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BIOREMEDIATION OF DIESEL BY SOIL FUNGI

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ABSTRACT

The study attempts to bioremediation of diesel as petroleum contaminants using fungi isolated from soil of different sites in Baghdad . The three isolated fungi: *Aspergillus fumigatus*, *Candida nonsorbophila* and *zygosaccharomyc*es *bailii* were identified by molecular classification (PCR) and selected for bioremediation experiments that were more predominant in the soil .The fungi tested to biodegradation of diesel in solid medium (PDA) , liquid medium (MSM) and in soil.

Results of PDA experiments showed *A. fumigatus* was resistant to diesel fuel in 5% concentration, the colony diameter reached up to 7.1cm after 14 days. Lowest diameter 1.4 cm appeared in *Z. bailii* after 28 days.Regarding MSM experiment, *A. fumigatus* showed highest bioremediation 69.50% after 28 days in 10g/L diesel, while *C. nonsorbophilii* showed high biodegradation 69.0% after 28 days. To test the ability of mix fungi in MSM media, mix *A. fumigatus* with *Z. bailii* showed higher degradation 59.8% in 10g/L diesel after 28 days. While at used three fungi *(A.fumigatus+ C.nonsorbophilii+ Z.bailii)* showed less degradation 48.0% in 10g/L diesel after 28 days.Experiments of diesel biodegradation in soil after 60 days incubation, *A. fumigatus* recorded high percentage of remediation 97.70% ,while 97.20% recorded by *C. nonsorbophilii.* , and 94.80% by *Z. bailii* that was not different significance among them, but significantly higher than the minimum bioremediation of the three fungal mixture (90.70%).The study concluded that *A. fumigatus* could be used in bioremediation being better than the rest of the fungi on diesel pollutant biodegradation in solid, liquid and soil medium.Ability of mixed fungi to diesel biodegradation was less than using alone depended on competition and antagonisms between the fungi in the growth medium.

Keywords: bioremediation, diesel, fungi, soil

INTRODUCTION

Petroleum-based products like diesel are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products (Kvenvolden *et al.*, 2003). Soil contamination with hydrocarbons causes extensive damage of the local system since accumulation of pollutants in animals and plant tissue may cause death or mutations. The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion, and washing. However, these technologies are expensive and can lead to incomplete decomposition of contaminants. The process of bioremediation, defi-ned as the use of microorganisms to detoxify or remove pollutants owing to their diverse metabolic capabilities is an evolving method for the removal and degradation of many environmental pollutants including the products of the petroleum industry. Biodegrada-tion by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollu-tants can be removed from the environment. Hydrocarbons in the environment are biodegraded prim-arily by bacteria, yeast, and fungi. Recently, many researchers studied the importance of fungi in biodegradation of pollutants and the most common fungi which have been recorded as a biodegrades belongs to following genera: *Aspergillus, candida, zygosaccharomyc*es, *Fusarium, Penicillium, Amorphoteca* (Mandal, *et al*. 2007, Quan *et al*., 2009 and Dhaker & Jain 2011).

The study focused on the biodegradation of diesel contaminating soil by three soil fungi *A.fumigatus, Candida nonsorbophila* and *Zygosaccharomyces bailii* growing through 14, 21 and 28 days in different concentrations of diesel (10, 20, 30 g/L) at different environmental media such as agar (solid) and mineral salt medium MSM (liquid) in addition to soil.

MATERIALS AND METHODS

Source of Diesel: Obtained from station refinery of Al-Qanat in Baghdad.

Samples of Soil: The soil samples were obtained from Baghdad, 3 samples taken from contaminated soil surrounding the fuel stations of (Al-Qanat) in Al-Rasafa side. Soil samples were collected from location weighed 50 g, just 10 cm below the soil surface by hand trowel. Samples were taken carefully in plastic bags to avoid contamination, and then transported to the mycology laboratory immediately. Plastic bag samples were stored

under refrigeration at 4ºC until fungal separation and identification, which was no later than 48 hours after sampling, Isolation and identification of fungi.

Classification of morphological properties: Isola-ted fungi were identified on the basis of their morp-hological characters by observing colony advantage (colonial morphology, color, texture, shape, diameter and appearance of the colony) and observed under microscope for the conidiopho-res, conidia, and arrangement of conidia, fungi were described, identified and classified as depen-dent on taxonomic keys (Watanabe, 2002).

Molecular identification: The genomic DNA of fungi was isolated from the cultures according to (Geiser*et al*., 2004). The ITS gene was amplified using the universal primer ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (White *et al*., 1990).

The PCR product was sent for sanger sequencing using ABI3730XL, automated DNA sequencer by macrogen corporation, Korea. The results were received by email then analyzed using genious soft-ware.

Bioremediation of Dieselby Fungi in Solid Media (PDA): 1000 ml of PDA was prepared as manual method and autoclaved at (121°C, 15 Ibs/In² pressure for 20 minutes). 20ml PDA was put in each of 42 Petri dish.5% (1ml per dish) of diesel was poured in each of dish as used by (Sakineh, *et al*., 2012). Fungal disc inoculums (piece fungi 1cm plug) were added to dishes which ordered as follows: 7 dishes considered as control (containing dextrose without diesel) inoc-ulated with the fungus *A. fumigatus* (F1) as a first dish, second dish inoculated with the fungus *C. nonsorbophila* (F2), third inoculated with the fun-gus *Z. bailii*(F3), the forth with (F1+F2), the fifth with (F1+F3), the sixth with (F2+F3), and the final seventh with mixture of all three fungi (F1 + F2+F3). Other 7 dishes PDA (with-out dextrose, containing diesel) inoculated with the same fungal inoculums arranged previously. The same method of the previous 14 dishes were grown at 3 periods of 14, 21, and 28 days, the outcome of total dishes equaled to 42. These dishes were prepared into triplicate.

Bioremediation of Diesel by Fungi in Liquid (MSM): Mineral Salt, Medium (MSM) was pre-pared in according to (Ahmed, *et al* 2016) by using Bushnell Hass Mineral Salts medium cont-aining $MgSO_4$ (0.2g/l), $CaCl_2$ (0.02 g/l), KH_2PO_4 (1 g/l), K_2HPO_4 (1 g/l), $FeCl_2$ (0.05 g/l) and NH_4NO_3 (1 g/l). In which dissolved1000 ml distilled water and shake. The pH was adjusted to 5.5 then added anti-biotic chloramphenicol (250 mg/ L), each 50 ml of MSM was put in 250-ml flask and added 250 mg/L chloramphenicol, inoc-ulated by 4% (v/v) 2ml of inoculums (2.4 x 10^5 CFU/ml). The flasks were incubated at 30°C for 14, 21 and 28 days. Experi ments were performed in (72) Erlenmeyer flasks. Of each group a set made up as following: 9 flasks of control with Initial diesel (10, 20, 30 g/L) had no fungi, incubated at three periods (14, 21 and 28 days). Another 7 sets prepared by same diesel treatment previously but inoculated with fungi arranged as 9 flasks for each of(F1), (F2), (F3), (F1+F2), (F2+ F3), (F1+F3),and (F1+F2+F3), the flasks were done in triplicate.The growth was stopped after incubation by adding 1N HCl 1% followed by extraction of diesel that estimated by gravimetric glass as used by (Ahmed, et al., 2016). The per-centage of Bioremediation rate of diesel was calc-ulated in the tables by the following equations:

CR = IC – FC, PR = (CR / IC) X 100

Bioremediation Rate % = PR Fungi – PR Control

Where: CR=Concentration of remediate diesel (%),

IC = Initial Concentration of diesel (%),

FC = Final Concentration of diesel (%),

PR = percentage of remediate diesel

Bioremediation of Diesel by Fungi in soil: The soil was collected from Alrasafa, Baghdad in December 2016 and was characterized as a clay-ey sand. Soil has an organic matter of 5.9%, total nitrogen content of 136.0 ppm, phosphor total content of 89.0 ppm in the soil was determined according to (Mohanan *et al*., 2005). The soil for laboratory experiments was put 50g on each of 5 bottles, then autoclaved.10g (20% v/w) of diesel was uniformly placed on the soil under steriliza-tion, it was allowed to be adsorbed during 30 minutes as used by (Facundo, 2001). One bottle lets as a control (without inoculation). 3 bottles were Inoculated by an equal amount of 2.4x10^5 (CFU /g soil) of fungi (F1, F2, and F3) in each bottle separately. Fifth bottle inoculated by the same amount of 2.4x10^5(CFU /g soil) of mixed fungi (F1+ F2+ F3) together. The experiment was done triplicate.All bottles were incubated in room temperature for 60 days.

Measurement of residual petroleum hydrocarbons in soil: After 60 days percentage residue of diesel in soil samples was measured. The extraction of diesel from soil was conducted according to the method used by (minai-Tehraniand Herfatmanesh, 2007). With slightly modified 2g of soil was mix-ed with 10ml of CH_2Cl_2 and shaken firmly. The sam-ple was centrifuged ICE centra-8R (USA) 3000g for min to precipitate the soil, and the sol-

vent phase was removed. This solvent extraction was repeated twice. The solvent was vaporized during 24 h and the amount of crude oil and diesel was measured using the gravimetric method and its reduction was compared with control sample. The percentage of Bioremediation Rate of diesel in samples were cal-culated according to (Sakineh*et al.*, 2012), using above formula.

Statistical Analysis: All treatments in the study were completed with three replicates. SAS (2014) program was used to effect of different factors in study parameters. Least significant difference-LSD test (ANOVA) was used to significant compare between means.

RESULTS AND DISCUSSION

Molecular Identification: The phylogenetic trees that were drawn based on partial PDA gene sequence homologies between species enables us to understand the inter-species relation.

Geneious program with Neighbor-Joining tree building method was used to construct phylogenetic trees. The tree in (appendix 1-A) shows the relationship of the species of the genus *Aspergillus* based on the partial DNA sequences of cytochrome *b* gene.

The fungi were divided into two clusters with a high similarity of sequences, and then the species identified as *Aspergillus fumigatus*, the nucleotide alignment consensus tree is showing in (appendix 1-B). In the same method, the phylogenetic tree of *Candida nonsorbophila* showing in (appendix 2-A) and nucleotide alignment in (appendix 2-B), while appendix 3-A and 3-B represent the phylo-genetic tree and nucleotide alignment of *Zygosacc- haromyces bailii* respectively.

Biodegradation of Diesel by Fungi in PDA Solid Medium: The growth assay was used to find the resistant fungal species to diesel fuel contamination. Test dishes were prepared by adding diesel fuel into warm liquefied PDA in order to have 1ml (5%) concentration of diesel fuel in all plates (without dextrose), except control without diesel, sho-wed that *A. fumigatus* was utilized diesel fuel in solid medium and the colony diameter of this fungus reached up to 7.1cm after 28 days, lowest diameter appeared in *Z. bailii* that dropped to 1.4 cm after 14 days as in figure 1. The present results in the study were similar to the findings of (Nilanjana and Preethy, 2011).

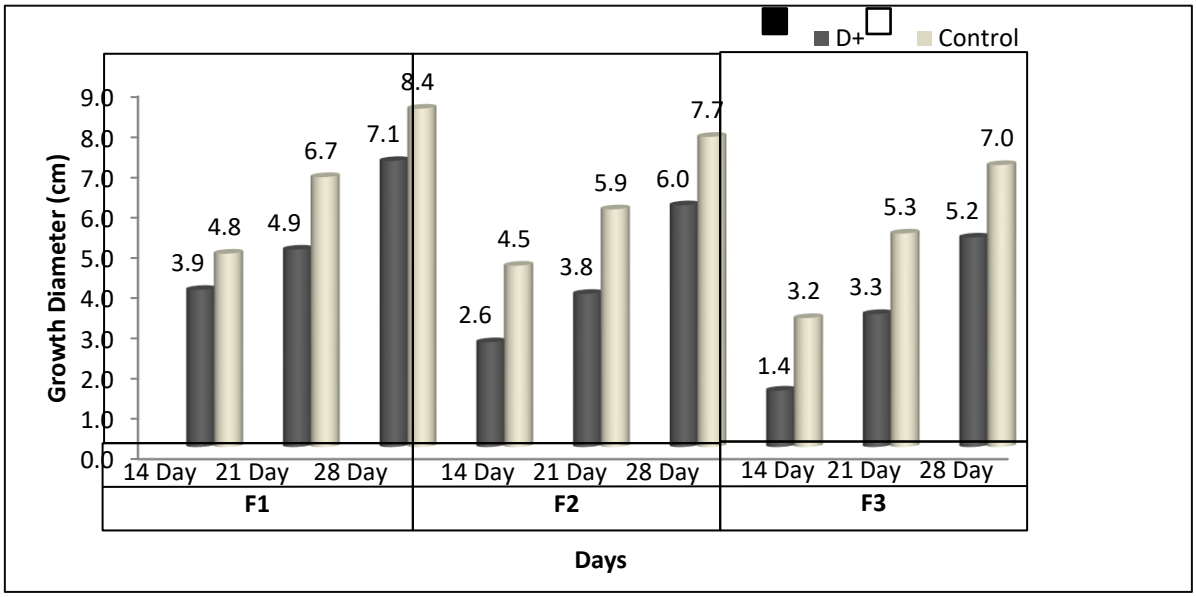

Figure 1 : Fungal diameter (cm) growth by PDA medium incorporated with Diesel . F1 (*A. fumigatus*), F2 (*C. nonsorbophila*), F3(*Z. bailii*) CONTROL = PDA medium ; D+ = PDA (without dextrose) incorporated with 1 ml Diesel (5%) in Petri dish.

Figure 2 showed mixed of fungi grown inPDA media without dextrose to utilize diesel (5%) as a sole carbon source. High diameter (1.9 cm) appeared after 28 days in mixed two fungi (F1+F2) but the lowest diameter (0.4cm) appeared in fungal mixture (Fm) after 14 days because of many factors such as competition and antagonisms (Mancera *et al*., 2007).

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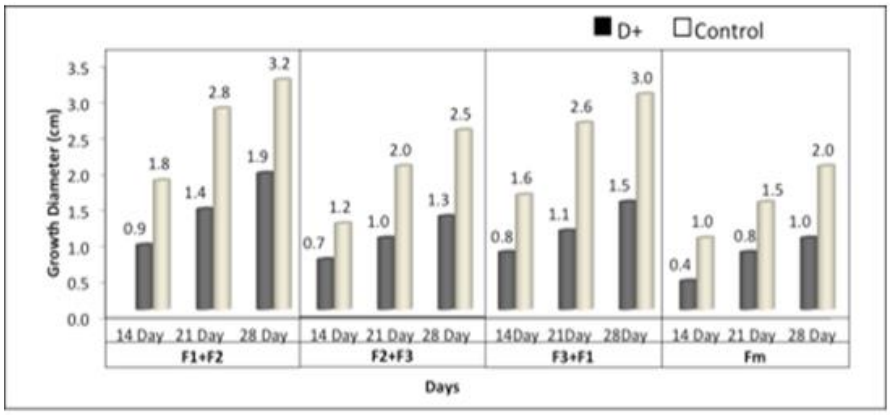

Figure 2 : Fungal diameter (cm) growth by PDA medium incorporated with Diesel.
F1 (*A. fumigatus*), F2 (*C. nonsorbophila*), F3 (*Z. bailii*.).
CONTROL = PDA medium. ; Fm = Fungal mixture (F1+F2+F3).
D+ = PDA (without dextrose) incorporated with 1 ml diesel (5%) in Petri dish.

Biodegradation of Diesel by Fungi in MSM Liquid Medium: The growth of *A. fumigatus* isolates to bioremediation of diesel in the bottles contained MSM was shown in figure 3. Table 1 showed the highest bioremediation rate percentages of diesel after 28 days were 69.50%, 64.15% and 67.67% in diesel concentrations 10g/L, 20g/L, and 30g/L respectively. The period 28 days of 10g/L diesel had more value than 14 and 21days but not significant differences, therefore can use 10g/L diesel in 14 days to reduce time. This result was similar to the findings of (George *et al*., 2000).

Figure 3. *A. Fumigatus* growing in MSM added diesel (10, 20 and 30 g/L) and control during (14, 21 and 28 days)

The highest bioremediation rate percentage of diesel concentration by *C. nonsorbophila* illustrated in table 2 that recorded as 69.0% at 28 days in 10g/L diesel concentration, which follows shows convergent biodegradable value amounted 65.75% and 67.07% in concentration 20g/L and 30g/L respective.

Table 1: Biodegradation of **Diesel** (g/L) in Mineral Salt Medium (MSM) medium by *A. fumigatus* for three Periods incubation .

Fungi		**Control**			***A. fumigatus***			**LSD value**
Day		**14**	**21**	**28**	**14**	**21**	**28**	
Initial Diesel (10 g/L) (IC)	Final Conc. (FC)	7.41	7.22	7.20	0.71	0.37	0.25	---
	remediation conc. (CR)	2.59	2.78	2.80	9.29	9.63	9.75	---
	Remediation (PR)%	25.9	27.8	28.0	92.9	96.3	97.50	---
	Bioremediation Rate %	0	0	0	67.0	68.5	69.50	3.76 NS
Initial Diesel (20 g/L) (IC)	Final Conc. (FC)	14.10	13.52	13.03	1.60	0.73	0.20	---
	remediation conc. (CR)	5.90	6.48	6.97	18.40	19.27	19.80	---
	Remediation (PR)%	29.50	32.40	34.85	92.00	96.35	99.00	---
	Bioremediation Rate %	0	0	0	62.50	63.95	64.15	3.65 NS
Initial Diesel (30 g/L) (IC)	Final Conc. (FC)	23.40	22.10	21.12	3.65	2.00	1.82	---
	remediation conc. (CR)	6.60	7.90	8.88	26.35	28.00	29.18	---
	Remediation (PR)%	22.00	26.33	29.60	87.33	93.33	97.27	---
	Bioremediation Rate %	0	0	0	65.33	67.00	67.67	3.09 NS
LSD value		---	---	---	4.38 *	4.52 *	4.85 *	---
Total : LSD value		---	---	---	4.86 *			---

* ($P<0.05$)

It was observed not significant differences between 14, 21 and 28 days in all diesel conc, therefore can use 14 days to be economically and reduce time,this result agreed with the results of (Gesinde *et al*.,2008) with variation in species of fungi.

Table 2 : Biodegradation of Diesel (g/L) in MSM medium by *C. nonsorbophila*for three Periods incubation .

FUNGI		CONTROL			*C. nonsorbophila*			LSD value
DAY		14	21	28	14	21	28	
Initial Diesel (10 g/L) (IC)	Final Conc.(FC)	7.41	7.22	7.20	0.66	0.45	0.30	---
	remediation conc. (CR)	2.59	2.78	2.80	9.34	9.55	9.70	---
	Remediation(PR)%	25.9	27.8	28.0	93.4	95.50	97.0	---
	Bioremediation Rate %	0	0	0	67.5	67.70	69.0	3.71NS
Initial Diesel (20 g/L) (IC)	Final Conc.(FC)	14.10	13.52	13.03	1.41	0.70	0.18	---
	remediation conc. (CR)	5.90	6.48	6.97	18.59	19.30	19.92	---
	Remediation(PR)%	29.50	32.40	34.85	92.95	96.50	99.60	---
	Bioremediation Rate %	0	0	0	63.45	64.10	65.75	3.88NS
Initial Diesel (30 g/L) (IC)	Final Conc. (FC)	23.40	22.10	21.12	3.44	2.10	1.00	---
	remediation conc. (CR)	6.60	7.90	8.88	26.56	27.90	29.00	---
	Remediation(PR)%	22.00	26.33	29.60	88.53	93.00	96.67	---
	Bioremediation Rate %	0	0	0	66.53	66.67	67.07	3.36NS
LSD value		---	---	---	3.92 NS	4.36 NS	4.52 NS	---
Total : LSD value		---	---	---	5.07 *			---

* (P<0.05)

Table 3 showed the highest bioremediation rate percentage by *Z. bailii* in concentration 30g/L at 28 days 62.67% more than 61.90% and 58.95% in oncentration 10 g/L at 28 days and 21 days respectively, the different result was obtained by (Obire and Anyanwu, 2009).

Table 3 : Biodegradation of **Diesel** (g/L) in MSM medium by *Z. bailii*for three Periods incubation .

FUNGI		CONTROL			*Z. bailii*			LSD value
DAY		14	21	28	14	21	28	
Initial Diesel (10 g/L) (IC)	Final Conc. (FC)	7.41	7.22	7.20	1.82	1.33	1.01	---
	remediation conc. (CR)	2.59	2.78	2.80	8.18	8.67	8.99	---
	Remediation (PR)%	25.9	27.8	28.0	81.8	86.70	89.90	---
	Bioremediation Rate %	0	0	0	55.9	58.95	61.90	4.65 *
Initial Diesel (20 g/L) (IC)	Final Conc. (FC)	14.10	13.52	13.03	3.97	3.20	2.01	---
	remediation conc. (CR)	5.90	6.48	6.97	16.03	16.80	17.99	---
	Remediation (PR)%	29.50	32.40	34.85	80.15	84.00	89.45	---
	Bioremediation Rate	0	0	0	50.65	51.60	54.60	4.35 NS
Initial Diesel (30 g/L) (IC)	Final Conc. (FC)	23.40	22.10	21.12	7.60	6.70	2.31	---
	remediation conc. (CR)	6.60	7.90	8.88	22.40	26.30	27.69	---
	Remediation (PR)%	22.00	26.33	29.60	74.67	87.67	92.30	---
	Bioremediation Rate %	0	0	0	41.67	54.67	62.67	5.84 *
LSD value		---	---	---	5.32 *	4.95 *	5.21 *	---
Total : LSD value		---	---	---	5.79 *			---

* (P<0.05)

There is no significant value at 28 days between 61.9% (in 10 g/L) and 62.67% (in 30g/L), therefore the fungus appeared able to treat 30g/L of diesel pollutant better than 10 g/L like stated by (Shallu 2014).

Table 4 showed *A.fumigatus* and *C. nonsorbophila* together had the highest percentage bioremediation rate of diesel concentration 10g/l after 28 days was 59.4% while 57.7% after 21 days. It was no significant difference when using separately, and a different result was obtained by (Ghanem *et al*., 2015) because they used different genus with kerosene.

Table 4: Biodegradation of **Diesel** (g/L) in MSM medium by **Mixing** soil fungi F1 (*A. fumigatus*) & F2 (*C. nonsorbophila*) for three Periods incubation .

SOIL FUNGI		CONTROL			F1+F2			LSD value
DAY		14	21	28	14	21	28	
Initial Diesel (10 g/L) (IC)	Final Conc. (FC)	7.41	7.22	7.20	1.80	1.44	1.26	---
	remediation conc.(CR)	2.59	2.78	2.80	8.20	8.56	8.74	---
	Remediation(PR)%	25.9	27.8	28.0	82.0	85.6	87.4	---
	Bioremediation Rate %	0	0	0	56.1	57.7	59.4	4.12NS
Initial Diesel (20 g/L) (IC)	Final Conc. (FC)	14.10	13.52	13.03	3.81	3.20	2.60	---
	remediation conc. (CR)	5.90	6.48	6.97	16.19	16.80	17.40	---
	Remediation (PR)%	29.50	32.40	34.85	80.95	84.00	87.00	---
	Bioremediation Rate %	0	0	0	51.45	51.60	42.45	5.52 *
Initial Diesel (30 g/L) (IC)	Final Conc. (FC)	23.40	22.10	21.12	6.21	5.08	3.80	---
	remediation conc. (CR)	6.60	7.90	8.88	23.79	24.92	26.20	---
	Remediation (PR)%	22.00	26.33	29.60	79.30	83.07	87.33	---
	Bioremediation Rate %	0	0	0	51.30	56.74	57.73	4.89 *
LSD value		---	---	---	4.022 *	4.19 *	5.08 *	---
Total : LSD value		---	---	---	5.86 *			---

* (P<0.05)

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In table 5 the results showed the highest percentage bioremediation rate of diesel by using *C. nonsorbophila* and *Z. bailii* in concentration 10g/ L was 58.5, 57.0% after 28 days and 21days respectively, but it observed that these fungi achieved a higher bioremediation rate when work alone and there was no significant difference for the three-time period at three concentration. The same result was obtained by Ghanem *et al*., (2015).

Table 5 : Biodegradation of **Diesel** (g/L) in MSM medium by **Mixing** soil fungi . F2 (*C. nonsorbophila*) & F3 (*Z. bailii*) for three for Three Periods incubation .

SOIL FUNGI		CONTROL			F2+F3			LSD value
DAY		14	21	28	14	21	28	
Initial Diesel (10 g/L)	Final Conc. (FC)	7.41	7.22	7.20	1.72	1.51	1.35	---
	remediation conc. (CR)	2.59	2.78	2.80	8.28	8.49	8.65	---
	Remediationpercent (PR)	25.9	27.8	28.0	82.8	84.9	86.5	---
	Bioremediation Rate %	0	0	0	56.9	57.0	58.5	3.71 NS
Initial Diesel (20 g/L)	Final Conc. (FC)	14.10	13.52	13.03	3.91	3.30	2.20	---
	remediation conc. (CR)	5.90	6.48	6.97	16.09	16.70	17.80	---
	Remediationpercent (PR)%	29.50	32.40	34.85	80.45	83.50	89.00	---
	Bioremediation Rate %	0	0	0	50.95	51.10	55.15	5.007 *
Initial Diesel (30 g/L)	Final Conc. (FC)	23.40	22.10	21.12	6.77	5.40	5.11	---
	remediation conc. (CR)	6.60	7.90	8.88	23.23	24.60	24.89	---
	Remediationpercent (PR)%	22.00	26.33	29.60	77.43	82.00	82.97	---
	Bioremediation Rate %	0	0	0	55.43	56.67	53.37	3.18 NS
LSD value		---	---	---	3.67 NS	4.25 *	4.44 *	---
Total : LSD value		---	---	---	5.97 *			---

* (P<0.05)

Table 6 indicated that mixed of *A. fumigatus* and *Z. bailii* had the highest bioremediation rate 59.8% in diesel concentration 10g/L after 28 days and 57.1% after 21 days,a less result was obtained by (Ghanem *et al*., 2015) and a less result was obtained when work alone fungi. It was known that when using mix *A. fumigatus* and *Z. bailii* the result appeared less efficiency in hydrocarbon degradation because of competition for carbon source.

Table 7 act three mix fungi *A.fumigatus*, *Z.bailii* and *C. nonsorbophila*, the highest percent of bioremediation rate of diesel was 48.0 % in concentration 10g/L at 28 days, while the less percent of bioremediation rate of diesel was recorded to 33.73% after 28 days in concentration 30 g/L. The results had a significant difference in three concentrations which appeared the best in concentration 10g/L at 28 days, but the result of three fungi mixture was no more decomposition compared with using alone fungi because secretion of fungal metabolites inhibits growth of other fungi (Mohsenzadeh *et al*., 2012).

Table 6 : Biodegradation of **Diesel** (g/L) in MSM medium by **Mixing** soil fungi F1 (*A. fumigatus*)& F3(*Z. bailii*) for three Periods incubation .

SOIL FUNGI		CONTROL			F3+F1			LSD
DAY		14	21	28	14	21	28	value
Initial Diesel (10 g/L) (IC)	Final Conc. (FC)	7.41	7.22	7.20	1.98	1.50	1.22	---
	remediation conc. (CR)	2.59	2.78	2.80	8.02	8.50	8.78	---
	Remediationpercent (PR)%	25.9	27.8	28.0	80.2	85.0	87.8	---
	Bioremediation Rate %	0	0	0	54.3	57.1	59.8	4.06 *
Initial Diesel (20 g/L) (IC)	Final Conc. (FC)	14.10	13.52	13.03	4.79	3.50	2.60	---
	remediation conc. (CR)	5.90	6.48	6.97	15.21	16.50	17.38	---
	Remediationpercent (PR)	29.50	32.40	34.85	76.05	82.50	86.90	---
	Bioremediation Rate %	0	0	0	46.55	50.10	52.05	5.17 *
Initial Diesel (30 g/L) (IC)	Final Conc. (FC)	23.40	22.10	21.12	8.90	7.41	6.38	---
	remediation conc. (CR)	6.60	7.90	8.88	21.10	22.59	23.62	---
	Remediationpercent (PR)	22.00	26.33	29.60	70.33	75.30	78.73	---
	Bioremediation Rate %	0	0	0	48.33	48.97	49.13	3.66 NS
LSD value		---	---	---	4.07 *	4.35 *	5.31 *	---
Total : LSD value		---	---	---	5.68 *			---

* (P<0.05)

The results indicated that when used PDA solid medium or MSM liquid in experiments the fungi if used single or mix can utilize diesel as a sole carbon source and the process depends on the fungi characteristics and the environmental conditions required by the microorganisms (Bernal-Martínez, *et al.* 2009). The higher capacity to remove diesel depends on the adaptation of these fungi to the pollutant composition, as well as to the enzymatic systems of the fungi (Piola, and Johnston,2008). High degradation showed when used *A. fumigatus* or *C. nonsorbophila* while *Z.bailii* considered as the lowest.The degradation depended on long time, which showed in 28 days incubation whose percentage degradation, higher compared with 14 and 21 days.

Table 7: Biodegradation of **Diesel** (g/L) in MSM medium by **Mixing** soil fungi (Fm): F1 (*A. fumigatus*), F2 (*C. nonsorbophila*) and F3(*Z. bailii*) for three Periods incubation

SOIL FUNGI		CONTROL			Fm			LSD
DAY		14	21	28	14	21	28	value
Initial Diesel (10 g/L) (IC)	Final Conc.(FC)	7.41	7.22	7.20	4.14	3.15	2.37	---
	remediation conc.(CR)	2.59	2.78	2.80	5.86	6.85	7.63	---
	Remediation (PR)%	25.9	27.8	28.0	58.6	68.5	76.3	---
	Bioremediation Rate %	0	0	0	32.7	40.6	48.0	5.86*
Initial Diesel (20 g/L) (IC)	Final Conc. (FC)	14.10	13.52	13.03	8.91	7.20	5.50	---
	remediation conc. (CR)	5.90	6.48	6.97	11.09	12.98	14.50	---
	Remediation (PR)%	29.50	32.40	34.85	55.45	64.90	72.50	---
	Bioremediation Rate %	0	0	0	25.95	32.50	37.65	5.02*
Initial Diesel (30 g/L) (IC)	Final Conc. (FC)	23.40	22.10	21.12	16.01	14.13	11.00	---
	remediation conc. (CR)	6.60	7.90	8.88	13.99	15.87	19.00	---
	Remediation (PR)%	22.00	26.33	29.60	46.63	52.90	63.33	---
	Bioremediation Rate %	0	0	0	24.63	26.57	33.73	5.11*
LSD value		---	---	---	5.19 *	4.98 *	6.75 *	---
Total : LSD value		---	---	---				---

* (P<0.05)

It was found that the mix of (*A. fumigatus* + *C. nonsorbophila*), (*C.nonsorbophila* + *Z.bailii*), (*Z. bailii*+ *A.fumigatus*) and (*A.fumigatus*+ *C. nonsorbophila*+ *Z.bailii*) have reduced the growth of fungi because many factors such as the competition and antagonisms(Mancera-lopez, *et al*., 2007) leaded to decrease biodegradation of diesel.

Ability of fungi degradation of diesel in soil: This test tried to determine the capbilities of diesel degrading by fungi isolated from soil of Baghdad in order to reduce the problem of oil pollution of the soil. Soil samples contaminated with diesel from diff-erent sites of Baghdad were treated with the fungi for 60 days as shown in figure 4.

Figure 4: Growth of fungi (F1,F2,F3,Fm and control) in soil contaminated with diesel.

Table 8 showed the higher remediation percent in soil reached to 97.70 % by F1 (*A. fumigatus*) compared with other alone or mix fungi. The same result was obtained by (Farid, 2012). Result of mix three fungi (Fm) was the reduction of remediation (90.70%) because of many factors such as the competition and antagonisms (Mancera-lopez *et al*., 2007). When showing bioremediation of the higher rate in F1 compared with other fungi or with mix, there was no significant differences observed in F1 and other single fungi, but found significant differences between F1 and F2 compared with Fmwhose lowest value.

The study concluded that *A. fumigatus* was better than the rest fungi in biodegradation of diesel in solid and liquid medium in addition to soil. The data obtained the diesel hydrocarbon was less bioremediation in mixed culture of *A. fumigatus, C. nonsorbphila* and *Z. bailii* and their ability to remove diesel from contaminated environments depended on many factors such as the competition and antagonisms.

Table 8: Bioremediation Rate of Diesel(20%) in Soil by Fungi. F1 (*A. fumigatus*), F2 (*C. nonsorbophila*) and F3(*Z. bailii*) for 60 days incubation in soil.

Conc. / Fungi	Initial diesel (IC) %	Final diesel (FC) %	Remediation Conc. (CR) %	Remediation Percent (PR)%	Bioremediation Rate %
Control	20.00	4.84 4.74 4.82 4.80 0.39	15.20	76.00	0.0
Mean SD					
F1	20.00	0.46 0.44 0.48 0.46 0.26	19.54	97.70	21.70
Mean SD					
F2	20.00	0.58 0.54 0.56 0.56 0.24	19.44	97.20	21.20
Mean SD					
F3	20.00	1.02 1.02 1.08 1.04 0.31	18.96	94.80	18.80
Mean SD					
Fm	20.00	1.86 1.88 1.84 1.86 0.27	18.14	90.70	14.70
Mean SD					
LSD value	---	---	---	---	5.293 *

* ($P<0.05$).

CONCLUSION

The study concluded thatability of *A. fumigatus* was better than *C. nonsorbophila* while *Z. bailii* was the lowest in biodegradation of diesel in solid and liquid medium in addition to in soil.

Mix fungi with each other decreased the rate degradation because antagonisms and competition 20g/L concentration of diesel was the best for the activity of fungi to biodegrade than10g/L and 30g/L.

The degradation of fungi for diesel in soil environment had a higher percentage than liquid medium by *A. fumigatus* 97.70% , *C. nonsorbophilii* 97.20% and 94.80% by *Z. bailii*.

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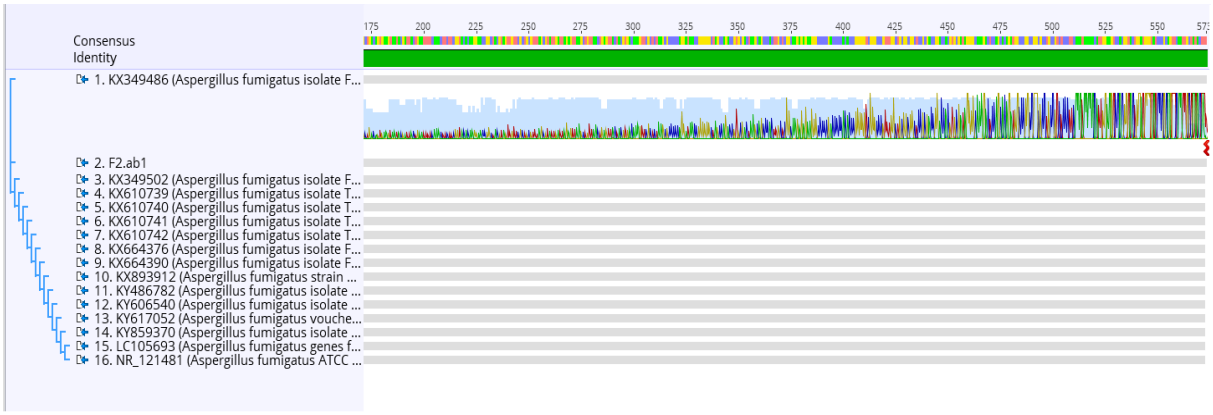
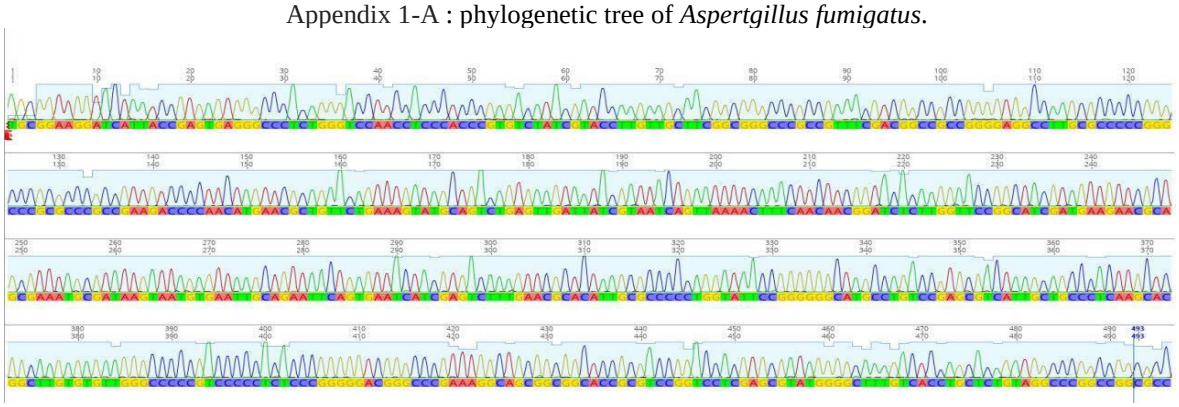
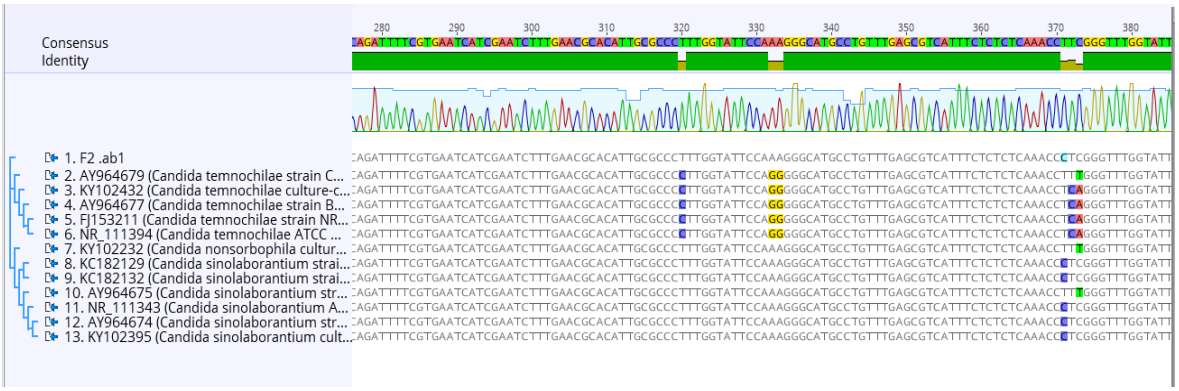
APPENDICES



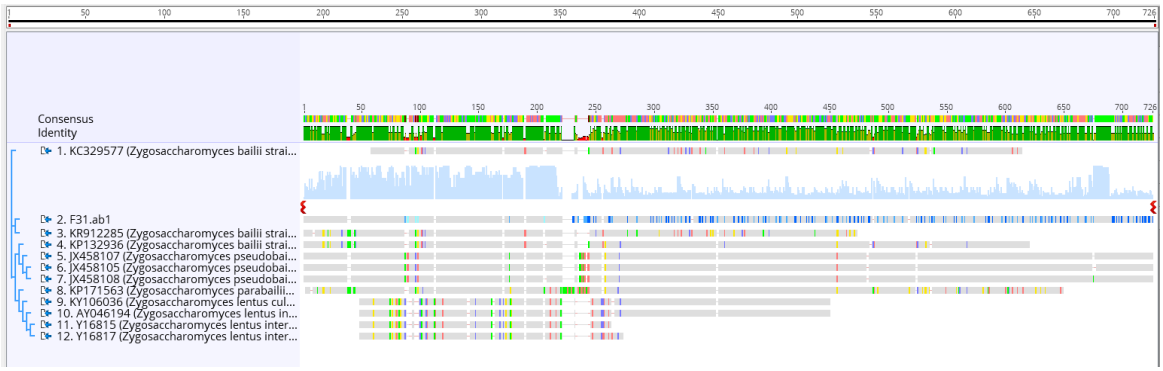
Appendix 1-A : phylogenetic tree of *Aspertgillus fumigatus*.



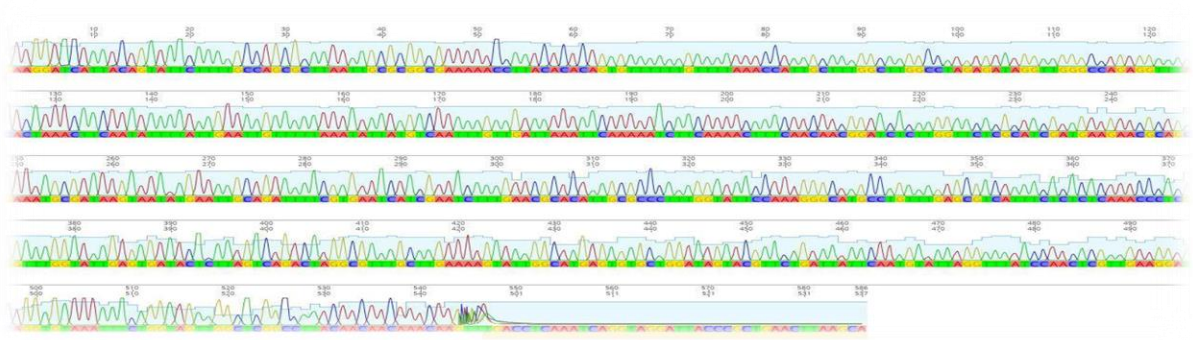
Appendix 1-B: Partial ITS1 gene sequence of the isolate *A.fumigatus*



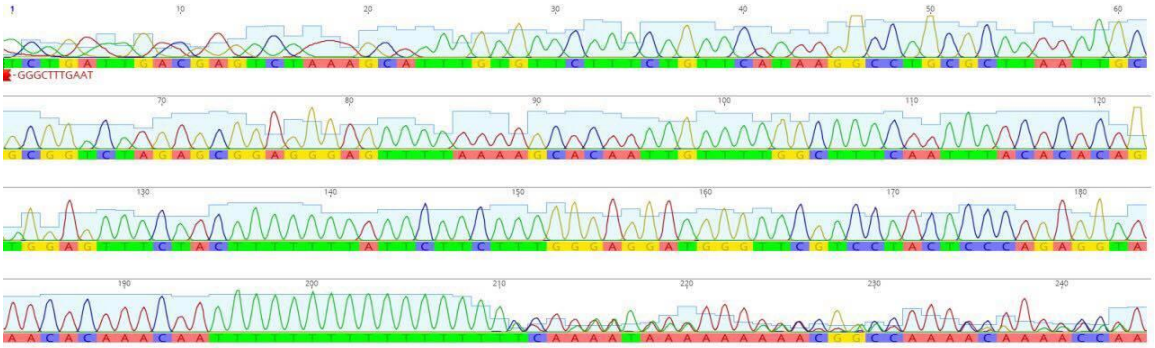
Appendix 2-A: phylogenetic tree of *Candida nonsorbophila*



Appendix 2-B: Partial ITS1 gene sequence of the isolate *C. nonsorbophila*



Appendix 3-A: phylogenetic tree of *Zygosaccharomyces bailii*



Appendix 3-B:Partial ITS1 gene sequence of the fungi isolate *Z. bailii*