The dynamic change of microbial communities in crude oil-contaminated soils from oilfields in China

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Abstract

To study the biodegradability of microbial communities in crude oil contamination, crude oil-contaminated soil samples from different areas of China were collected. Using polyphasic approach, this study explored the dynamic change of the microbial communities during natural accumulation in oilfield and how the constructed bioremediation systems reshape the composition of microbial communities. The abundance of oil-degrading microbes was highest when oil content was 3%-8%. This oil content is potentially optimal for oil-degrading bacteria proliferate. During a ~12 months natural accumulation, the quantity of oil-degrading microbes

increased from 10^5 to 10^8 cells/g of soil. A typical sample of Liaohe (LH, oil-contaminated site near Liaohe river, Liaoning Province, China) was remediated for 50 days to investigate the dynamic change of microbial communities. The average FDA (a fluorescein diacetate approach) activities reached 0.25 abs/h·g dry soil in the artificially enhanced repair system, 32% higher than the 0.19 abs/h·g dry soil in natural circumstances. The abundance of oil-degrading microbes increased steadily from 0.001 to 0.068. During remediation treatment, oil content in the soil sample was reduced from 6.0% to 3.7%. GC-MS analysis indicated up to 67% utilization of C_{10}-C_{20} normal paraffin hydrocarbons, the typical compounds that undergo microbial degradation.

Keywords: Crude oil; oil-contaminated soils; oil-degrading microbes; microbial dynamic change

1 Introduction

The negative impacts of oil contamination on the quality of soil and groundwater throughout the oil exploitation, transportation, preservation and utilization process, have been widely studied in recent years, as the development of the oil industry (Sun et al. 2016, Zhang et al. 2013). Hydrophobic petroleum hydrocarbons, filling the soil pore spaces and decreasing the aeration and water permeability of soils, play an important role in water infiltration and nutrient transportation. This results in the destruction of the natural environment of the oilfields from oil contamination (Xia 2001). In addition, sharply increased eco-toxicity, vulnerability and aberrance of the ecosystem, as well as physiological turbulence of plants, have been reported in several studies (Li et al. 2016). Microbial communities are essential for the ecological function of soils and the efficiency of natural bioremediation. The metabolic activities of indigenous or exogenous microorganisms ameliorate soil condition accelerate the decomposition of petroleum hydrocarbons and other pollutants (Wang et al. 2015), thus reshaping the microbial communities in adaptation to oil contamination (Van Elsas et al. 1998, Zhang et al. 2006).

Microbial population, activity and pollutant levels vary significantly among different soil samples (Van Elsas et al. 1998, Sandaa et al. 2001). Microbial communities, as one of the most important components in ecological soil systems (Jia et al. 2004), especially the subpopulations with petroleum degradability, are extremely critical for the bioremediation efficiency of contaminated soil (Pinholt et al. 1979, Jones et al. 1983). In general, indigenous microbes can be promoted by oil contamination in soils, which are capable of oil degradation by using petroleum hydrocarbons as their carbon source (Xu et al. 2016). Moreover, abiotic parameters such as oil content also influence the bioremediation process by promoting or inhibiting the activity of

microorganisms based on different mechanisms (Godbout et al. 1995, Oudot et al. 1998, Morasch et al. 2001). Therefore, the bioremediation efficiency can be achieved by adjusting the abiotic factors using the relationship between the microbial communities and the abiotic factors (Atlas 1995, Zhong et al. 2006). Previously studies and their corresponding discoveries are based mostly on the bioremediation experiments performed in the laboratory or on a single pilot study.

In this paper, with the aim to explore the potential of microbial degradation in oil-contaminated soils, 27 oil field oil-contaminated soils were sampled and analyzed. The changes of abiotic factors, including oil contents and components, and biotic factors, such as microbial quantity and activity, were compared under different degradation conditions.

2 Materials and methods

2.1 Crude oil-contaminated soil sampling

A total of 27 crude oil-contaminated soil samples (Table 1, Figure 1) from 7 sites were collected and analyzed, covering the whole life cycle of oil exploitation, oil well examination, and petroleum pipeline leakage in each oilfield.

Contaminated soils were sampled at the surface layer (0-20 cm) using aseptic sampling procedures. Samples were immediately sealed in aseptic plastic bags and transported to the laboratory. They were subsequently aliquoted into several containers, one of which (50-100 g) was stored at 4℃ for microbial analysis within one week (from the time of sample collection).

The soil samples were analyzed for three experimental settings (Table 1). The first aim (samples 1-27) was to study the level of soil contamination under ambient conditions in the 27 soils. The second aim was to study the level of soil contamination during natural accumulation. For this

purpose, we selected the samples from Shengli (samples 23-26) because they are silty soil and allow air permeability. The third aim was to test the effects of the artificial enhanced repair system. For this approach, the sample Liaohe (LH, one oil-contaminated site near Liaohe river, Liaoning Province, China) was selected (sample 27). The contamination level of Liaohe is 6%, an oil content that is suitable for the growth and multiplication of microorganisms. In addition, a large amount of soils for the artificially enhanced repair system was required, and sample LH could supply enough material for the test. During this study we collaborated with the Liaohe oilfield on the remediation of contaminated soil.

Soils were sieved over a 2 mm mesh. They were then divided into two different groups, one enhancement repair material each group: AC (activated carbon) and biochar B (a kind of processed fern, which is an organic adsorption material) (Table 2), A control treatment, without any repair material, was carried out at the same time. All the other parameters in the repair system were kept consistent and unchanged.

2.2 Basic physicochemical parameters

2.2.1 pH and particle size analysis

pH value was measured according to the revised methods described in previous study (Jia et al. 2004). The particle size was analyzed using a Mastersizer 2000 laser particle size analyzer.

2.3 Microbial properties analysis

Microbial density and activity were used to characterize the microbial communities in the soil samples. Microbial density was measured using the most probable number (MPN) method (Yu et al. 1990). A beef-extract-peptone medium (10 g/L beef extract, 3 g/L peptone) was used to

quantify the total aerobic microbial density, while minimum medium with 5% (v/v) liquid paraffin media was used to quantify the density of oil-degrading bacteria. MPN tubes were cultured for 72 h and 168 h at 37°C for the total and oil-degrading microorganisms, respectively (Jia et al. 2004).

Microbial activity was analyzed using a fluorescein diacetate (FDA) approach (Adam and Duncan 2001). Two grams of soils were added into a sterilized flask containing 50 mL of potassium phosphate buffer (pH=7.0). The flask was then placed in a shaker at 37°C for 15 min (200 rpm) and 1 mL of FDA (2 g/L, dissolved in acetone) was added to the flask and mixed thoroughly. The flask was then incubated for 1.75 h on the shaker. The soil particles were separated by centrifuge for 5 min (1200 rpm) at room temperature. Microbial activity was measured by reading the absorbance of fluorescein of the supernatant at 490 nm using a spectrophotometer (UV2501PC, Shimadzu Corporation, Japan).

2.4 Crude oil detection methods

2.4.1 Content detection of petroleum hydrocarbon by UV spectrophotometry

To apply the tests close to the real situation, the petroleum hydrocarbons extracted from soil samples were selected as the standard oil. Therefore, the composition in the standard soil and the soil samples is considered to be the same. The standard deviation caused by inconsistencies can thus be eliminated.

The standard curve of petroleum hydrocarbons was constructed as follows. A series of oil standard samples was prepared as 10, 20, 30, 40, and 50 mg/L. Petroleum ether was used as a blank reference, followed by the absorbance of the above-mentioned series of samples was

measured. All samples were loaded into a quartz cuvette (1 cm). A standard curve was then drawn and the oil content (mg/L) of the samples was calculated based on their absorbance.

2.4.2 Detection of petroleum hydrocarbons by GC-MS

To extract oil from the soil samples, the Soxlet extraction method was used. The samples were tested after extraction from solvent evaporation. The oil and gas industry standard "SY/T 5779-2008 crude gas chromatographic analysis methods" was used for the hydrocarbon analysis. Petroleum hydrocarbons were analyzed with an Agilent 7890A/5975C gas chromatograph (GC) with a DB5-MS column (30 m × 0.25 mm × 0.25 μm) and flame ionization detector (FID). Initial column temperature was set at 40°C for 10 min, with 3-10°C/min heating up to 310°C. Once the instrument reached to a steady state, 1.0 μL proceed sample was extracted using a micro-injector, testing began.

The technical control for the GC-MS applied inner standardization and recovery of the standards was achieved. The external standard method employed C13 deuterium as the standard substance. A comparison of the peak area and the peak retention time of the normal paraffin hydrocarbon and those of the C13 deuterium at the same concentration was performed. Subsequently, 1.0 mg/kg deuterium was added to the soil samples before the start of the analysis by the chromatograph. The qualitative calculation was based on the peak area.

3 Results and discussion

3.1 Microbial properties of crude oil-contaminated soils in oilfields

The analysis of oil content (Figure 2) showed large variation in the oil contamination levels among soil samples (samples 1-27) from different oilfields. Oil content varied from as low as

0.04%-0.4% in the light-contaminated oilfields to 0.2%-23% in the heavily-contaminated ones, with an average value of ~10%. Oil content of the investigated oilfields was 500-1000 times higher than the background level (0.002%-0.015% generally) in uncontaminated soils.

According to the microbial characteristics of crude oil-polluted soil samples, total microbial density differed widely from 10^5 cells/g to 10^8 cells/g dry soil among the sampled soils from different oilfields. The density of oil-degrading microorganisms was 2-4 orders of magnitude lower than that of the total microorganisms, generally from 10^3 to 10^5 cells/g dry soil. Besides microbial density, the activity of the microorganisms was also an important index for the assessment of oil-biodegrading ability. FDA activities of sampled soils (Figure 3) ranged from 0.02 to 0.98 abs/h·g dry soil. Indigenous microbial communities adapt to the changes caused in soil ecosystems by hydrocarbons (Balba et al. 1998, Hawari et al. 2001). The density of oil-degrading microorganisms increased with the oil content in the soils when oil content was less than 8%. The data suggests that oil contamination of 3%-8% may actually promote the growth and reproduction of oil-degrading bacteria while oil content higher than 8% could inhibit microbial activity. Under certain environmental conditions, 3%-8% oil content in the soil may acclimated microbes adapt to oil-contaminated environments. In addition, crude oil, being a hydrophobic substance, adheres to soil pores and reduces the effective porosity, thereby reducing the moisture content of the soil. As a restrictive ecological factor, decreased moisture results in reduced quantity of microorganisms. Only when a certain range of oil content brings sufficient carbon source but no toxicity, could it be suitable for microbial growth. Crude oil contamination may lead to selective enrichment of indigenous microbial populations with the ability to degrade

oil in the soil, while other microbes are suppressed or inhibited, because of the oil-altered environment.

3.2 The dynamic change of microbial properties during natural accumulation and enhanced remediation of crude oil-contaminated soils

3.2.1 Change of m*icrobial properties during natural accumulation*

Comparison of the sampled soils (samples 23-26) under different conditions (Figure 4) demonstrated that the oil contamination, over a 12 months accumulation, changed the soil dramatically. The number of oil-degrading microbes increased significantly from approximately 10^4 cells/g to 10^8 cells/g. Under simulated natural accumulation condition and with the absence of external influence, the soil became short of nutrients and there had a decline proportion of oil, especially after being cultured for a period without nutrient supplements. Native soil microbes adjust their adaptability and metabolic mechanisms in order to increase the chance of survival in their environment. Ultimately, a dominant population of petroleum hydrocarbon-degrading microbes is formed. In the natural environment, due to the breadth of the presence of microorganisms, a certain number of oil-addicted microbes will be produced by natural acclimation (Scherr et al. 2007). Study has shown that (Yu et al. 2009), under normal circumstances, hydrocarbon-degrading bacteria accounted for only 1% of the microbial community, however with oil pollution, the hydrocarbon-degrading bacteria can increase up to 10%.

3.2.2 The dynamic change of microbial properties during enhanced bioremediation

Sample LH (sample 27) from Liaohe (oil-contaminated site near Liaohe river, Liaoning Province, China) was divided into seven groups. In each group, different repair materials were added, including activated carbon and biochar B (Table 2). Activated carbon is a traditional adsorbent, with large specific surface area and strong adsorption capability. Biochar B is a processed fern, one type of biochar. Biochar is made up of carbon-rich biomass that proceeds after cracking under conditions of incomplete combustion (Guo et al. 2015). Turning biomass into carbon biomass increased the waste resource reuse. Biochar is mainly composed of single rings and polycyclic aromatic compounds, leading to high chemical and biological stability. Biochar mainly includes elements C, H, O and N, and it also contains numerous other nutrients needed by plants, for instance P, S, K, Ca and Mg (Tsai et al. 2012). They are rich in oxygen-containing functional groups on the surface, such as -COOH, -CHO, -OH. The negative charge on the surface has a high cation exchange capacity (CEC) (Wang et al. 2012, Yuan and Xu 2011). Therefore biochar has a strong adsorption capacity. Moreover complex pore structure and large surface area also features of this kind of material. Biochar is similar as activated carbon based on these characteristics but has advantages of a wide source of raw materials and the low production costs. Thus the remediation efficiency can decide whether it could become a substitute for activated carbon. Khokhlova et al. took advantage of its large surface area and explored the feasibility of biochar in oil-degradation efficiency (Khokhlova et al. 2010). First, oxidizing microorganisms were fixed on the surface of biochar, next the biochar was added to the oil contaminated soil. Then the oil degradation efficiency was studied. Oil degradation was 40%-50% after 10-14 days, exceeding 90% after 30-40 days. In addition, the added biochar in

the soil leads to changes in soil properties, which might be a positive force for soil remediation. Hua et al. used straw biochar and found a better retention of nutrients effectively reducing water erosion caused by nitrogen and phosphorus losses (Hua et al. 2010). Zhou et al. added straw biochar and pine twigs biochar into soil and cultivated for 45 days (Zhou et al. 2011). Experiments showed that the contents of organic carbon, humic acid, fulvic acid and available nutrients all have different degrees of increase.

Both the numbers of aerobic microbes and oil-degrading microbes increased after 50 days (Figure 5 a, b, c, d, e, f, g). The amount of oil-degrading microbes exceeded 10^5 cells/g, 267% of the original. The abundance of oil-degrading microbes increased steadily, with the highest abundance reaching 0.068 from 0.001. Meanwhile, the FDA activity of the samples (Figure 5 a, b, c, d, e, f, g) increased steadily too. Treated with different repair materials, FDA activities in LH artificially enhanced repair system had an average level of 0.25 abs/h·g dry soil, which is 32% higher than the 0.19 abs/h·g dry soil in natural circumstances. With the repair system running, the oil content in the soil sample was reduced to 3.7% from 6%. The petroleum hydrocarbon contamination was significantly reduced. The increase of FDA activities indicates an enhanced remediation with the combination of microbiology and repair materials. Since soil enzyme activity also reflects the fertility and biogeochemical cycles of elements in soil, there was also an improvement in the soil micro-ecological environment. The increase of activities and quantity indicates that the soil is more suitable for microbe growth.

Besides, by comparing the Figure 5 (a)(b)(c) and (d)(e)(f), the two types of repair materials did not show significant difference on oil removal efficiency. While the FDA in Figure 5 (a)(b)(c) and (d)(e)(f) showed difference between the two types of materials, and there is more substantial

growth on FDA in biochar B group (Figure 5 d, e, f). However, whether biochar can create a more suitable environment for microbial growth by its own degradation of organic compounds remains unclear.

3.3 Relationship among the crude oil components

Of the seven groups, group K was selected for further analysis. A GC-MS test was performed to quantify the petroleum hydrocarbons in sampled soil (Figure 6 a, b, c). This test demonstrated the mechanisms of microbial remediation in petroleum-contaminated soil. From the chart (Figure 6 a, b, c), the decline in oil content was followed by a decrease of components of petroleum, which is degradable by microbes.

According to our data, analysis of fresh soil allows for calculation of the average hydrocarbon abundance. The average abundance of C_{10}-C_{20} was about 900,000. After 30 days, the value decreased to 570,000, and reduced to 300,000 after 50 days. A great increase in the biodegradation activity led to a 67% utilization of microbial-degradable components.

Researchers (Yu et al. 2009) screened *Bacillus subtilis* and *Bacillus licheniformis* from oil-contaminated soil sampled from Liaohe oil field and Daqing oil filed. These two bacteria were applied for degradation on *n*-alkanes, aromatics and asphalt. And the removal efficiency was n-alkanes 62.96-78.67%, aromatics 16.76%-33.92%, asphalt 3.78%-15.22%. Also in Chen's work, 60% and 68% *n*-alkanes were degraded by *Streptomyces atrovirens* and *Pseudomonas monteilii*, respectively (Chen et al. 2016). In a similar work, 54%-58% of two-six-ring PAHs remained in sediment (Xu and Obbard 2004). While in Douglas' research, over 88% of the two-, three-, and

four-ring PAHs compounds of semivolatile hydrocarbon were degraded by 20 weeks (Douglas et al. 2012).

The literature (Jia et al. 2012) suggests that the degree of difficulty of alkane biodegradation can be summarized as: C5-C15 is very easily degradable, C15-C20 is easily degradable, and >C20 is hard to be degraded by microbial action. In a similar way, alkane composition is a direct factor affecting biodegradation as previously reported (Yu et al. 2009). When the carbon number is greater than 18, the decomposition becomes difficult. The degradation rate of medium-chain alkane (C_{10}-C_{22}) is the fastest (Lin 2009, Yang and Jiang 2011). In addition, Straight-chain alkanes are more likely to be oxidized than branched-chain alkane cycloalkanes.

About the unresolved complex mixture (UCM), in non-biodegraded oil the UCM may comprise less than 20% of the total area of the chromatogram, while in biodegraded oils this figure can raise to over 80%. The UCM in the gas chromatograms revealed a priority in molecular weight as biodegradation advanced (Douglas et al. 2012). One possibility of the mass UCM is generated during the microbial degradation. Another opinion (Killops and Al-Juboori 1990) concerned that it is unlikely biodegradation would produce such a wide range and number of complex hydrocarbons as appears to be present in the UCM. More likely is that the major part of the UCM is generated from the kerogen matrix as part of the natural petroleum generation process. An UCM would be anticipated to occur in all petroleum, but it is not observed at the concentrations involved in capillary GC analysis of non-biodegraded oils where *n*-alkanes and other resolved components are present.

4 Conclusions

Oil content is a critical factor affecting microbial communities in crude oil-contaminated soils in the 27 samples studied. In sampled soils containing less than 8% oil, the total and oil-degrading microbial densities were higher than 10^8 cells/g and 10^5 cells/g dry soil, respectively. The highest FDA activity was more than 0.3 abs/h·g dry soil and the average FDA activity was 0.19 abs/h·g dry soil, under natural circumstances.

In the repair system, the amount of oil-degrading microbes exceeded 10^5 cells/g, which is 267% of the original. The abundance of oil-degrading microbes increased, with the highest abundance of 0.068 from 0.001. With the repair system running, the oil content in the soil sample was reduced to 3.7% from 6%. The average level of FDA activities reached 0.25 abs/h·g dry soil in the artificially enhanced repair system, which is 32% higher than the natural condition. The abundance of the biodegradable oil component was reduced from 900,000 to 300,000.

In remediation progress, the structure of microbial communities in crude oil-contaminated soils is re-adjusted. In the oil-contaminated environment, the amount of total microorganisms stays steady while other microorganisms decrease in abundance. Those adapting to the oil-contaminated environment can survive and multiply, whereas the other un-adapted species are eliminated from such an environment. With a suitable level of crude oil pollutant as carbon source and the appropriate environmental conditions, the oil-degrading bacteria can be encouraged, resulting in high bioremediation efficiency. Currently over 100 genera and 200 species of microbial have the ability to oxidize and degrade petroleum hydrocarbons (Wang et al.

2014). To sum up, microbial remediation supplemented by enhanced materials is a promising remediation technology.

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Table 1. Basic physicochemical parameters of the sampling crude oil contaminated soils in six oilfields of China.

Number	Oilfield	Coordinator	Soil texture	pH	Contamination level (%)
1	Changqing	34°19’36.17’’N	silty soil	7.76	0.00
2		108°56’55.40’’E		7.87	0.06
3				7.73	0.07
4				7.87	0.26
5				7.55	0.32
6				7.46	0.36
7				7.49	0.40
8				7.54	0.43
9	Daqing	46°37’00.62’’N	silt loam	7.29	5.05
10		124°53’12.81’’E		7.82	4.67
11				7.92	5.53
12				7.96	2.05
13	Yumen	39°43’29.26’’N	silt loam	7.85	4.63
14		98°29’40.19’’E		7.93	1.59
15				7.86	10.93
16	Jianghan	30°26’51.37’’N	coarse	8.00	15.12
17		112°41’39.57’’E	sand	7.19	12.37
18				7.59	17.41
19				7.92	5.53
20	Huabei	38°40’36.5’’N	loam	7.82	6.07
21		116°5’55.59’’E		7.49	6.71
22				7.6	7.7.3
23	Shengli	37°25’20.92’’N	silty soil	7.32	10.26
24		118°22’04.27’’E		7.75	16.22

25				7.85	21.13
26				7.51	23.83
27	Liaohe	41°09’33.67’’N 122°05’58.98’’E	sandy loam	7.3	6

Table 2. Repair materials in each treatment.

Treatment	Sample weight/g	Oil content / %	Volume Portion (oil:material)	Material weight/g	Repair material
B4	800	6	4:1	12	B*
B8	800	6	8:1	6	B*
B15	800	6	15:1	3	B*
C4	800	6	4:1	60	AC**
C8	800	6	8:1	30	AC**
C15	800	6	15:1	15	AC**
K	800	6	-	-	-

B* for biochar, one kind of processed fern which is an organic plant adsorption material.

AC** for active carbon (cylinder particle with length 1 cm and radial 5 mm).

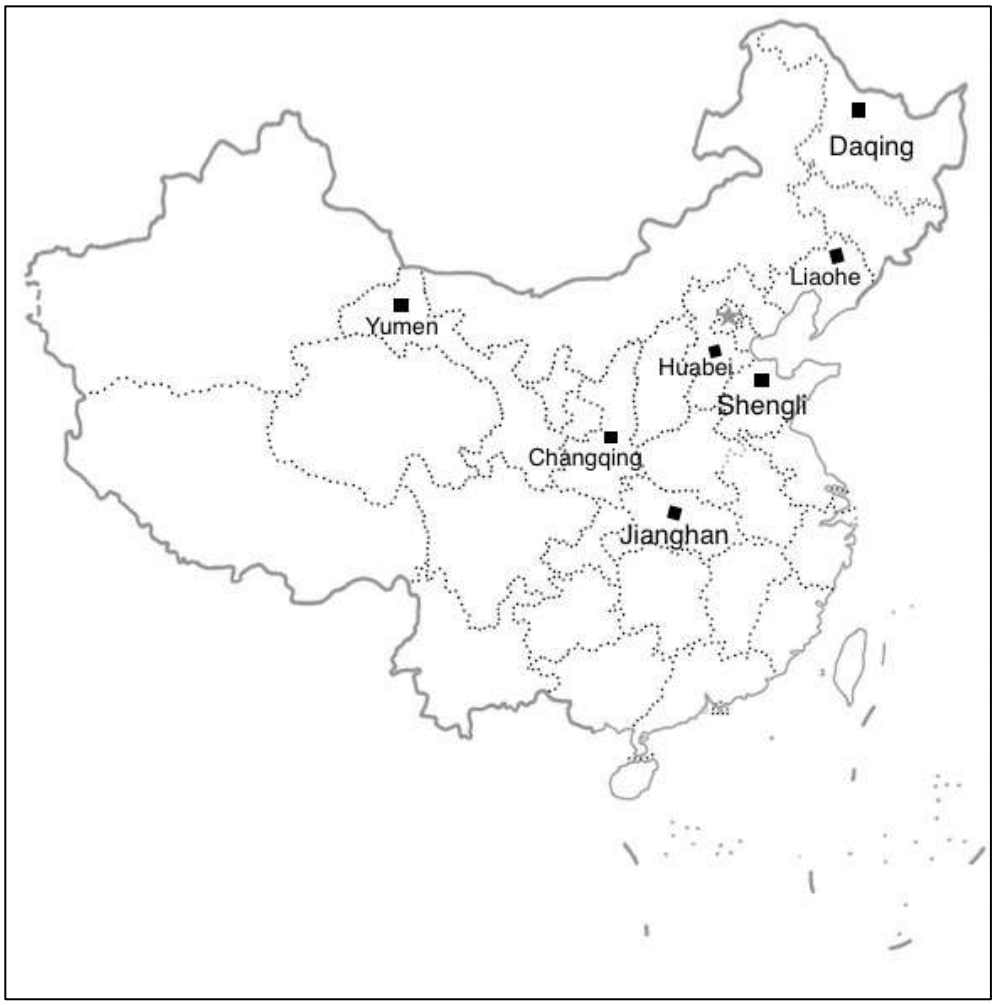


Figure 1. Sampling sites.

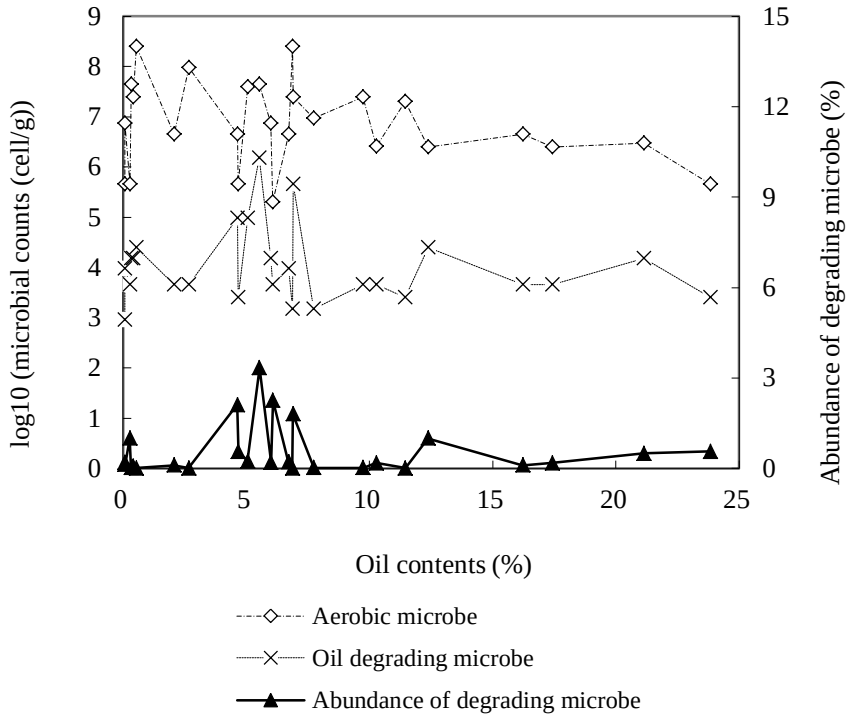


Figure 2. The abundance of oil degrading microbe in total aerobic microbe during the change of oil contamination level in oilfields soils.

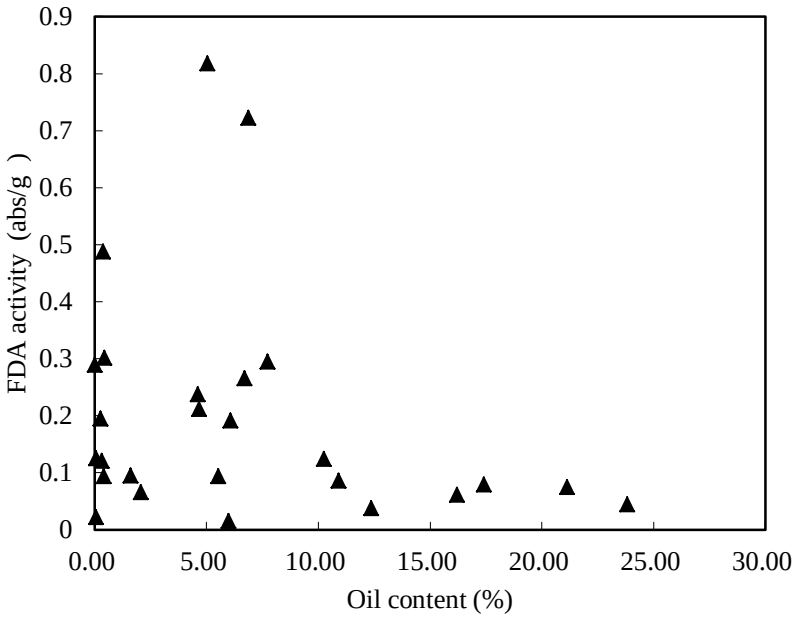


Figure 3. Microbial FDA activities in sampled soils

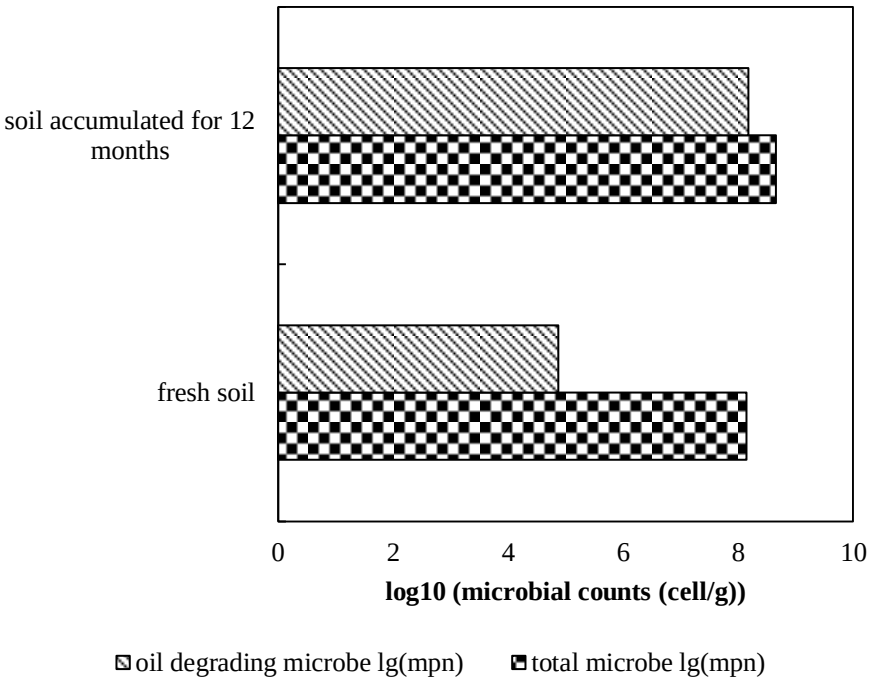
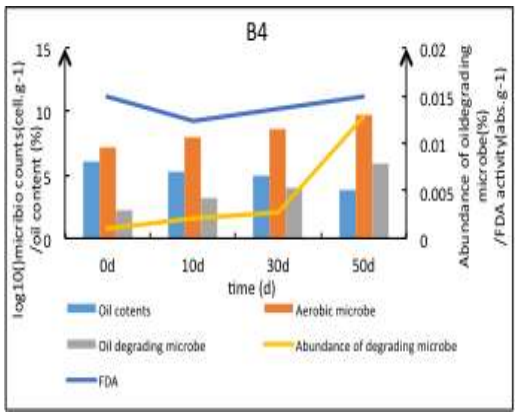
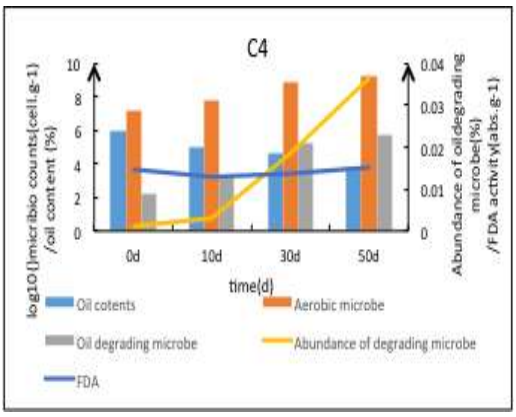


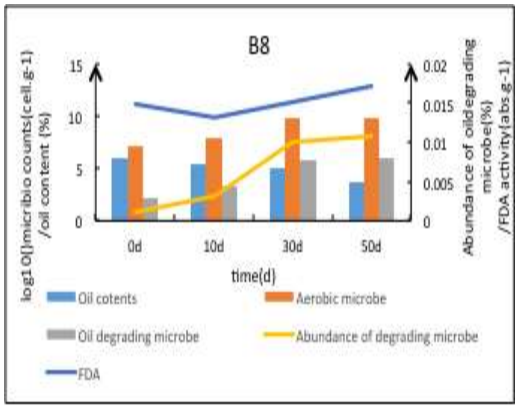
Figure 4. The change of soil microorganisms amount under natural accumulation during 12 months.


B4
log10(microbio counts(cell.g-1)
/oil content (%)
Abundance of oildegrading microbe(%)
/FDA activity(abs.g-1)
0d
10d
30d
50d
time (d)
Oil cotents
Aerobic microbe
Oil degrading microbe
Abundance of degrading microbe
FDA

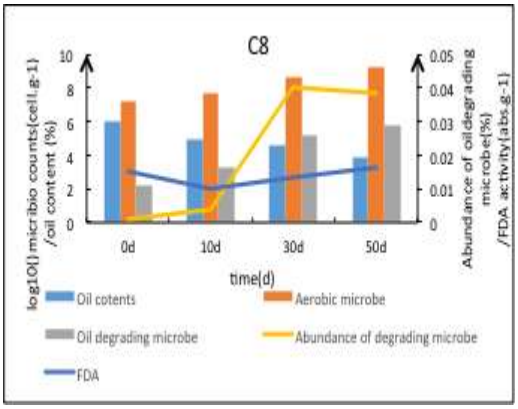
(a)


C4
log10(microbio counts(cell.g-1)
/oil content (%)
Abundance of oildegrading microbe(%)
/FDA activity(abs.g-1)
0d
10d
30d
50d
time(d)
Oil cotents
Aerobic microbe
Oil degrading microbe
Abundance of degrading microbe
FDA

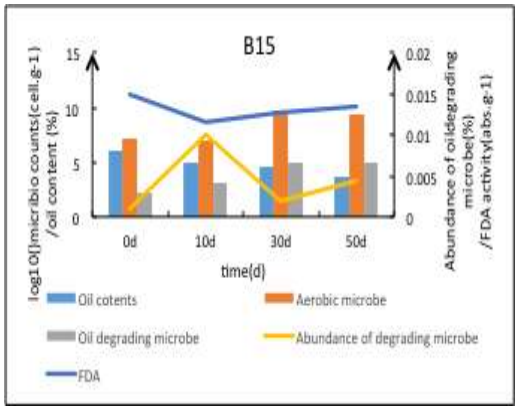
(d)


B8
log10(microbio counts(cell.g-1)
/oil content (%)
Abundance of oildegrading microbe(%)
/FDA activity(abs.g-1)
0d
10d
30d
50d
time(d)
Oil cotents
Aerobic microbe
Oil degrading microbe
Abundance of degrading microbe
FDA

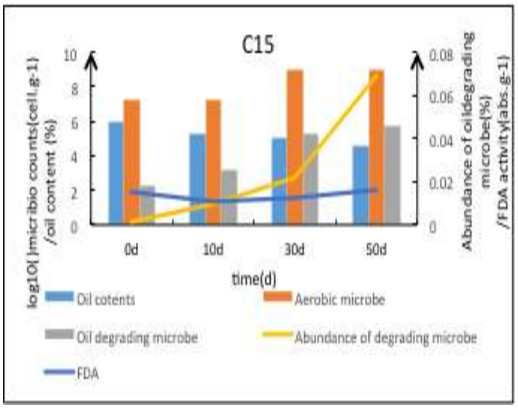
(b)


C8
log10(microbio counts(cell.g-1)
/oil content (%)
Abundance of oildegrading microbe(%)
/FDA activity(abs.g-1)
0d
10d
30d
50d
time(d)
Oil cotents
Aerobic microbe
Oil degrading microbe
Abundance of degrading microbe
FDA

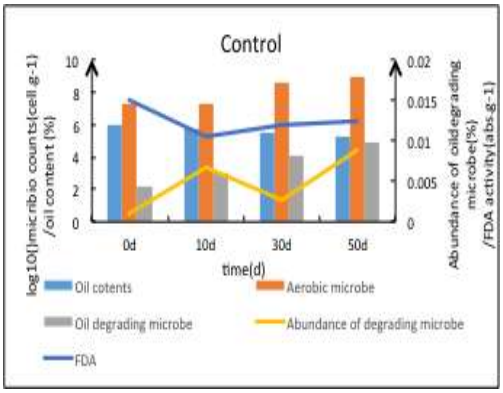
(e)


B15
log10(microbio counts(cell.g-1)
/oil content (%)
Abundance of oildegrading microbe(%)
/FDA activity(abs.g-1)
0d
10d
30d
50d
time(d)
Oil cotents
Aerobic microbe
Oil degrading microbe
Abundance of degrading microbe
FDA

(c)

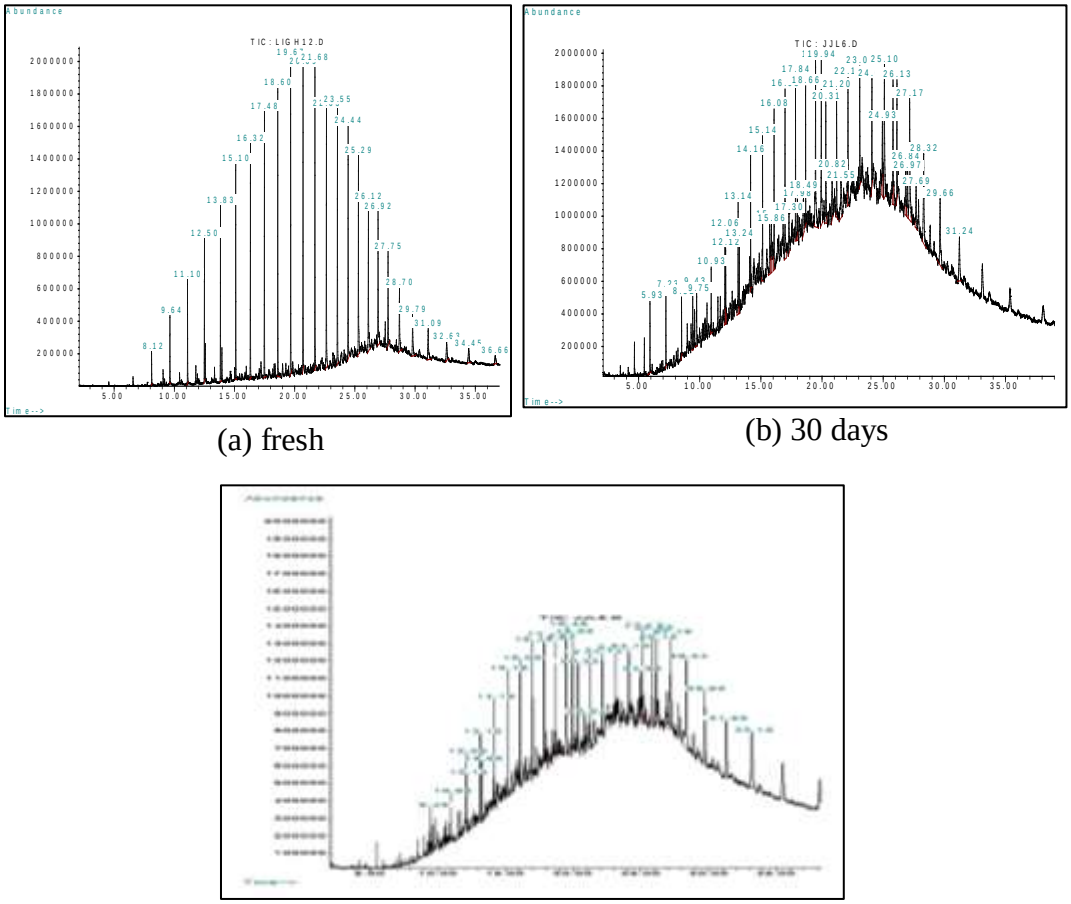

C15
log10(microbio counts(cell.g-1)
/oil content (%)
Abundance of oildegrading microbe(%)
/FDA activity(abs.g-1)
0d
10d
30d
50d
time(d)
Oil cotents
Aerobic microbe
Oil degrading microbe
Abundance of degrading microbe
FDA

(f)



(g)

Figure 5. The dynamic change of microbial properties during enhanced bioremediation.



(a) fresh (b) 30 days

(C) 50 days

Figure 6. The dynamic change of the crude oil components.