



# Bioremediation of Oil Contaminated Soil

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## Abstract

**Objective:** In order to study the microbial remediation of oil contaminated soil. **Methods:** The method of soil composting was adopted. G-40, G-94 and G-40 + G-94 were added into the soil with 2% oil, adding 20% bran and adding distilled water to keep the soil water content in 35%, and it was incubated at 35°C. After sampled, the alkali hydrolysable nitrogen, available phosphorus and oil content were measured. **Result:** The oil removal rates of G-40, G-94 and G-40 + G-94 treatment groups were 29.08%, 31.09% and 32.68% on 100<sup>th</sup> day, respectively. **Conclusion:** This study provides a reference for microbial remediation of petroleum contaminated soil.

## Subject Areas

Environmental Science

## Keywords

*Brevibacillus laterosporus*, *Candida tropicalis*, Oil Degrading

## 1. Introduction

Petroleum hydrocarbons are a major energy source. However, environmental contamination by petroleum hydrocarbons has become a serious problem all over the world. The leakage of petroleum hydrocarbons to nature causes the disruption of the natural ecosystem since petroleum hydrocarbons contain many kinds of toxic compounds [1]. Fortunately, the degradation of oils in the environment is possible through several techniques: physical, chemical or biological [2]. Compared with biological methods, physical and chemical methods may produce secondary pollution to repair oil contaminated soil [2]. There are a lot of reports about microbial remediation of oil contaminated soil [3] [4] [5]. Thus, the present study focused on the ability of G-40, G-94 and G-40 + G-94 to repair

oil contaminated soil.

## **2. Materials and Methods**

### **2.1. Experimental Sample**

Soil sample: From the experimental base of the Agricultural College of Yangtze University, the samples were collected, and after the air-dry, 40 mesh sieves were grinded, and the follow-up experiment was left to be used.

### **2.2. Media**

Beef peptone liquid medium, potato sucrose liquid medium, according to reference [6].

### **2.3. Strain**

Degrading microorganism: G-40 and G-94 were isolated from Qiangjiang Guanghua Oilfield on June 2015, stored in the laboratory of College of Life Science, Yangtze University [7] [8]. G-40 and G-94 were activated in beef peptone liquid medium and potato sucrose liquid medium, respectively.

### **2.4. Petroleum Contaminated Soil**

The oil was dissolved in petroleum ether, and the soil was added to the soil to dry the 7 d, during which the petroleum ether was completely volatilized, that was, the soil containing 2% of the oil.

### **2.5. Alkali Hydrolysable Nitrogen**

Alkaline solution diffusion method is for alkali hydrolysable nitrogen [9].

### **2.6. Available Phosphorus**

NaHCO<sub>3</sub> extraction method is for available phosphorus [10].

### **2.7. Oil Removal Rate**

The oil removal rate was determined by gravimetric method [11].

### **2.8. Experimental Design**

In this experiment, 3 treatments and 1 control (no inoculation) were set up: G-40 (inoculation 8%), G-94 (inoculation amount 4%), G-40 (inoculation amount 8%) + G-94 (inoculation amount 4%). The experiment was carried out in tissue culture bottles. 200 g bottles of petroleum contaminated soil were added to each bottle, plus 20% bran. After adding the activated strains, the distilled water was added to make the soil moisture content reach 35%. After autoclaving, the degrading bacteria were inoculated and placed at 35°C incubator. The soil samples of 1<sup>th</sup>, 5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup>, 20<sup>th</sup>, 30<sup>th</sup>, 40<sup>th</sup>, 100<sup>th</sup> were used to determine soil alkali hydrolysable nitrogen, available phosphorus and oil content. The experimental design is shown in **Table 1**.

**Table 1.** Experimental design table.

Treatment	Soil (g)	Strain	Inoculation amount
1	200	G-40	8%
2	200	G-94	4%
3	200	G-40 + G-94	8% + 4%
CK	200	-	-

-: no.

### 3. Results and Analysis

#### 3.1. Alkali Hydrolysable Nitrogen

The content of alkali hydrolysable nitrogen is shown in **Figure 1**. As can be seen from **Figure 1**, with the increase of time, the content of alkali hydrolysable nitrogen in the 3 treatment groups increased from 1<sup>th</sup> to 10<sup>th</sup> d. The content of alkali hydrolysable nitrogen was 0.839, 0.832, 0.872, 0.968 g/kg of CK, G-40, G-94 and G-40 + G-94, on the 10<sup>th</sup> d, respectively. After that, the change of the content of alkali hydrolysable nitrogen was not obvious until 100<sup>th</sup> d. The content of alkali hydrolysable nitrogen reached 0.848, 0.905, 0.905, 0.980 g/kg on 100<sup>th</sup> d, which was 1.05%, 8.78%, 3.75%, 1.33% higher than that of CK, G-40, G-94 and G-40 + G-94, on the 1<sup>th</sup> d, respectively. From the beginning of 10<sup>th</sup> d, the content of alkali hydrolysable nitrogen of the G-40 + G-94 treatment group was higher than that of the G-40 and the G-94 treatment groups.

#### 3.2. Available Phosphorus

The content of available phosphorus is shown in **Figure 2**. P standard curve can be seen from the **Figure 3**. As can be seen from **Figure 2**, the content of available phosphorus in the 3 treated groups did not change basically from 1<sup>th</sup> to 40<sup>th</sup> d. After 40<sup>th</sup> d, the content of available phosphorus rose sharply. The content of available phosphorus was 6.105, 6.924, 2.569, 2.870 g/kg of CK, G-40, G-94 and G-40 + G-94, on the 1<sup>th</sup> d, respectively. The content of available phosphorus reached 27.158, 30.247, 31.887, 31.997 g/kg on 100<sup>th</sup> d, which was 344.85%, 336.84%, 1141.22%, 1014.88% higher than that of CK, G-40, G-94 and G-40 + G-94 on the 1<sup>th</sup> d, respectively.

#### 3.3. Oil Removal Rate

The result of oil removal rate is shown in **Figure 4**. As can be seen from **Figure 4**, microbes have a strong adaptability to the environment. At the time of 5<sup>th</sup> d, the oil removal rates of 3 treatments were 4.85%, 4.95% and 5.23%, respectively. The oil removal rate of G-40 reached 5.04% on 15<sup>th</sup> d, which was 3.92% higher than that of 5<sup>th</sup> d. The oil removal rate of G-94 reached 6.86% on 15<sup>th</sup> d, which was 38.59% higher than that of 5<sup>th</sup> d. The oil removal rate of G-40 + G-94 reached 13.68% on 15<sup>th</sup> d, which was 161.57% higher than that of 5<sup>th</sup> d. The oil removal rate of the 3 treatments is increasing, and the oil removal rate of the

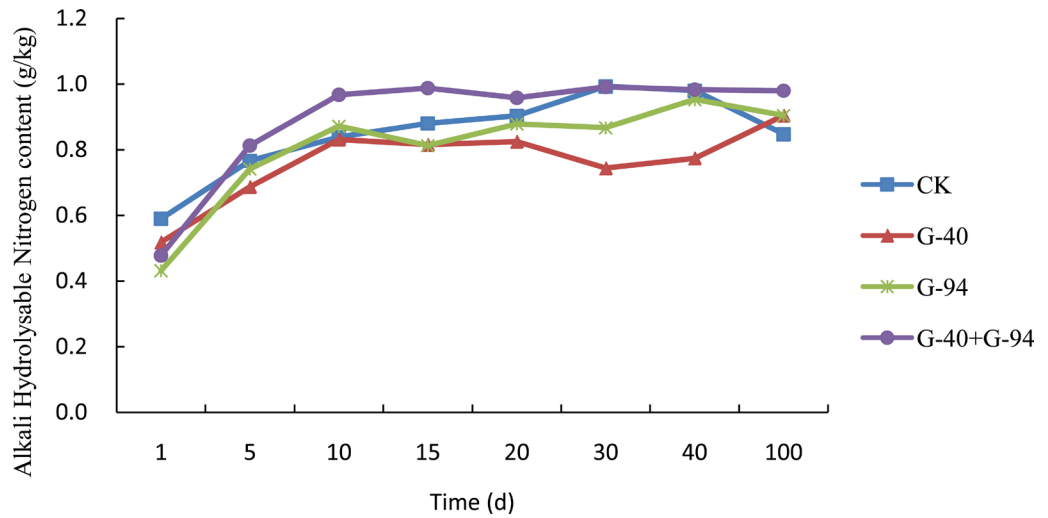


Figure 1. Alkali hydrolysable nitrogen content on different times.

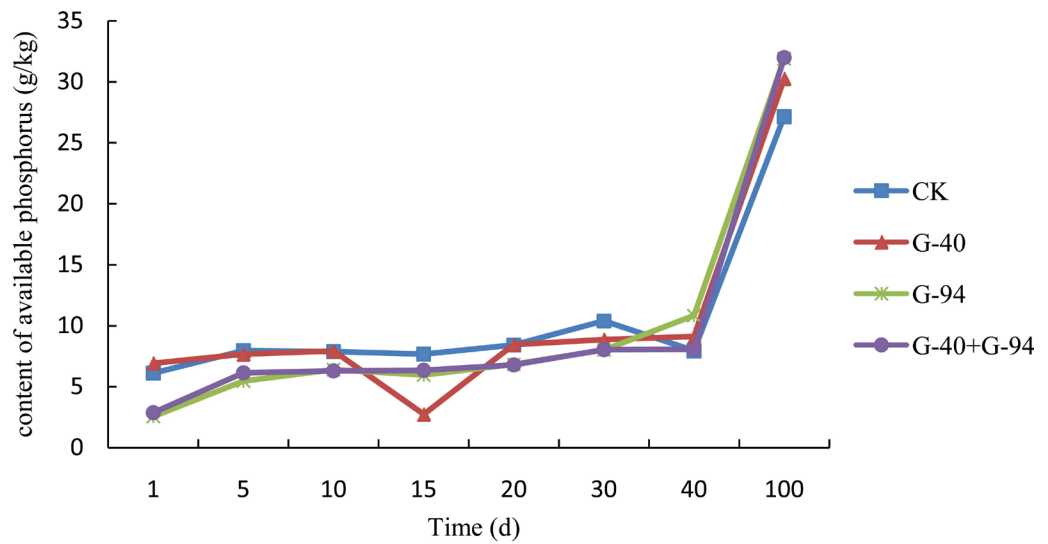


Figure 2. Available phosphorus content on different times.

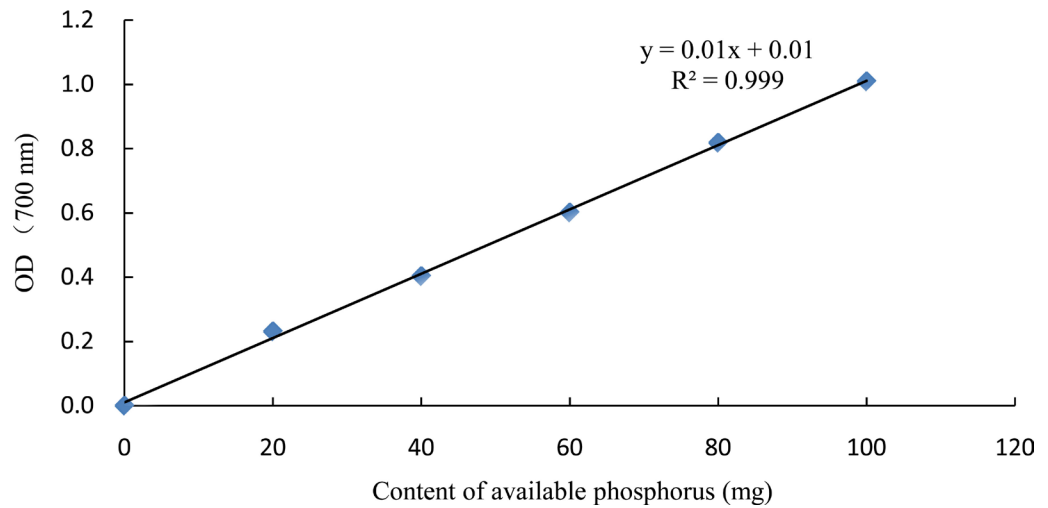
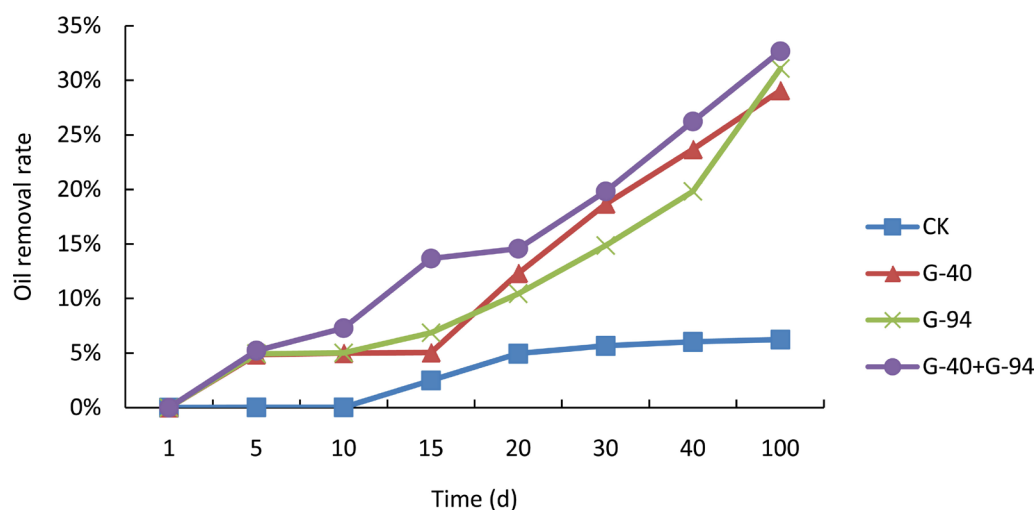


Figure 3. P standard curve.



**Figure 4.** The oil degrade rate affected by different times.

G-40 + G-94 treatment group is higher than that of the G-40 and the G-94 treatment group from 15<sup>th</sup> to 100<sup>th</sup> d. The oil removal rates of G-40, G-94 and G-40 + G-94 treatment groups were 29.08%, 31.09% and 32.68% on 100<sup>th</sup> d, respectively.

## 4. Discussion and Conclusion

### 4.1. Discussion

The process of oil removal rate on microorganism is very complex. Its oil removal rate depends not only on the microbial community and composition, but also on the number and status of TPH, the surrounding environment and many other factors. Soil structure, parent material and moisture content also have great influence on the oil removal rate of microorganism. Appropriate nutrients can be added, and proper amount of water is added to ensure the rapid growth and propagation of microorganisms, so as to achieve high oil removal rate [12].

The composition of oil is complex, and it cannot be restored by one or two microbes alone. In this experiment, microbial degradation of petroleum efficiency is low, and can be considered to increase the degradation of petroleum by different kinds of microorganisms in order to achieve higher degradation efficiency [2].

### 4.2. Conclusion

The oil removal rate of G-40 + G-94 (32.68%) treatment was higher than that of G-40 (29.08%) and G-94 (31.09%), but the synergistic degradation of oil between G-40 and G-94 is not obvious.

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