Journal of Environment and Earth Science
ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online)
Vol.4, No.8, 2014

Biodegradation of Gasoil by Fungal Isolates from Petroleum Contaminated Soils

Sajid Salahuddin Al- Saeedi[1*] Tariq Abdul Jalil Mandeel [2] Bihar Moqdad Al-Ani[1]

1. Department of Biology , Faculty of Science – Anbar. Univ , Iraq
2. Department of Chemistery , Faculty of Science – Anbar. Univ , Iraq
[*] E – Mail : saggedalseaidy@yahoo.com

Abstract

Biodegradation of gasoil can be improved using various fungi isolated (Aspergillus sp . and Alternari sp) from chronically diesel-oil-polluted soil . Samples were collected from power generators locations in Ramadi city. In this present investigation, bioremediation was carried out through growth of individual fungicide strains on gasoil and examined for its ability to degrade gasoil hydrocarbon at $26C^0$ was accomplished and assessed by using Gas Chromatography (GC). Evaluated of biodegradable observed the short-chain alkanes C_9H_{20} greater extent than that for the long-chain $C_{21}H_{44}$. In addition, from the biodegradation efficiencies obtained, it can be inferred that gasoil was amenable to Bioremediation**.** Results pointed that bioremediation depends on the kind of used fungus such as *Alternaria sp*. Strain which are more effective in the degrade of gasoil than the *Aspergillus sp* Fungi strain

Key Words : Biodegradation , Fungi , *Aspergillus* SP , *Alternaria* sp **,** Petroleum

Introduction

Microbial degradation has emerged as the most significant natural mechanism for removal of nonvolatile hydrocarbon pollutants from the environment. Although biodegradation occurs at a distressingly slow rate, it can be enhanced by inoculation with microbial species that will degrade the oil waste more efficiently, and/or by introducing air and nutrients into the environment, (Wanga *et al* .2010; WuM *et al* . 2010). The toxicity of crude oil or petroleum products varies widely depending on their composition , concentration , environmental factors and the biological state of the organisms at the time of the contamination. Petroleum distillates up to and including gas oils are more severely toxic on a short time scale than the other components of crude oil . In strongly polluted areas, there are immediate detrimental effects on plant and animal life (Tehrani *et al* . 2009 ; Wanga *et al* .2010) . Chemical, physical as well as biological methods are used for gasoil remediation. Above all, biological methods are favored because of their good results and low costs. Microbial biodegradation is a friendly and effective means to remove gasoil and polycyclic aromatic hydrocarbons (PAHs) from the environment and has been extensively use (Xia 2002) **.** At present, various microbial genera have been detected in petroleum-contaminated soil or water, which strongly suggests that each has a role in the hydrocarbon transformation process. The most frequently found microorganisms are bacteria and fungi, with bacteria assuming the dominant role in marine ecosystem and fungi in terrestrial environments . It has been reported that adapted communities previously exposed to hydrocarbons exhibit higher biodegradation rates than communities with no hi-story of hydrocarbon contamination. Organic compounds of low molecular weight and simple molecular structure are preferred by many (PAHS) and over 65 % degradation rate was achieved with Phenanthrene and Nphthalene . Many studies concluded that most filamentus fungi species are excellent hydrocarbons degraders . (Gadd 2001 ; Okoh 2003)

The aims of this research is to isolate , identity and study, some of the indigenous fungal of gasoil contaminated soils and evaluate the biodegradation efficiencies of the potent isolates from polluted soils including fuel.

1 . Materials And Mmethodes

1.1. Sample Collection

The soil samples contaminated with Gasoil were collected from different sites around an electric power generators from the center Ramadi for Fungi isolation. Soil samples were obtained from depths of (0 –15cm) in a contaminated area, sterile polyethylene bags were used to packing soil samples in order to use it in subsequent operations.

1.2. Isolation Of Fungi.

Fungi that have ability to consume Hydrocarbons compounds were isolated by plating out at low dilutions methods (10^{-1}-10^{-3}) on mineral salt medium (M.S.M) (mills *et al* , 1978). 0.25ml of prepared dilution from studied soil were added separately to filter paper in base of petri dish Soaked with Gasoil, M.S.M. Medium (modified after Okoro 2010)added and gently shacked . Cultures incubated on $29C^0$ for 15 days till fungal colonies were obvious. In order to get a pure fungal isolates a series of inoculation Potato Dextrose Agar medium (P.D.A) were made. (Raper & fennel 1965). Pure cultures then preserved on slants of P.D.A medium. All media autoclaved at 120^0C for 20 min.

1.3. Identification Of Fungi

Fungal isolates Diagnosed According to characters of culture and appearance using taxonomic keys (Watanabe 2002) **.**

1.4. Screen Test For The Ability Of Fungal Isolates To Utilize Diesel

In order to study the ability of fungal isolates to consume and degrade diesel Potato Sucrose Broth mixed with 0.0, 0.5, 1.0, 3.0, 5.0 % of diesel inoculated by disc (6mm) of culture token from each fungal culture in 5 – 7 days old . Cultures were incubated at 29 ^{o}C in shaker incubator in speed shook 200 cycle/min and for 7 days and 14 days . Cultures filtrated using sterile filter paper (Watman No.1.) . Supernatant which represent p.s.b and the residuals of diesel was taken for further analysis (Modified from Obire & Anyanwu 2004)

1.4. Biodegradation Process

500 mL flasks were used to carry out Biodegradation,for this purpose two strain of specified isolates fungal (***Aspergillus sp . and Alternaria sp***), were grown on the gasoil samples (diesel) from pajji refinery. According to the (Obire *et al* , 2009) method, Then incubated in the incubator shaker at $29C^0$ for 15 days.

1.5. Biodegradation Tests

The bioremediation test were realized using two strain of isolated fungi. Then been calculated all the incubated fungi were cultivated in flasks in comparison with standard sample.Table.1 Which shows the consumption of fungi through decreasing of the numbers due to gasoil degradation, and follow-up of the biodegradation tests by (GC), through retention time, peak aria and gasoil concentration for each one compound, by using hydrocarbon compounds which present in gasoil (C_9H_{20} , $_{12}H_{26}$, $C_{16}H_{34}$, $C_{17}H_{34}$, $C_{21}H_{44}$) as standards and reference to follow up the biodegradation.

Table 1. Composition of samples.

NO	Fungi's	Gasoil %	Incubation time
1	*Aspergillus sp.*	0% Control	14 days
2	*Aspergillus sp.*	0.5%	14 days
3	*Aspergillus sp.*	1%	14 days
4	*Aspergillus sp.*	3%	14 days
5	***Aspergillus sp.***	**5%**	**14 days**
6	***Alternaria sp***	**0% Control**	**14 days**
7	***Alternaria* sp**	**0.5%**	**14 days**
8	***Alternaria sp***	**1%**	**14 days**
9	***Alternaria sp***	**3%**	**14 days**
10	***Alternaria sp***	**5%**	**14 days**

1.6. Analytical Methods

The analytical methods were carried out by using (GC) kind PACKARD MODEL 433A(USA) . And detector kind FID, detector temperature $300C^0$, the temperature of the injection port $270C^0$, furnace temperature 100 – 300 C^0 and column kind SE / 30, OC^0 / min with capillary column (DB-5, 3M , diameter 1/ 8 , helium (He) used as the carrier gas of samples and flow rat 20m/ min. Spectroscopic charts of GC was recorded for 12 samples and **C_9, C_{12}, C_{16},C_{17} C_{21}.**

2 . Results And Discussion

Gasoil consists of mixture of hydrocarbons compounds mainly of C_{15} – C_{22} and other compounds have negligible concentration contain less than C_{15}. The growth of fungi on the gasoil led to obtain degradation processes were formation a new compounds and disappearance of other compounds. Which refers to the ability of fungi due to braking the carbon bonds in hydrocarbons then change the long chains to short and change the short chains to methane and CO_2 gas (Walworth .2003 ; Daniel *et al* . 2007**)** Which show in GC studying of treated samples with fungi and compare them with virgin sample.Table.2 shows five of standard compounds and two compound were choice with unknown structure but known of the retention time. **Fig 1**

Table 2. Peak aria, Concentration, anti- Retention time of compounds for Gasoil sample

No	Compounds	Peaks aria	Concentration	Retention times
1	C_9H_{2O}	2922	0.144	2.714
3	$C_{12}H_{26}$	87814	4.088	8.141
5	$C_{16}H_{34}$	10502	7.082	11.680
6	$C_{17}H_{36}$	12080	5.972	12.902
7	$C_{21}H_{44}$	10830	5.354	16.098

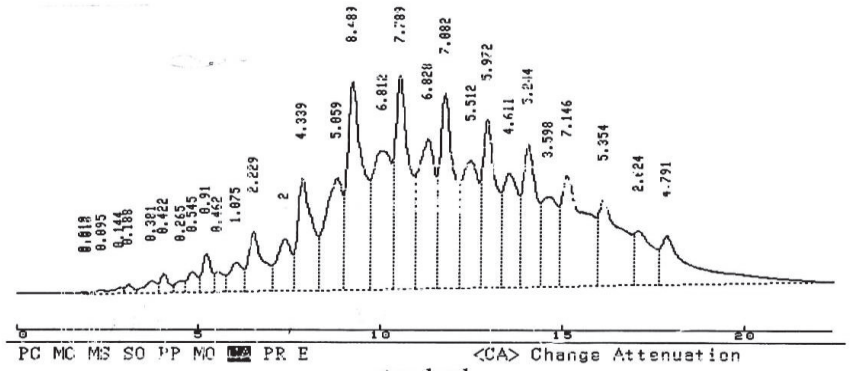

Fig.1. GC profile of Gasoil for stander sample

2.1 . Gas Chromatographic Analysis Of Samples:

Biodegradation of gasoil Result have been Followed-up through GC with several charts for all samples and which carried out by different strains of fungi were grown on the fuel. As can be seen from Table.3 The results of gasoil degradation indicate potential application of fungi for hydrocarbon bioremediation activity. The ability of isolated fungi for bioremediation of gasoil led to disappear of short chains C_9H_{20} (completely degradation) because of C_9 isn't that small, hence most of theories suggested its decomposition and transformation to CO_2 and CH_4 and other gaseous compounds or volatile compounds and decrease the concentration of compounds that contain long chains such as C_{16}, C_{17}, C_{21}. Table 3 shows the composition of control sample .

Table 3. Peak aria, Concentration and anti- Retention time of compounds forsample.1. (*Aspergillus sp.)*

No	Compounds	Peaks aria	Concentration	Retention times
1	C_9H_{2O}	….	….	2.714
3	$C_{12}H_{26}$	….	....	8.141
5	$C_{16}H_{34}$	….	….	11.680
6	$C_{17}H_{36}$	….	….	12.902
7	$C_{21}H_{44}$	….	….	16.098

Table 4 Shows the results of gasoil degradation indicate potential application of fungi for hydrocarbon bioremediation activity. The ability of isolated fungi for bioremediation of gasoil led to disappear of short chains C_9H_{20} (completely degradation) because of C_9 isn't that small, hence most of theories suggested its decomposition and transformation to CO_2 and CH_4 and other gaseous compounds or volatile compounds and decrease the concentration of compounds that contain long chains such as C_{16} , C_{17} and C_{21}. While the fungi *Aspergillus sp.* The consumption of $C_{12}H_{26}$ from 4.088 to 0.0513 and a peak aria which converted from 15565 to 1329

.

Table 4. Peak aria, Concentration, anti- Retention time of compounds for sample.2.treated with fungi *Aspergillus sp.* With 0.5 % Gasoil.

No	Compounds	Peaks aria	Concentration	Retention times
1	C_9H_{2O}	……	……	2.714
3	$C_{12}H_{26}$	1329	0.0513	8.141
5	$C_{16}H_{34}$	1329	0.0708	11.680
6	$C_{17}H_{36}$	482	0.0257	12.902
7	$C_{21}H_{44}$	151	0.0080	16.098

The compound $C_{16}H_{34}$ behaved similarly which contains `higher percentage of C_{12} which its concentration converted after treatment with fungi from 7.082 to 0.0708 which resulted in the reduction of the peak from 10502 to 1329 .The fungi was able to destroy the $C_{17}H_{36}$ and converting its concentration from 5.972 to 0.0257 reducing the peak area from 1208 to 482 besides the destruction of the compound $C_{21}H_{44}$, and changing the concentration from 5.354 to 0.0080 resulting and decreasing the peak area from 1083 to 151.Fungi had been succeeded in the decomposition gasoil with different percentage. The variation of the added compounds as references among them the following five (C_9, C_{12}, C_{16}, C_{17}, C_{21}). (Khorasani *et . al* 2013**) ,** and this differ from the idea that fungi cant degrade compound with more complex structure such as PAHs , with more than five benzene rings are more resistant to microbial break down . (Mancera – Lopez *et . al* 2006) .Table.5. Fungi showed clearly that degradation processes of gasoil was good enough, but the destruction of C_9H_{20}, was the highest due to the short chains of this compound. The ability of fungi varied in its capability of destruction of diesel compounds. Samples 7 , 8 , 9 and 10 were treated with fungi *Alternaria sp.* are more degradative comparing with 2 , 3 , 4 and 5 which treated with *Aspergillus sp.* Peaks height and concentration of compounds are sketched in tables 2 –11 which show that the sample 10 was of high potency of destruction for diesel fuel while sample.**6** was the lowest, generally fungi ability of destruction samples may be arranged as following: 10 > 7 > 8 > 9 > 4 > 2, 3, 5. It's clear that the fungi in sample 10, were able to destroy the hydrocarbonic which are highly resistable to atmospheric condition and vaporization , and as a consequence its remain into air and soil for along time.
(Abdulsalami *et. al* 2013 ; Agamuthu , 2013) **.** Also Table. 6 – 11 and Fig 2 – 4 .Shows each fungicide for destruction of diesel fuel contents.

Table 5. Peak aria, Concentration and anti- Retention time of compounds for sample.3. *Aspergillus sp.* With 1 % Gasoil.

No	Compounds	Peaks aria	Concentration	Retention times
1	C_9H_{2O}	134	0.0084	2.714
3	$C_{12}H_{26}$	6287	0.2871	8.141
5	$C_{16}H_{34}$	1804	0.0824	11.680
6	$C_{17}H_{36}$	3445	0.1574	12.902
7	$C_{21}H_{44}$	49	0.0022	16.098

Table 6. Peak aria, Concentration and anti- Retention time of compounds for sample.4. *Aspergillus sp.* With 3 % Gasoil.

No	Compounds	Peaks aria	Concentration	Retention times
1	C_9H_{2O}	861	0.0746	2.714
3	$C_{12}H_{26}$	5485	0.4756	8.141
5	$C_{16}H_{34}$	5595	0.4852	11.680
6	$C_{17}H_{36}$	3673	0.3184	12.902
7	$C_{21}H_{44}$	1287	0.1118	16.098

Table 7. Peak aria, Concentration and anti- Retention time of compounds for sample.5. *Aspergillus sp.* With 5 % Gasoil.

No	Compounds	Peaks aria	Concentration	Retention times
1	C_9H_{2O}	…..	……	2.714
3	$C_{12}H_{26}$	4029	0.2599	8.141
5	$C_{16}H_{34}$	1336	0.0862	11.680
6	$C_{17}H_{36}$	1042	0.0573	12.902
7	$C_{21}H_{44}$	256	0.0165	16.098

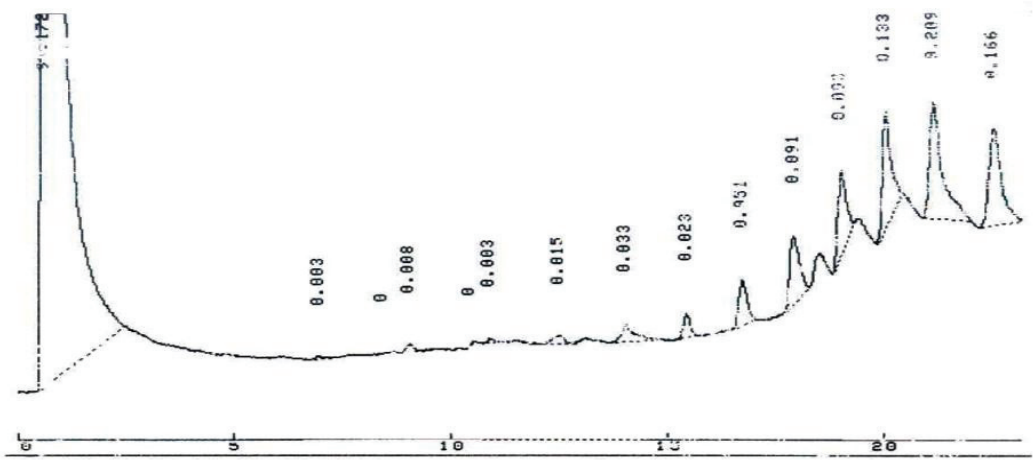

Fig.3. GC profile of sample.7.

Table 8. Peak aria, Concentration and anti- Retention time of compounds for sample.7. *Alternaria sp* with 0.5% Gasoil.

No	Compounds	Peaks aria	Concentration	Retention times
1	C_9H_{2O}	…..	……	2.714
3	$C_{12}H_{26}$	…..	…..	8.141
5	$C_{16}H_{34}$	76	0.0011	11.680
6	$C_{17}H_{36}$	228	0.0149	12.902
7	$C_{21}H_{44}$	354	0.0231	16.098

Table 9. Peak aria, Concentration and anti- Retention time of compounds for sample.8. *Alternaria sp* with 1% Gasoil.

No	Compounds	Peaks aria	Concentration	Retention times
1	C_9H_{2O}	…..	……	2.714
3	$C_{12}H_{26}$	…..	…..	8.141
5	$C_{16}H_{34}$	…..	….	11.680
6	$C_{17}H_{36}$	250	0.0954	12.902
7	$C_{21}H_{44}$	…..	…..	16.098

Table 10. Peak aria, Concentration and anti- Retention time of compounds for sample.9. *Alternaria sp* with 3% Gasoil.

No	Compounds	Peaks aria	Concentration	Retention times
1	C_9H_{2O}	…..	……	2.714
3	$C_{12}H_{26}$	147	0.0110	8.141
5	$C_{16}H_{34}$	544	0.0407	11.680
6	$C_{17}H_{36}$	205	0.0153	12.902
7	$C_{21}H_{44}$	161	0.0120	16.098

Table 11. Peak aria, Concentration and anti- Retention time of compounds for sample.10. *Alternaria sp* with 5% Gasoil

No	Compounds	Peaks aria	Concentration	Retention times
1	C_9H_{2O}	11	0.0006	2.714
3	$C_{12}H_{26}$	…..	…..	8.141
5	$C_{16}H_{34}$	370	0.0226	11.680
6	$C_{17}H_{36}$	106	0.0065	12.902
7	$C_{21}H_{44}$	72	0.0044	16.098

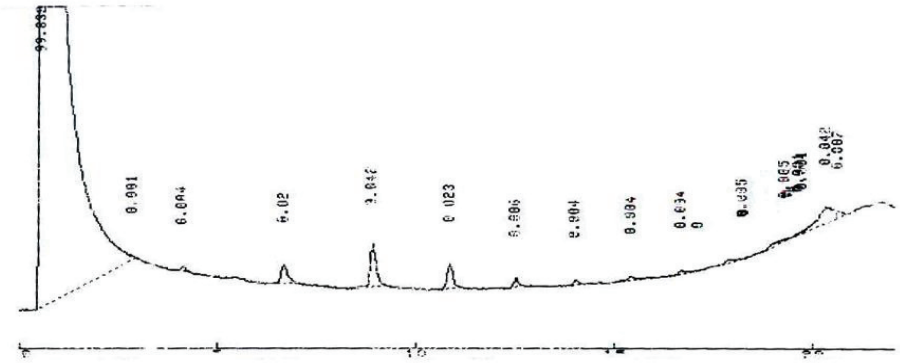

Fig.4. Show the ability of fungi for degradation the compounds which involved in the gasoil composition. 'GC profile of sample.10 '

Table12. Peak aria of gasoil compound before and after biodegradation**.**

Comp.	Before	Peak aria of compound in samples after biodegradation									
	stander	Control	2	3	4	5	Control	7	8	9	10
C_9H_{20}	2922	`….	……	134	861	…..	….	…..	…..	…..	11
$C_{12}H_{26}$	13814	….	1329	6287	5485	4029	….	…..	…..	147	…..
$C_{16}H_{34}$	10502	….	1329	1804	5595	1336	….	76	….	544	370
$C_{17}H_{36}$	12080	….	482	3445	3673	1042	….	228	250	205	106
$C_{21}H_{44}$	10830	….	151	49	1287	256	….	354	…..	161	72

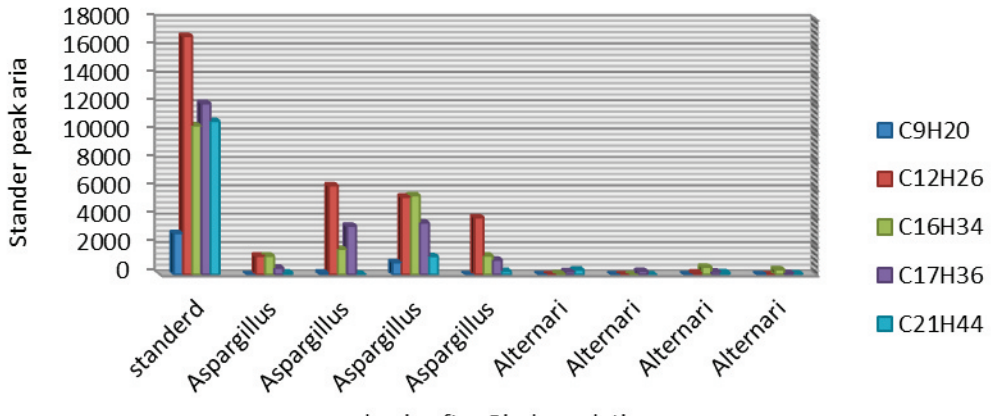

Fig.5. peaks aria of gasoil compound after degradation.

Conclusion

Using fungi as Biodegradation agents for diesel- fuel hydrocarbons gave good results, especially for long chains which polluted of environment . Its cheap method, simple techniques and ecofriendly. In the current study, it was found that all fungi tested could degradation of gasoil, also showed that the biodegradation correlated significantly positive with the numbers of microorganisms and based on the results obtained in this study the biodegrade depends on the kind of used fungus such as *Alternaria sp*. Strain are more effective in the degrade of gasoil than the *Aspergillus sp* Fungi strain.

References

Daniel, D. , Emilien. P . , Frederic C.(2007) . The influence of temperature on bacterial assemblages during bioremediation of a diesel fuel contaminated sub Antarctic soil. *Cold Regions Science and Technology*, 48, (2) : 74-83

Abdulsalami, B., anjuma , Y. D. , and Amen , I.A. (2013) . Bioremedation of soil contaminated with crude oils *Journal of Applied Phytotechnology in Environmental Sanitation*, 2(1): 15-24.

Agamuthu A. Dadrasnia . (2013) . Potential of biowastes to remediate diesel fuel contaminated soil *Global NEST Journal*, 15, (4) : 474-484

Gadd ,G,M . (2001) . Fungi In Bioremediation , Cambridge university , (1st ed) , press , 472 pp .

Okoh, A.I.(2003) . Biodegradation of Bonny Light Crude Oil in Soil Microcosm by Some Bacterial Strains Isolated from Crude Oil Flow Stations Saver Pits in Nigeria. *African Journal of Biotechnology*.. 2, 104 – 108.

Khorasani Ali reza Chackoshian, Mansour Mashreghi, and SoheylaYaghmaei . (2013) . Study on biodegradation of Mazut by newly isolated Enterobacter cloacae1 *Iranian Journal of Environmental Health Sciences & Engineering* 10, 2 - 7

Mancera-Lopez, M.E., Esparza-Garcia, F., Chavez-Gomez, B., Rodriguez-Vazquez, R., Saucedo-Castaneda, G., Barrera-Cortes, J. (2008) . Bioremediation of an aged hydrocarbon-contaminated soil by a combined system of biostimulatione bioaugmentation . *International Biodeterioration & Biodegradation* 61 , (2), 151–160

Mills, AL. , Breul, C. , Colwell, RR . (1978) . Enumeration of Petroleum Degrading Marine and Estuarine Microorganisms by the most probable number method. Can. J. Microbiol. 24(5):522-7.

Obire O., Anyanwu E. C. (2009) . Impact of various concentrations of crude oil on fungal populations of soil , *Int. J. Environ. Sci. Tech.,* 6 (2), 211-218 .

Okoro , Chuma C and Olukayode O Amund .(2010) . Biodegradation of Produced Water Hydrocarbons by Pure Cultures of *Alcaligenes* sp. *Report and Opinion* , 2(11):54-59

Tehrani , Minai ; Dariush, Minoui, Saeed ; Herfatmanesh, Ali. (2009) . Effect of salinity on biodegradation of polycyclic aromatic hydrocarbons (PAHs (of heavy crude oil in soil, *Bulletin of Environmental termination and Toxicology*, 82(2), 179–184 .

Walworth, J. L., Woolard . C. R . , Harris . K . C. (2003). Nutrient amendments for contaminated peri-glacial soils :Use of cod bone meal as a controlled release nutrient source, *Cold Regions Science and Technology,* 37 (2) , 81–88

Wanga. C. , Wanga . F. , Wanga. T., Biana. Y., Yanga. X. , Jianga X . (2010). PAHs biodegradation potential of indigenous consortia from agricultural soil and contaminated soil in two-liquid-phase bioreactor (TLPB). *J. Hazard. Mate.,* 176: 41– 47.

Watanabe, Tsuneo . (2002). Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species . (2nd . ed)

Wu M, Nie M, Wang X, Su J, Cao W (2010). Analysis of henanthrene biodegradation by using FTIR, UV and GC–MS. *Spectrochimica Acta* 75: 1047–1050. with filamentous fungi. *International Biodeterioration and Biodegradation* 61, 151-160

Xia Beicheng. Biodegradation of Pollutants in Environment, Beijing. (2002) ,chemical industry press, 179–180(in Chinese)

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