresh mineral salt liquid media need to be replaced, because degradation of petroleum products by G-94 may be toxic.

Oil degradation is limited by many factors in the soil [14] [15]. The development and utilization of the genetic resources of G-94 for petroleum degradation and their application in the remediation of petroleum contaminated soils should be further studied.

4.2. Conclusion

The optimal conditions of oil removal rate of G-94 were 28˚C, pH 7.8, oil concentration 0.3%, salt concentration 0.5%, inoculation amount 4%, N and P was $(NH_4)_2SO_4$ and K_2HPO_4. Under orthogonal optimum conditions, the oil removal rate of G-94 reached to 25.83%, 28.56% and 30.90% on the 10th, 20th and 40th day, respectively.

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