Status quo of soil petroleum contamination and evolution of bioremediation

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Abstract: Along with the rapid development of oil industries internationally, petroleum prospecting and exploitation activities are growing intensively. Especially in China, with the fastest economic growth in the world and shortage of petroleum resources, we are leading the practices of petroleum deep exploitation. Obviously, the risk of damage to the natural environment from these activities is high. Oil contamination in soils and groundwater is becoming a big issue along with pesticide pollution, which makes organic pollution prevention and control (OPPC) much more complex. In this paper, based on recent research on oil-contaminated soil at home and abroad, we make comments on the remediation technologies for polluted soil, emphasizing bioremediation techniques and degradation mechanisms in order to push forward research into bound organic pollution prevention and control (OPPC), especially in China.

Key words: Soil pollution, crude oil, bioremediation, organic pollution, biotransformation, polycyclic aromatic hydrocarbons

1 Introduction

In the last thirty years, petroleum prospecting, exploitation and refining activities have soared with the rapid increasing development of oil industries, synchronously with China's world-leading economic growth. Simultaneously, the environment pollution is catching the public's and scientists' attention because the limited resources inland force the petroleum and petrochemical industries into profound exploitation to meet China's oil thirst. For example, wells are being drilled at higher temperatures and at deeper depths, and adding much more drilling mud additives increased from the former 20 types to more than 100 types (but it was out of this paper's scope), leaving us to face increasingly higher pressure for environment protection. Though oil pollution in soil and groundwater was reported in the beginning of 1940s, the issues have been only paid serious attention in China in recent years, one big pollution problem after pesticide pollution. Recently, most countries in the world begin to focus on bioremediation of oil-contaminated soil and groundwater to recover from polluted ecology and to protect their agriculture and living environment, while avoiding secondary pollution.

2 The petroleum-caused pollution in the soil and its sources

Processes in oil industries, mainly including prospecting, exploitation, gathering and transportation, refining, and even sales and trading of oil products, cause different kinds and extents of contamination to the surrounding soils (Fig. 1). These are generally classified into three sorts: pollution from prospecting and exploitation, pollution from petroleum and petrochemical industries' production and those from facilitating and auxiliary processes (Chen, 2005). We could cover a large range of chemicals, such as variable additives and auxiliaries, but in this paper, we focus on the pollution of petroleum (consisting components of saturates, aromatics, resins, asphaltenes, and unresolved complex mixture (UCM or hump)). It is obviously and publicly accepted that aromatics (such as benzene, toluene, ethylbenzene and xylenes (BTEX) and polycyclic aromatic hydrocarbons (PAHs)) pose high risks of ecological and environmental toxicity (He et al, 2009). Asphaltenes and UCM are similarly or much more harmful, especially if they are weathered (unpublished data).

Now, the issues about the solid wastes of oilfields (including both oil- and gas-fields in this paper) have provoked public recognitions and the dangerous wastes are entirely listed in the National Hazardous Waste Inventory (CMEP and NDRC, 2008). The emission solid sources in
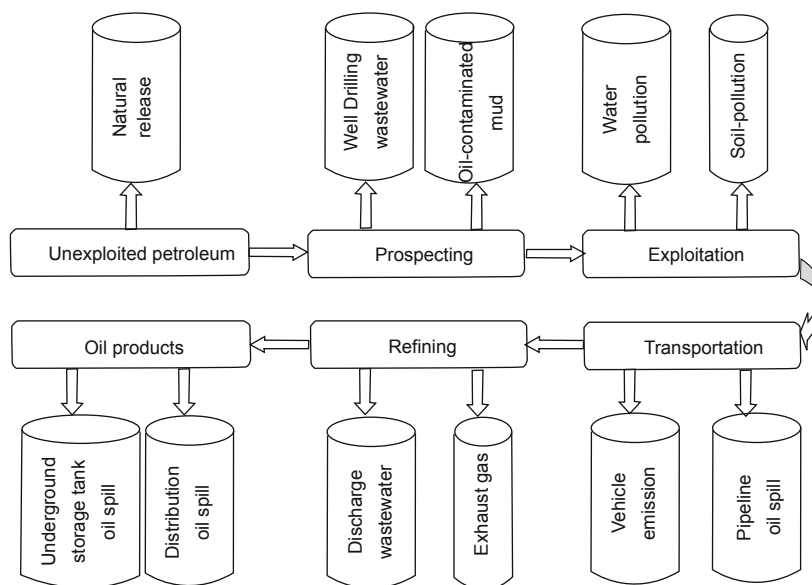
Fig. 1 Process Flows and discharges of waste materials in petroleum industries

oilfields mainly include fugitive/organized drilling waste mud (DWM), rock debris (RDs), crude oil-contaminated soil (COCS), oil sludge and oil sandstones (OSOS) (Table 1). For example, the basic compositions of oily solid waste in Jidong Oilfield are: water (5%–30%), oil (5%–21%), solid (60%–90%that includes clay (28%–58%), sand particles (14%–39%), grits and steel slag (21%–28%). For this kind of oil, the physical parameters of its heavy oil fractions are: initially boiling point 270 ºC, 309 ºC of 10% distillates, 336 ºC of 20% distillates, colloid and asphaltene (25.7%) with density of 941kg/m^3 (20 ºC), and viscosity of 394 mPa·s (40 ºC). According to the data above, the amount of oil in the solid waste is very high, and it can be not only chemically cleared away, but also microbially degraded (Sun et al, 2003).

Table 1 The sources and yields of solid wastes in the Chinese oilfields

Solid wastes	Characteristic description	Source	Yield (ten thousand ton/year)/ Emission ratio, %
Drilling waste mud	Oily, COD, Cr^{6+}, Pb^{2+}, Cd^{2+}, polymer materials, abnormal pH	Formation change; discarded after drilling completion; leaked in the mud circulatory system	100/40
Rock cuttings & debris	Oily, COD, Cr^{6+}, Pb^{2+}, Cd^{2+}, polymer materials	Cutting brought back by the mud	116/25
Crude oil-contaminated soil	High concentrated oil	Spilling during operation; accidents	70/10.5
Oil sludge and oil sandstone	Oil, sludge, sandstone and water (99%) fully emulsified and not easily separated. Complex trace components.	Transferring operation	22/0.44
Domestic waste	Abandoned packaging, abandoned cotton yarn, and thermal insulation and anti-freezing materials	Domestic waste	/

Three sources of drilling waste mud (DWM) are: (1) generated by the replacement of the mud system when the formation properties change; (2) disposed after drilling; (3) leaked in the mud circulatory system. The amount of DWM in oilfields can be up to 1000,000 t each year, and the components are very complex (Table 1) because with the differences of drilling fluid systems, each type of drilling waste fluid (DWF) has its own characteristics. The drilling fluids used in various oilfields in China are mainly water-based drilling fluids, oil-based drilling fluids and gas-based drilling fluids. Different types of drilling fluids contain different additives which consist of inorganic salts, heavy metals, oil-products and a lot of organic polymers (Du et al, 2011). Some of these components with high toxicity such

as Cr are carcinogens, and public concerns have arisen. In China, the enterprise standard to detect the biological toxicity of drilling fluid and its waste for onshore oilfields has been enacted in 2004.

As far as oilfield prospecting and exploitation is concerned, crude oil-contaminated soils (COCS) are found around the oil pools, and some spills scattered near depot stations, others from the downhole operations, oil production testing, the fracturing and/or workover processes. It has been reported that totally the crude oil spilled to the ground is up to 2100−8400 tonnes each year in Daqing Oilfield (Qi and Wang, 2002). In addition, accidents (such as well blowouts or man-made sabotage) induced, unpredictable, sudden and emergency crude oil contamination cannot be ignored either (unpublished data). Oil sludge and oil sand (OSOS) from the cleaning processes of devices including transferring stations, united/jointing stations, oil tanks, settling tanks and/or others were usually composed of oil, oily sand with water content reached to 99%, forming a fully emulsified mixture that is difficult to separate. The oil content of oily sludge is generally 10%−15%. In the petrochemical industries of China, there are 800,000 t bottom mud generated from tanks and pools annually. In Shengli Oilfield, that is more than 100,000 t, and about 150,000 t in Dagang Oilfield, 5×10^4 m^3 in the Henan Oilfield, relatively. Often the typical oily sludge is just piled up outdoor without any treatment. The percentage of oil, silt (solids with the particle size>0.1 mm) and water respectively is roughly 10%, 85% and 5%, in which a lot of harmful and odorous components exist, such as benzene series, phenols, carboxylic acids, PAHs (such as anthracene and pyrenes), sulfides and nitrides. If it is disposed of inappropriately, the oily sludge will not only contaminate the environment, but also waste resources. It is always a tough problem in most oilfields (http://solidwaste. chinaep-tech. com/subject/oil-sludge/8935. shtml). In terms of lots of old oilfields coming into the "Three-High" (high water-cut, high recovery, high production speed) exploitation periods, the production of oil sludge and oil sand proliferates, and it becomes increasing important to develop harmless, environmentally friendly and effective treatment technologies.

3 The pollution situation of petroleum hydrocarbons in the soil and the progress of the treatment technologies

Since the beginning of 1980s, people have realized the seriousness of the issues about the petroleum hydrocarbons soil pollution (Atlas, 1981, 1984; Stegmann et al, 2001). Because of producing, transporting, storing and using petroleum products massively, soil has become seriously polluted since then in America (Kostecki and Calabrese, 1988). Presently, dry oily sludge amounts to 10,000,000 t in America, and more than 6,600,000 t in Europe, and about 2,400,000 t in Japan (Liu et al, 2004). In Netherlands, there are around 100,000 contaminated sites, with the polluted soil over 200 Mt (Rulkens, 2001). In Russia, with the oil and the gas pipelines more than 1.5 million kilometers, there

were about 700 massive leakage accidents (each spilling greater than or equal to 25,000 barrels) and more than 60,000 small ones annually. Just in Eastern Siberia, there was about 3−10 Mt oil leaked each year (Philp, 2005). In China, the situation is also not optimistic (Du et al, 2011). In order to dispose the pollutants and wastes, global emphases have been put on them for many years, accumulated rich experience, some of which has been used in the industrial stage combating these problems. Main measures taken are: 1) Leave it be for the soil that has been polluted, but keep it out of any utilities; 2) Completely or partly trap the polluted soil; 3) Excavate the contaminated soil, and refill with borrowed soil for amelioration; 4) treat in situ (for example without excavation) or ex-situ, that is to say on site or in a processing center. The main disposal methods include backfilling, reclamation, extrusion, solid-liquid separation, solidification and stabilization, incineration, recovery and utilization and biodegradation. Some specific processing techniques appear (Calabrese and Kostecki, 1992; Stegmann et al, 2001), such as carbon dioxide extraction, soil vapor extraction (SVE), bioventing (BV), bio-slurry(BS) and soil washing (SW)(Barlow and Philp, 2005; USEPA, 2001; He et al, 2006). Reviews on soil vapor extraction (SVE) (Li et al, 2001b) and bioventing (BV) (Sui et al, 2003) are available (Korda et al, 1997; USACE, 2002). Other new techniques and methods have been around, but cost-efficiency is one important factor for technique selections, and sometimes we have to make compromising decisions between them. Table 2 shows the costs of various techniques used for soil remediations. It should be noted that the cost was varied not only with different techniques but also with various factors of solid conditions. Because of limited cases, cost data and so-called "commercial secrets", the cost evaluation could be exclusively a reference of an actual application.

In the Exxon Valdez oil spill, it cost one million dollars each day just for cleaning the oil-polluted rocks with water, not to mention physical cleaning, however, instead, the cost of bioremediation for the several hundreds of kilometers coastline was totally no more than one million dollars. According to variously statistical reports, the costs of various bioremediation are varied from one case to another, generally between USD 2 and 268 per ton, having advantages both price and environmental benefits, compared to physical and chemical methods. Looking at the total market value and the taxation proportion of remediation market, we can see that the bioremediation market is expanding (Table 3 and Fig. 2), which indicates that people are optimistic about the prospects of this technique around the world.

Due to increasing public attention to environmental qualities and the demand of environment protection, the bioremediation market is expanding gradually. In 1997, the total bioremediation market value was 870 million dollars, which soared to $1.1 billion in 2002. Even so, it just accounted for less than 10% of the total cost for market remediation that is 25 billion although the data varies among different reports (Boopathy, 2000; Ward, 2004), but which means it still has a broader development space.

Table 2 Costs of various soil-remediation techniques

Remediation	Cost[a], ton	Cost[b], m³	Comments
Excavation/disposing ex-situ	7-50	53-134	Simple but with long processing procedures
Soil washing	50-250	26-71	For poor and/or dry soil, water consuming, higher after cost
Physicochemical cleaning	50-170	200-300[c]	For poor and/or dry soil, solvent consuming
in situ stabilization/solidification	60-100	17-178	Destroy soil structure
in situ electrokinetic technique	40-120		Since 1980s, automatic, power consuming
Engineering seal-capping		26-62	Without eliminating pollution
Bio-slurry	50-80		
Biopile	15-45		
Land farming	10-100		
Turning over dry windrow	5-60		
in situ chemical oxidation		71-152	Probably producing by-products, such as DBPs
Biotreatment in situ	5-160		Relative longer time
Bioventing	15-80		Highly stable and trustable
Thermal treatment	40-700	178-715(incineration in site)	Heating speed slowly, complete/ loses most soil functions
Incineration	50-1200	400-1200[c]	Destroying soil functions, causing secondary pollution
Lime/concrete /volcanic ash solidification	20-170		Pollution was not eliminated
Solvent extraction	30-600		Massively consuming solvents
Encapsulation with plastic membrane		71-107	Probably inducing new pollutants
Kiln-based vitrification	30-500		High energy-consuming

Notes: a- UK in 1997, unit: pound (£), modified from (Barlow and Philp, 2005);

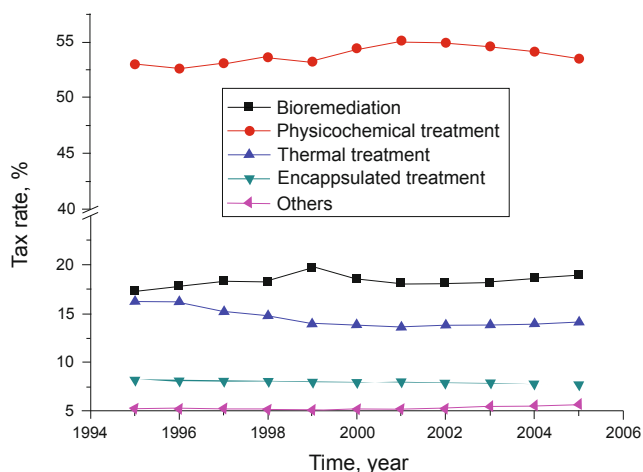b- Industrial Market Researches of MSI, unit: US dollar ($), modified from (Philp, 2005); c- unit: ton

Fig. 2 Remediation markets of polluting fields in Europe

Table 3 The bioremediation markets in the world

Markets	Dollar/million		
	1994	1997	2000
United States of America	160−450	225−325	375−600
Europe	105−175	180−300	375−600
Germany	70−100	100−150	250−350
Netherlands	10−20	15−35	30−60
Scandinavia	10−20	15−35	30−60
England	5−10	7.5−20	15−30
Others	10−25	42.5−60	50−100
Canada	15−35	30−50	50−100

4 The biological techniques in the treatment of oil-contaminated soils

Among various remediation techniques, the biological treatment (biotreatment) no doubt at present is the researchers' highlight and top priority. Biotreatment is a controlled and spontaneous process that uses organisms, especially microorganisms for biocatalytic degradation of environmental pollutants to decrease or eventually destroy them. It is an emerging technique for environmental protection which is based on microorganism degradation and represents the development direction of future (Shen, 2000). The advantages of biological techniques are as follows (Agathos and Reineke, 2002; Fass et al, 1999; Ward, 2004): (1) The pollutants are degraded and cleared up on site; (2) It is very simple and interferes little with the surroundings for on-site processing; (3) Least cost, which just accounts for 30%−50% of those of the traditional chemical and physical remediations; (4) The possibility that people are exposed directly to the pollutants is significantly reduced; (5) There is no or less secondary pollution and consequent problems.

Although this method which has provoked international attention is just in its preliminary stage at present, with the increasing severity of the pollution and the seriousness of the environmental protection situation, the disadvantages of traditional ways show up gradually. In 1989, the bioremediation techniques have successfully been used to help dispose of oil spilled from the giant oil tanker "Exxon Valdez" on the coast of Alaska (until the 2010 Deepwater Horizon oil spill, the Exxon Valdez spill was the largest and most hazardous ever in USA waters (NIH and NLM, 2010; USEPA, 2009)). For the first time in the United States of America, bioremediation research began to be the hotspot and cutting edge of environment science, at least in bound fields. In 1997, 1.2 Mt soils was remediated via bioremediation in Germany, which accounted for 55.8% of all repairing amount remediation (850,000 tons remediated by chemical/physical ways, 100,000 tons by thermal treatment) (Miehlich, 2001). Comparing with thermal treatment (which mostly loses all the soil functional diversity) or washing (just for dry and barren soils) (Table 2), natural/intrinsic remediation which organisms play main roles has been paid great attention in scientific communities, because of its extensive applicability and limited adverse side effects.

In nature, microorganisms which can degrade oil exist widely in soil, ground water, oceans and lakes. Many of them can live with oil as their only carbon source. Now more than 70 genera and 200 strains have been found that can oxidatively biodegrade one or more types of petroleum hydrocarbons (Englert et al, 1992; Feng et al, 2007; Li et al, 2001a; Li and Feng, 1991; Liang et al, 2005). Some of bacterial and fungal genera (Chaineau et al, 1999; 1995; Korda et al, 1997; Pointing, 2001) reported in the literature are listed in Table 4. In addition, some algae can biodegrade oils as well (Semple et al, 1999; Walker et al, 1975). It should be pointed that enhanced bioremediation through rhizosphere interactions (Su and Yang, 2009; Toyama et al, 2011) is also very important but this is at its early stages and needs further investigation (Robertson et al, 2007; Wan, 2011a).

Comparison with physical and chemical methods, although having its weak points, the advantages of biotreatments (Table 2 and Table 5) are obvious, (Agathos

Table 4 Some of reported microorganism genera that can biodegrade petroleum hydrocarbons

Bacterium		Fungus	
Achromobacter	Pseudomonas	Acremonium	Humicola
Acinetobacter	Pseudobacterium	Aspergillus	Monilia
Aeromonas	Rhodococcus	aureobasidium	Mortierella
Agrobacterium	Sarcina	Basidiomycete	Paecilomyces
Alcaligenes	Serratia	Beauveria	Penicillium
Arthrobacter	Spirillum	Candida	Phoma
Bacillus	Streptomyces	Chrysosporium	Pichia
Brevibacterium	Vibrio	Cladosporium	Rhodotorula
Chromobacterium	Xanthomonas	Cochliobolus	Saccharomyces
Coryhebacterium		Cryptococcus	Scolecobasidium
cytophaga		Cylindrocarpon	Selenotila
Desulfovibrio		Debaryomyces	Sporobolomyces
Erwinia		Endomyces	Spicaria
Flavobacterium		Fusarium	Tolypocladium
Micrococcus		Geotrichum	Torulopsis
Micromonospora		Gliocladium	Trichoderma
Mycobacterium		Gongronella	Trichosporon
Nocardia		Graphium	Verticillium
Proteus		Hansenula	

Table 5 The comparison of common treatment approaches for oil pollutants
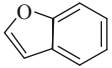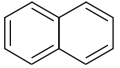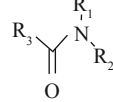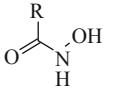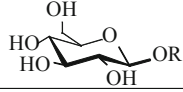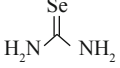
Treatment approaches	Advantages	Disadvantages
Physical	Destroy most pollutants	Need devices/equipment at high cost of destruction of soil diversity and energy consuming
Chemical	Good at high efficiency of oil removal	Produce secondary pollutions at high cost
Biological	1) High efficiency of oil removal 2) Low energy, low cost 3) Little impact to environments, no or less secondary pollutions 4) Wide applicable ranges 5) High public acceptability	1) No accessible to some artificial pollutants 2) Special microorganisms just biodegrade the specific pollutants 3) Easily affected by surrounding conditions 4) Possibly with residues 5) No oil could be recycled

and Reineke, 2002; Fass et al, 1999). It will be the sole choice if other methods are not accessible.

Oil is a highly complicated mixture with a combination of hydrocarbons consisting predominantly of aliphatic, alicyclic and aromatic hydrocarbons, and small amounts of nitrogen, oxygen, and sulfur compounds (NOS components), trace amounts of metals and/or metallic compounds. Until now some components are bulked as unresolved complex matter (UCM) (Table 6). Though it is still too early to understand the real-time and in-situ mechanisms of oil degradation, quite a lot of research has been done on the mechanisms of several typical components, such as the predominant components of alkanes, alicyclic hydrocarbons, aromatics and polycyclic aromatic hydrocarbons (Semple et al, 1999), and possible pathways of biodegradation of these compounds have been recently reviewed (van Hamme et al, 2003).

Table 6 Bio-transformable organic molecular fragments and microbial dissociation

Molecular fragment	Organic molecular fragment	Structure	Molecular fragment	Organic fragment	Molecular	Structure
C–OH	Alcohol		$R–N=N^+=N^-$	Organicazide		
–CHO	Aldehyde		$RCONH_2$	Amide		
	Alicyclic hydrocarbon	△	R_4N^+	Quatenary amine		
$C–CH_{2-}$	Saturated aliphatic comound		$R_1(R_2)NR_3$	Amine		
–CH=CH–	Unsaturated aliphatic compound		Cyclic amide	(α~ε)lactam		
	(simple substituted)Aromatic		R–C≡N	Nitrile		
	Heterocyclic aromatic		$C–NO_2$	Nitrile		
–COOH	Carboxylic acid		$R_1N(–R_2)–N=O$	Nitrosocompound		
	Polycyclic aromatic hydrocarbon		$R_3C(=O)NR_1(R_2)$	N-substituted amide		
–RCOOCR	Ester	RC(O)OR'-->RC(O)OH	R–CO–NH–OH	Hydroxamic acid		
RC–O–CR	Ether	ArOR--ArOH; ROCH₂R'--ROH	$–NH_2–OH$	Hydroxylamine		
$C_6H_7(OH)_4OR$	Glucopyranoside		$R_1(R_2)=NOH$	Oxime		
RX	Halide	R=carbon atoms	$R_1–CS–NR_2(R_3)$	Thioamine		
C–Si–	Organosilicone		$(NH_2)_2CO$	Urea		
C–As bond	Organoarsenicals			(α~ε)lactone		
	Selenourea		R–C(=O)–R	Ketone		
C–P bond	Organophosphorous compound		$R–S(=O)_2–OH$	Sulfonic acid		
C–Sn bond	Organotin		R–SH	Thiol		
$R–O–SO_2–O–R$	Organic sulphate			Heterocyclic compound		
C–Hg bond	Organomecuricals		–C–Te–C–	(Dimethyl)telluride		

Abbreviation: R (including those R_1, R_2, R_3 with same or different groups)-organic fragment; Ar-aryl group; Alk-alkyl group; Het-heterocyclic group; X-halogen atom. The abbreviations in Table 7 are same with these.

(1) Degradation of normal alkanes

General recognition at present believes that the normal alkanes with carbon atoms up to about 44 (Haines and Alexander, 1974) can be oxidized via monoterminal and/or diterminal oxidation which can also be named as ω-oxidation and sub-terminal oxidation. The alkane (n-C) first was usually oxidized to be primary alcohol via terminal-oxidation, then to an aldehyde and a carboxylic acid. Carboxylic acids are further transformed to acetyl coenzyme A (AcCo A) and shorter dicarboxylic acids (by removal of carbon dioxide) via β-oxidation. The chain-length of dicarboxylic acids is continuously decreased to become two-carbon acetic acid (Atlas, 1981; Singer and Finnerty, 1984). Acetic acid is broken off from the alkane chains and finally decomposed to carbon dioxide via the central metabolic pathway. Alkanes with branched chains can be biodegraded by α-oxidation, ω-oxidation and/or β-basic groups elimination, although the branched chains can increase the resistance at certain degrees to be oxidatively degraded by microorganisms (Singer and Finnerty, 1984; Xia et al, 2006; Wang and Wen, 2000) (Fig. 3).
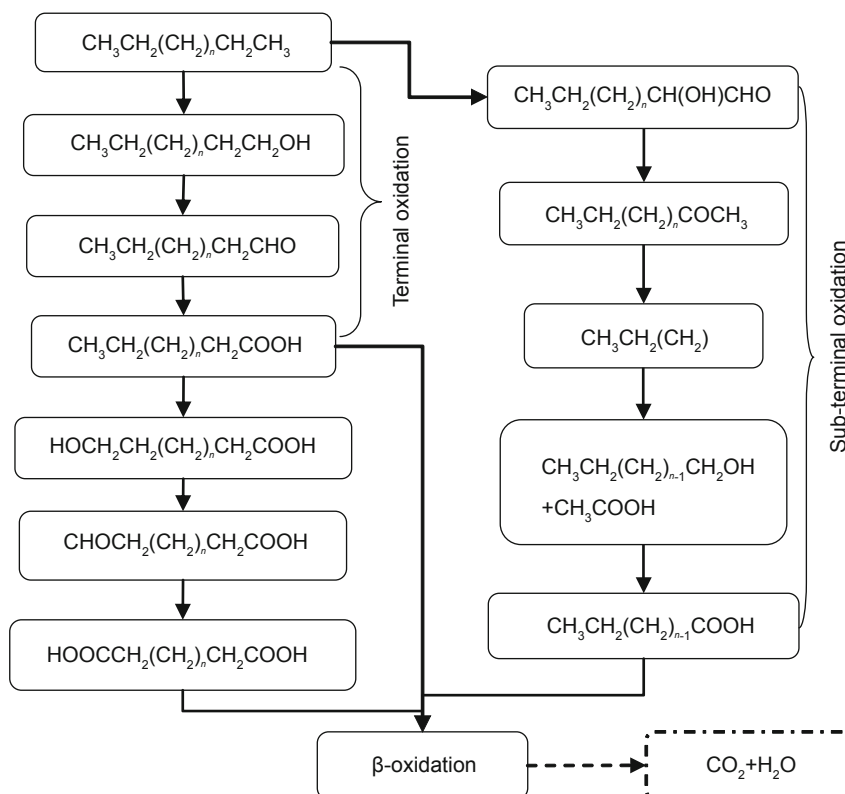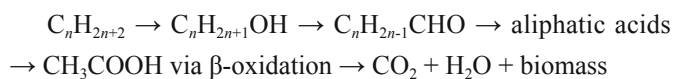

Fig. 3 The aerobic biodegradation processes of n-alkanes

The biodegradation of alkanes of petroleum hydrocarbons can be concisely summed up as:

$$C_nH_{2n+2} \rightarrow C_nH_{2n+1}OH \rightarrow C_nH_{2n-1}CHO \rightarrow \text{aliphatic acids} \rightarrow CH_3COOH \text{ via } \beta\text{-oxidation} \rightarrow CO_2 + H_2O + \text{biomass}$$

(2) Degradation of cyclic hydrocarbons

Cyclic hydrocarbons are any chemical compounds with one or more rings of carbon atoms in the chemical structure of their molecules, including cycloalkanes, aromatics and heterocyclic compounds. Cycloalkanes (naphthenes) exist extensively in the biosphere and crude oil (in the boiling range of 40 to 200 ºC, with a content of 20% to 70%; in the boiling range of 350–500 ºC, with a content up to 50%–60%) (Perry, 1984), but most often they can be ignored easily. Presently, it is believed that the degradation of cycloalkanes is similar to the sub-terminal degradation pathways of alkanes (paraffins). Many microorganisms which can oxidize the acyclic alkanes can also hydrolyze the naphthenic hydrocarbons because of their broad specificity.

Actually, co-metabolisms of naphthenic hydrocarbons are much more common because of lack of microorganisms in nature those can consume naphthenic hydrocarbons directly (Perry, 1984). Hydroxylation is the crucial stage for the degradation of these compounds. As shown in Fig. 4, steroids, cyclic terpenes, indane (benzocyclopentane) and cyclohexane all have similarly metabolic processes. The intermediates in the pathway of cyclohexane biodegradation includes cyclohexanol, cyclohexanone, ε-caprolactone, and then open loop to generate hydroxycarboxylic acid. The processes between cyclohexane and cyclohexanol (or cyclic alcohols in other conditions) have the relationships of co-metabolism (Fig. 4) (Atlas and Bartha, 1998). To the alkyl-substituted alicyclic compounds, two initial positions that can be potentially oxidized are on the side chains and the alicyclic rings, which can be affected by properties of compounds, genera of microorganisms, and others factors. Research into the typical biodegradation of naphthenic acids found that the naphthenic acids with substituent groups were biodegraded much more slowly than those unsubstituted (Clemente and
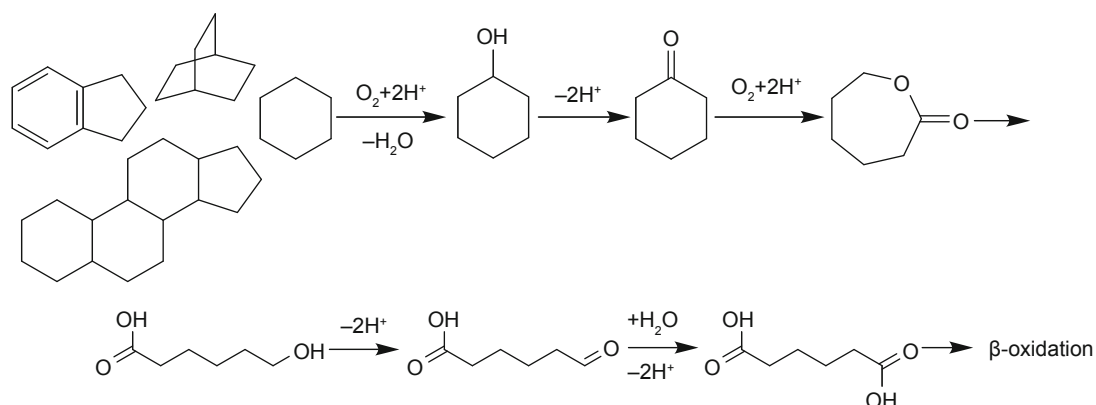
Fig. 4 Aerobic degradation reactions of cyclic hydrocarbons

Fedorak, 2005).

(3) Degradation of arenes

Fungi and microorganisms (bacteria and archaea) can oxidize aromatic substrates from benzene to benzanthracene. Initially, bacteria incorporate the two oxygen atoms of an oxygen molecule to substrates under enzymatic oxidation of dioxygenase, oxidizing arenes to cis-dihydrodiphenols. The cis-dihydrodiphenol can be further oxidized to catechols which will be further oxidatively decomposed by another dioxygenase which can cleave aromatic rings. On the contrary, fungi oxidize the arenes to trans-configuration dihydrodiphenol under the enzymatic degradation of monooxygenase and cyclohydrolase. The typical pathways of microbial degradation of typical arenes are shown in Fig. 5 (revised from Cerniglia, 1984), actually which could be the prime differences between prokaryotes and eukaryotes response to environmental pollutants (xenobiotics) (Wackett and Hershberger, 2001; Su et al, 2001; Jin, 1997). Anaerobic degradation of arenes has been recently invoked as the most important in deep subsurface oil degradation (Head et al, 2003; Jones et al, 2008; Roling et al, 2003), but it is beyond the scope of this paper because the oil pollution mostly happens on the earth's surfaces where aerobic reactions are thought to be predominant (Venosa et al, 2010). The aerobic degradation which takes place in petroleum hydrocarbons forms a diphenol intermediate (Fig. 5, upper right), and one of the anaerobically degrading intermediates of BTEX is benzoylcoenzyme A (Wackett and Hershberger, 2001) (Fig. 5, lower right) and then it can be further degraded to cyclic acids (Aitken et al, 2004).

(4) Degradation of polycyclic aromatic hydrocarbons

Naphthalene is the simplest PAH. Initially, it is catalyzed by dioxygenase to produce cis-naphthalene dihydrodiol (1), then it will be dehydrogenated to produce 1,2-dihydroxynaphthalene (2). The cis-O-hydroxyl benzalacetone acid (4) may be generated from 2-hydroxychromene-2-carboxylic acid (3) by its isomerase (Philp, 2005). The compound 4 is further cleaved of the side-chain pyruvate (5) to form salicylaldehyde (6) and salicylic acid (7). The salicylic acid is further transformed to catechol (8) or gentisic acid (2,5-dihydroxybenzoic acid) (9) by different enzymes, which separately enters into the analogous metabolic pathway of nitrobenzene metabolism or intermediary metabolism (Fig. 6). Fluorene and anthracene and some heterocyclic compounds or derivatives are biodegraded with similar pathways via compound 7 (Fig.
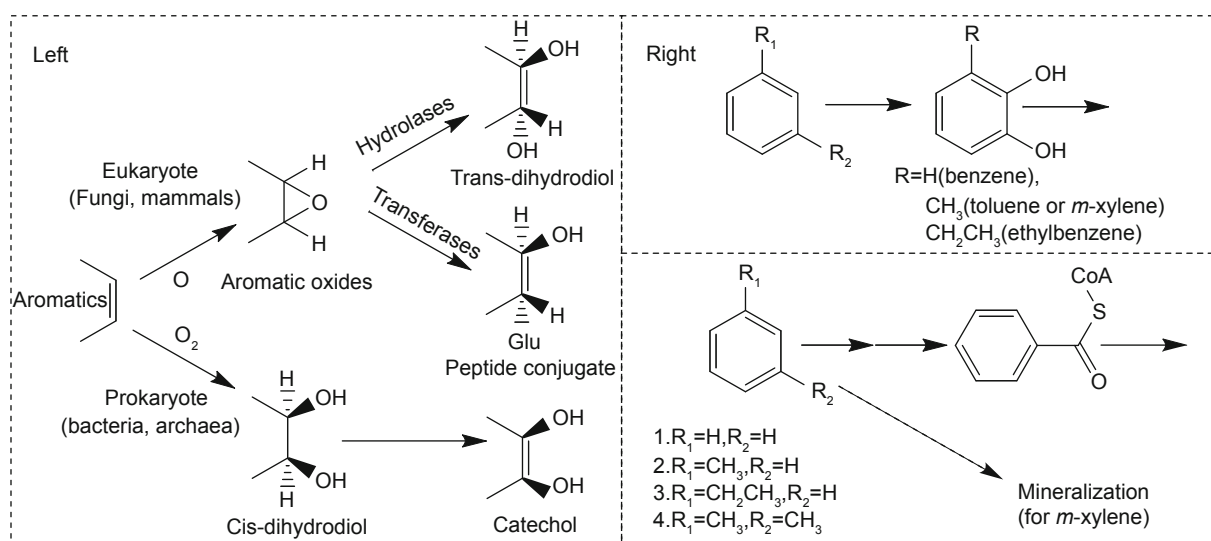

Fig. 5 The aerobic (left and upper right) and the anaerobic (lower right) reactions of aromatic hydrocarbons.
Right, 1: benzene, 2: methylbenzene, 3: ethylbenzene, 4: m-xylene

6). Many kinds of enzymes are involved in the metabolic processes (Fig. 5 and Fig. 6).

Three ring PAHs have similar biodegradation processes. The first step is still to generate cis-dihydrodiol by dioxygenase catalysis, then dehydrogenation to the corresponding diol. Thirdly, one ring is oxidatively decomposed to a side chain which is further cleaved from the rings. The remaining degradation of two ring diol goes as the way of naphthalene described above (Fig. 6) to catechol and/ or gentisic acid until they are absolutely degraded.

As for four- or greater ring PAHs, the catabolic metabolism processes are unclear yet, but the initially oxidative process is similar. The following induction of biodegradation are general rules which refer to the majority of PAHs.

In both aerobic and anaerobic conditions, the degrees of biodegradable ease/difficulty are dependent on the properties of PAHs, such as solubility, numbers of rings, type and position and number of substituent groups and properties of any heteroatoms (common in NOS) (Cookson, 1995;
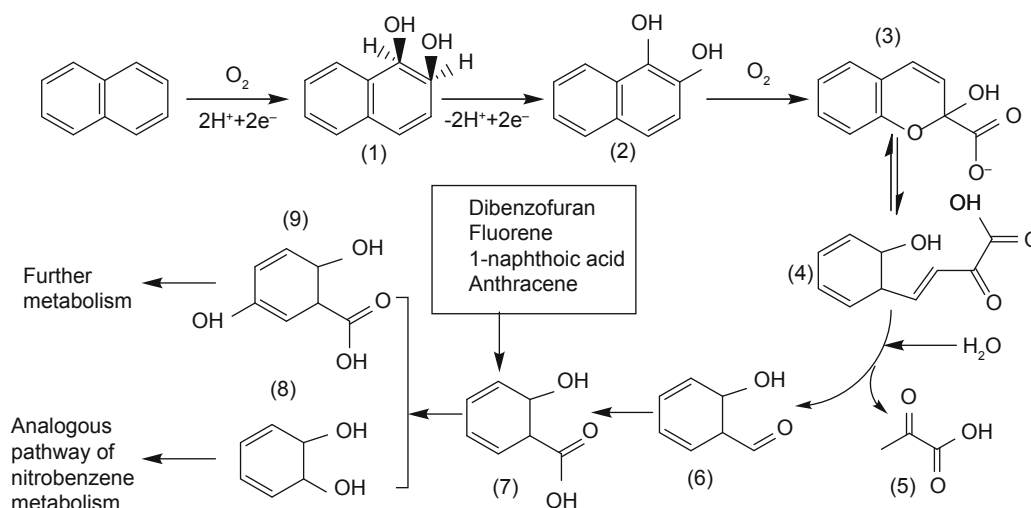

Fig. 6 The aerobic degradation of naphthalene and other analogous compounds by bacteria

Wackett and Hershberger, 2001). Generally speaking (Fig. 7), (1) there are distinctly different biodegradations of various microorganisms to different PAHs. (2) Usually, two or three ring PAHs can be degraded easily by bacteria. (3) Four or five ring PAHs are resistant to biodegradation, and some are even tend to persistent. (4) Adding three methyls to the structure of benzene ring seriously reduces the biodegradability. (5) Increasing the saturation of PAHs will significantly reduce the biodegradability. (6) Biodegradation of PAHs of four/five or more rings is dependent on co-metabolism with their analogies. (7) The bio-synergy and bio-diversity of microorganisms are beneficial to biodegradation and bioremediation. (8) The rate of the incipient oxidation of rings is a crucial step, and subsequent steps go rapidly for three or more ring PAHs. (9) Adding PAHs-oxidizing bacteria to the pollution sites probably increase the biodegradation speed and favors bioremediation. (10) The anaerobic degradation of PAHs has not been extensively studied and no uses on sites are reported yet. (11) PAHs with two or three rings can be transformed in the conditions of denitrification, sulfate reducing, methanogenesis or fermentation.

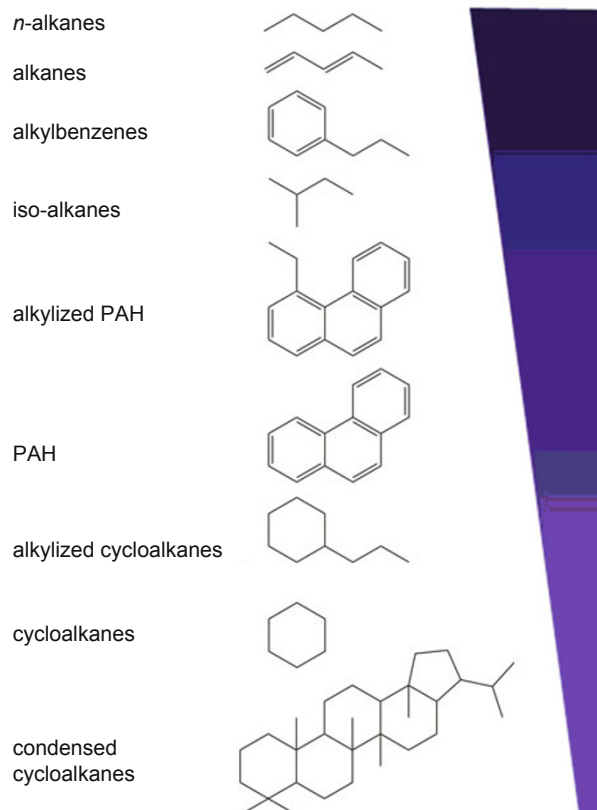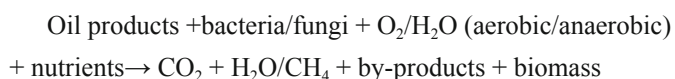Generally, the biodegradation process for oil can be expressed as the formula:

Oil products +bacteria/fungi + O_2/H_2O (aerobic/anaerobic) + nutrients→ CO_2 + H_2O/CH_4 + by-products + biomass

Fig. 7 General biodegradation rates of petroleum hydrocarbons

where probably nitrogen is a limiting nutrient (Atlas and Bartha, 1998; Atlas, 1995) and oxygen is a predominant factor for aerobic degradation (Venosa et al, 2010), but phosphorus supply instead of others could be a rate-limiting nutrient for subsurface biosphere (Head et al, 2003; Larter et al, 2006; Larter et al, 2003) .

From the view of the length of carbon chain, although longer or shorter chains with carbon atoms up to 36–44 or less than 10 were reported (Feng et al, 2007; Haines and Alexander, 1974), n-alkanes, n-alkyl aromatics and aromatic compounds with 10–20 carbons have the lowest bio-toxicity, can be biodegraded easiest. n-alkanes and (alkyl) aromatics with 5–6 carbons can be biodegraded in low concentration, but actually most of which are removed via volatilization not by biodegradation. n-alkane with 1–4 carbons with higher bio-toxicity can be biodegraded, but only by some specific microorganisms. The low water solubility of n-alkane, alkyl aromatics and aromatic compounds with more than 20 carbons makes them much more difficult be biodegraded.

As far as branched chains are concerned, asphalts with branched chains generally reduce the biodegradation rate, and it is slower for aromatic compounds than that for alkanes. Branched tertiary carbon and quaternary carbon can hinder β-oxidation. Biodegradation of branched alkanes and cycloalkanes with 10–20 carbons are much more difficult than the corresponding straight-chain homologous series. Biodegradation of cycloalkanes requires synergistic actions of two or more microorganisms. Furthermore, there is higher membrane toxicity of cycloalkanes equal or less than 10 carbon atoms. The biodegradation rate of condensed series of aromatics and cyclic waxes, such as tar oil, bitumen and asphalt which have the components of four or more condensed rings, and some partly oxy-substituted condensed aromatics, is very slow. The mineralization of condensed PAHs, cyclic waxes and high molecular weight alkanes is very slow as well (Englert et al, 1992). The general rate of biodegradation has been shown in Fig. 7 (Steinhart et al, 2001). Peters and Moldwan categorized petroleum hydrocarbons into ten levels according to their biodegradabilities (Peters et al, 2005; Peters and Moldowan, 1995). The bioremediation of spilled oil then should be cautiously based on the properties of oil

Table 7 Biotransformation and reaction types

Transformation type	Reaction
Dehalogenation	$RCH_2Cl \rightarrow RCH_2OH$; $ArCl(F) \rightarrow ArOH$; $ArCl \rightarrow ArH$; $Ar_2CHCH_2Cl \rightarrow Ar_2C=CH_2$; $Ar_2CHCHCl_2 \rightarrow Ar_2C=CHCl$; $Ar_2CHCCl_3 \rightarrow Ar_2C=CCl_2$; $RCCl_3 \rightarrow RCOOH$ $HetCl \rightarrow HetOH$
Deamination	$ArNH_2 \rightarrow ArH$
Decarboxylation	$ArCOOH \rightarrow ArH$; $Ar_2CHCOOH \rightarrow Ar_2CH_2$; $RCH(CH_3)COOH \rightarrow RCH_2CH_3$; $ArN(R)COOH \rightarrow ArN(R)H$
Methoxylation	$RCH_3 \rightarrow RCH_2OH$ and/or$\rightarrow RCHO$ and/or$\rightarrow RCOOH$
Hydroxylation and ketogensis	$ArH \rightarrow ArOH$; $RCH_2R' \rightarrow RCH(OH)R'$ and/or$\rightarrow RC(O)R'$ $R(R')CHR'' \rightarrow R(R')CH(OH)R''$; $R(R')(R'')CCH_3 \rightarrow R(R')(R'')CCH_2OH$
β-oxidation	$ArO(CH_2)_nCH_2CH_2COOH \rightarrow ArO(CH_2)_nCOOH$
Epoxidation	$RCH=CHR' \rightarrow \overset{O}{\overset{\displaystyle \bigwedge}{RCH\text{-}CHR'}}$
Nitrogen oxidation	$R(R')NR'' \rightarrow R(R')N(O)R''$
Sulfur oxidation, =S to =O	$RSR' \rightarrow RS(O)R'$ and/or$\rightarrow RS(O_2)R'$ $(AlkO)_2P(S)R \rightarrow (AlkO)_2P(O)R$; $RC(S)R' \rightarrow RC(O)R'$
Sulfoxide reduction	$RS(O)R' \rightarrow RSR'$
Alkyne reduction	$RC \equiv CH \rightarrow RCH=CH_2$;
Alkene reduction	$Ar_2C=CH_2 \rightarrow Ar_2CHCH_3$ $Ar_2C=CHCl \rightarrow Ar_2CHCH_2Cl$
Double bond hydration	$Ar_2C=CH_2 \rightarrow Ar_2CHCH_2OH$
Nitro metabolism	$RNO_2 \rightarrow ROH$ $RNO_2 \rightarrow RNH_2$
Oxime metabolism	$RCH=NOH \rightarrow RC=N$
Nitrile-amine metabolism	$RC=N \rightarrow RC(O)NH_2$ and/or$\rightarrow RCOOH$
Organotin	$R_3SnOH \rightarrow R_2SnO \rightarrow RSnO_2H$
Organomercuricals	$RHgR' \rightarrow RH$ and/or Hg

and microorganisms with corresponding enzymes.

In addition to normal hydrocarbons, there are many kinds of additives in oil products, especially as presently the production wells of most countries in the world have reached to their middle or late stages. In order to enhance their recoveries, lots of different additives have been unscrupulously/versatilely added in, for examples multifunctional detergents, anti-icing additives, dispersants, flow improvers, pour point depressants (pour point reducers), antioxidants, corrosion inhibitors, octane improvers, antiknock agents, combustion improvers, antistatic agents, antimicrobial agents, dyes, dehydrators, and agglomerating agents and so on. All these additives have multitudinous chemical components and structures. Luckily, however, there are lots of bacteria and fungi in soil which can bio-transform those additives to a certain extent. Organically functional fragments found in nature so far known to be bio-transformed are listed in Table 6 and Table 7 (Englert et al, 1992; Wackett and Hershberger, 2001; Wan, 2011b), but which may be only parts of our ignorance-enlarging universe.

Abbreviation: R (including those R_1, R_2, R_3 with same or different groups)-organic fragment; Ar-aryl group; Alk-alkyl group; Het-heterocyclic group; X-halogen atom. Partly bound with UCM. The abbreviations in Table 7 are same with these.

Comprehensively, as seen in Tables 6 and 7, to date, linkages found to be enzymatically cleaved by microorganisms include ester bonds, C−N bonds, C−S bonds, C−Hg bonds, C−Sn bonds, C−O−P bonds, P−S bonds, S−N bonds, S−S bonds, sulfonic esters, polypeptides and carbamates. The reaction types involved are methylation, etherification, N-acylation, nitration, N-nitrosification and dipolymer reaction (such as RSH produces RSSR) (Englert et al, 1992; Wackett and Hershberger, 2001; Wan, 2011b). Microbial diversity is such that presently only 10% or less is recognized (Whitman et al, 1998), so much more new fragments and reaction types (Heider, 2007) will be identified in future.

5 Perspectives

Although petroleum pollution of waters, such as oil spills and oil-well explosions, has widely led to public consideration and debate, the problems of petroleum-contaminated soil/sediments are less investigated and clarified. Recently anaerobic biodegradation of petroleum has been recognized and made a little progress (Jones et al, 2008; Lovley, 2001), however, most investigations reported in the literature in the past half century have been focused on or limited to aerobic degradation. No research has systematically considered and investigated the impacts of environmental conditions, especially in-site temperature, pH, oxidation-reduction potential (Eh), surface and subsurface hydrology, on aerobic degradations.

As a most complex mixture, petroleum pollutants represent a very difficult challenge (Du et al, 2011). For example, even after 20 years, and in spite of a great deal of human effort and millions and billions of money investment, the fishery and wild animals in the Prince William Sound have not recovered from the pollution caused by the massive oil spill of Exxon Valdez oil tanker (Raloff, 2009; WWF-US, 2009) and are still being exposed to it (Esler et al, 2010) although it is the largest and most thoroughly studied case (Atlas and Bartha, 1998; Atlas, 1995). As above mentioned, researchers put emphasis on some parts of petroleum, i.e., normal alkanes, cyclic hydrocarbons, arenes and aromatics those are easiest to be analyzed. The residual portions of petroleum, such as non-hydrocarbons (NOS-containing compounds), asphaltene and UCM, are less determined and analyzed, not to mention their biodegradation and mechanisms. However it should be pointed out that, in our opinion, it is not enough for microbes alone to deal with these matters. It is a top priority and scientists must work together with various methods, , to tackle it most urgently.

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