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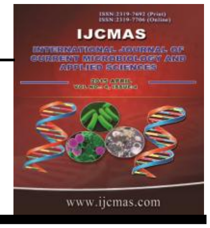

Original Research Article

Efficiency of some filamentous fungi to treatment of effluent petroleum wastewaters from refinery

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A B S T R A C T

Nine filamentous fungi isolated from the soil contaminated with petroleum hydrocarbon and wastewaters from refinery located in the East part of AI-Nasiriya city by using dilution method. The results showed that *Aspergillus niger* were more dominant with 94% and *A.flavus* was 85% , the percent of *Penicillium sp.* reached to 72% while the percent of *A.fumigatus* reached to 69% . The remaining 5 fungal species isolated with a very low frequency and the percent reached to 11% . The results showed that mixed four fungi with high freqancy were more efficient to decrease Total Suspended Solid (TSS) and Chemical Oxygen Demande (COD) with 95% in 20c^{o}, P^{H} 4 and 0.5gm /I salt concentration(Nacl), 30c^{o} ,p^{H} 6,1.0gm / I Nacl respectively. The treatment with *A.fumigatus* was found to be more efficient to decrease Biological Oxygen Demande (BOD) from 500mg /I to 263 mg /I . In the same time the results showed that the total acidity in wastewaters treatment with mixed fungi (high frequency were increased to 140 mg /I in 20 c^{o} ,P^{H} 4 ,0.5 gm / I salt concentration (Nacl). The results showed that the Total Petroleum hydrocarbon(TPH) was removel with 99.99%, and the statistical methods obtained a significantly with temperature in wastewater treatment with mixed fungi .

Keywords

BOD,
COD,
TSS,
Filamentous fungi,
Soil,
Total acidity,
Wastewater

Introduction

Large a mount of water are used for industrial activities and consequently siginificant volumes of wastewaters are generated. Based on the type of industry,various levels of pollutants are deliberately relased and discharged into the environment directly. A mong these industries that discharge their effluents into the aquatic environments are the petroleum oil refineries. As not all refineries have the same processes, the effluents that are produced will have different chemical compositions depending on the type of treatment they received(Hernandez et al.,1998 ; Wake,2005). Wastewaters released by oil refineries contain large a mounts of toxic derivatives such as oil and grease, Phenols, Sulphides, Cyanides, Suspended solids, nitrogen compounds as well as heavy metals such as iron, nickel, copper, selenium, zink, molybdenum, etc.(Burks, 1982).Due to the ineffectiveness of purification systems, wastewaters from the refineries may become seriously dangerous, leadind to the accumulation of toxic products in the receiving water bodies with potentially serious consequences on the ecosystem (Otokunefor and Obiukwu, 2005). Thus the discharge of these effluents containing persistant chemicals into a receiving water body may result in the long term effects to aquatic biota (Tisler et al.,1999). The toxicity of oil refinery effluents to aquatic organisms have being reported in many literatures. Toxicity of petroleum refinery depends on a number of facters which include quantity, volume and variability of discharge.

The different components of the effluents may have varing effects and toxicity (Saha and Konar, 1985).Atlas(1981)showed that the petroleum did not persist for long periods in the most soils even when relatively large quantities of petroleum have spilled. This is probably due to large part to the initial degradation by the action of sun light followed by microbial attack when the oil sink.Fungi and other microorganisms have the a bility to degrade several pollutants including crude oil in the aquatic ecosystem and utilize them as a nutrient source (Davis and Westlake,1979).

They may also metabolize such as pollutants to substrates with low harmful effect on the environment (Cerniglia et al.,1985; Sutherlands,1999; Boonchan et al., 2000).The efficiency of fungi isolated from soil and wastewaters to treatment of effluent petroleum wastewaters from AI-Nasiriya refinery has not been investigated, and the present study investigated the quality of the refinery effluent discharge.

Materials and Methods

Characterization of Petroleum oil refinery wastewaters

Non treated Petroleum oil refinery wastewaters were collected from inside and outside refinery by using 5 litres polyethylene containers. The containers were rinsed several times with the effluent sample at the point of collection.The sample were taken to the laboratary within 1h of collection,BOD, COD, TSS, TPH, Total acidity, Cations (Cd,Pb,Ni,Zn,Fe and Cu)in wastewaters samples were determined according to standard methods (APHA,1998).

Fungi strains and cultures conditions

The fungi strains used in this study was isolated from soil and wastewaters samples collected from effluent discharged to refinery in AI-Nasiriya city.One media was applied to isolated fungi from soil and wastewaters by using Potato Dextrose Agar (PDA),the media was supplemented with 250 mg /I Chloramphenicol to suppress bacterial growth. Plates and media were incubated at 25 c^{o} in the dark.Single colonies were picked from the plates under a dissecting microscope and transferred to appropriate media to allow fungal development. Stock cultures were maintained on the Potato Dextrose Agar slant, subcultured periodically and stored at 4 c^{o}.Fungal specimens were examined under light microscope after preparations and identified using morphological characters and taxonomical keys provided in the mycological keys (Watanabe, 2000).

Preparation of standardised inoculums

Spores suspensions were prepared by adding 15 mL of sterile distilled water to mature (4-

5 days) fungal colonies on PDA plates to dislodge the spores from the mycelium.The spores were counted using a haemocytometer (Neubauer,Germany) to obtain a spore concentration of a bout 10^5 spores / mL. These suspensions were then used to inculate 100 mL of wastewaters after sterilized in 500 mL Erlenmeyer flasks (Kendrick and Ratledge, 1996).The cultures were incubated at 25 c^o in an incubator and shaking three time in day.This resulting culture was then used as standard inoculum for further experiments.

Determination of biodegradation potential fungi

To determine the biodegradation potential of oil degrading isolate fungi. Add 500 mL of wastewaters from AI-Nasiriya refinery after sterilized by autoclave to Erlenmeyer flasks and incubated with 10 mL from axenic and mixed cultures of fungi spores (10^5 spore / mL), All flasks were coverd with non cotton wool absorbent and incubated at (10,20,30,40 c^o) with P^H (2,4,6,8) by using HCL or NaOH and add (0.0,0.5,1.0,2.0 gm / I) Nacl. Control flasks were left without microbes and these experiments were duplicate. The flasks were shaken manually at regular intervals to allow adequate mixing and homogeneity of the contents.1 mL of liquid culture was examined with Haemocytometer under light microscope and culculated spores.

A nalysis of the fungal biodegradation activity was made using a computerized capillary gas chromatography with flame ionized GC- FID (Backard 438 A) and flame ionization detector set at 325 c^o. The carrier gas was Helium at flow rate of 30 mL / min. The column length 3m, internal diameter 1/ 8 mm. The temperature was programmed to increase from 100 to 300 c^o at 10 c^o / min. Individual compounds present in the fractions were determined by matching the retention time with authentic standards.

Statistical analysis

The present study conducted on Anova (analysis of variance) which was performed on all the treatments and done using the SPSS (version 10.0) package to determine whether or not, a significance difference.

Results and Discussion

The results of the chemical characterization of the oil refinery wastewater sample are shown in Table 1. The wastewaters sample have elevated levels of organic compounds as indicate by the concentrations of BOD, COD, TPH.

Table 1. showed a reduction in the values of Zn,Cd,Pb,Ni,Fe in outside the refinery but Cu was increased.

Table 2. Showed the fungal strain isolated from soil and wastewaters from AI-Nasiriya refinery. *A.niger* was more dominant with 94% and *A.flavus* was 85%, the percent of *Penicillium sp.* reached to 72% while the percent of *A.fumigatus* reached to 69%. The remaning 5 fungal species isolated with a very low frequency while the percent reached 11%. to

These results were similar to the findings of (Keren et al.,2006) which showed that increase in the fungal population when presence of oil in the soil. In the same time the similar results were record by (AI-Jawhari, 2015) in their study obtained that *A.niger* were more frequency with 100% in all samples, but *A.fumigatus* and *Penicillium funiculosum* were 83%. Similar results reports have indicate the increase of microbial population in

The presence of oil contaminated soil (Minai – Tehrani et al., 2007) in their study about the effect of oil spill on the composition of microbes in a soil, they found that the soil was dominant by a diversity of oil degrading fungi including *Penicillium sp.,Rhizopus sp.*, *Thamanidium sp.*, *Cunninghamella* and *Candida.* The hydrocarbons may inhibited or caused by a death of certain microorganisms. On the other hand, there will also be increasing in numbers of certain microorganisms especially those capable of depending the hydrocarbons (Margesin et al.,2003), The same result was obtained by (Farid,2012) in this study showen that the numbers of oil degrading fungi were more than the numbers of oil degrading bacteria in the soil and changes in the flora of soil fungi following oil waste application and the study of (Oudot et al.,1993) on the biodegradation potential of hydrocarbon assimilating tropical fungi. The statistical methods obtained significantly with temperature. This result was similar to findings of (Ainon et al.,2010) which show that the growth of *Trichoderma virens* UKMP-IM was highest under acidic conditions and was able to grow in a relatively wide range of P^H from 5 to 7 and suggesting that this isolate could degrade oil under not only acidic but also neutral conditions.Among the parameters that could affect biomass production is temperature, generally considered the most important facter (Delille et al.,2004).

Anion et al.,2010) refer that heterotrophic organisms degrading the oil are increasing in number.The increase in numbers of fungi in present study in 0.5gm Nacl due to the adaptation of osmosis and because to produce Polyols and glycerol compounds and these compounds work to osmosis bufferering and transport positive sodium ion systems (Brown, 1990 ; Petrovic et al., 2002).

In the same time the high concentrations of salt produced increasing of spores production in some fungi (Dias-Munoz and Montalvo – Rodriguez, 2005). In the present study the total acidity was increased week after week in wastewaters treatment with fungi cultures Table. 3,4,5,6,7. Highly values of total acidity was recorded in wastewaters treatment with mixed fungi, the total acidity reached to 140 mg / I in fourth week with 20 c^o and 0.5 gm Nacl, and in the same time the statistical methods observed significant differences with temperature. The increase of total acidity in wastewaters treatment with fungi refer to chemical changes of hydrocarbon substrates and must have been precipitated by microbial enzymes (Atlas and Bartha, 1972). Hydrogen ion concentration is a major variable govering the activity and composition of fungi. Microbial degradation of hydrocarbon often leads to production of organic acids and other metabolic products (Nwachukwu and Ugoji, 1995).

Fungi consumed organic compounds as a sole of carbon and energy depend on dissolved oxygen in water and increases of growth of microorganisms produced reduction of dissolved oxygen during oxidation process of dissolved hydrocarbons, the results showed that the culture of *A.fumigatus* consumed high value of dissolved oxygen with 263 mg / I during 5 days Table. 8. No significant differences were observed with axenic fungi, but significant differences were observed when compared with control.

The most complete degradation of the mainly of organic materials was occurred under aerobic conditions. The initial intracellular attack of organic materials was an oxidative process and oxygen consumption (Najafi and Kashi, 2012). Fig. 1 Showed that the values of Chemical

Oxygen Demande (COD) were decreased in wastewaters treatment and highest decreases were recorded with mixed fungi in 30 c^o, P^H 6, 1.0 gm / I Nacl and the removal percentage reached to 95% in fourth week when compared with control, this result due to the complete of chemical oxidation to all chemical compounds and the values of (COD) were higher than (BOD). No significant differences were observed with all fungi and temperature. These results were similar to the findings of (Najafi and Kashi, 2012) which showed that the P^H were the effect on reduction of (COD), PAHS and TPH and the reduction increased with increasing P^H value and the optimum a mount of P^H for reaching to complete minerlization was 7.5.

Najafi and Kashi (2012) refer that temperature had a significant role in biodegradation of hydrocarbons with direct effecting on the chemistry of the pollutants, and effecting the diversity of the microbial flora. Temperature also effected on the solubility of hydrocarbons and the activity of the bio- enzyme. The biodegradation constants of removal (COD), PAHS and TPH reduction manily reduced with decreasing temperature. The highest biodegradation rates that happenes in the range 15- 20 c^o in marine environment. At 20 c^o temperature the viscosity of the oil decreased, the volatility of the low molecular weight hydrocarbons were increased, bio- enzymatic oxidation were increased, and the accelerating the onset of biodegradation.

In the same time (Najafi and Kashi, 2012) found that (COD), PAHS and TPH reduction was increased by an increase in the reaction's duration from 1 day to 10 days, and the percentage removal of (COD) reached to 100 % in 10 days with the initial concentration of 440 mg MI-1. Fig. 2 Showed decreased of Total Suspended Solids (TSS) in wastewaters treatment with axenic and mixed culture of fungi. The highest decreased were recorded with mixed fungi in 30 c^o, P^H 6, 1.0 gm / I Nacl and the value reached to 20 mg / I as well as the removal percentage reached to 95% Fig. 2 in fourth week when compared with control, these results due to that these fungi can degradation to suspended solids substrates, the statistical methods obtained significantly with temperature 10 c^o and 30 c^o and in the same time no significant differences were observed in *Penicillium sp.* with different temperature.

This result was similar to findings of (AI-Jawhari, 2015) which show that the highest percentage loss of crude oil concentration by mixed cultures of *A.niger* and *A.fumigatus* reached to 90% after 28 days of treatment. This result refer to that the greater capacity to remove crude oil due to the adaptation of these fungi to the pollutant composition as well as to the enzymatic systems of the fungi (Mancera – Lopez et al.,, 2007). It means that the fungal strains were able to degrade crude oil and consumption of its components. Crude oil consists of saturated and aromatic hydrocarbons and asphaltic compounds of varing molecular weight, complexity, and degree of susceptibility to microbial oxidation (Raymond and Davis, 1990). Mycellial organisms can penetrate insoluble substances such as crude oil and this increase the surface are available for microbial attach (Davis and Westlake, 1979). Fig 3. showed that the reduction of TPH was increased by an increase in the reaction's duration and increased temperature, P^H and Nacl concentration in wastewaters treatment with mixed fungi cultures from 1 week to 4 week and the percentage removal of TPH reached to 99.99% when compared with control. These figures showed decreased concentration and appear many Peaks when compared with control.

Table.1 Chemical characterization of the oil refinery wastewaters

Element	Inside Refinery	Outside Refinery
Zn	0.885*	0.460
Cd	0.137	0.083
Pb	0.121	0.093
Ni	0.283	0.062
Fe	2.299	1.160
Cu	0.128	0.164

* PPm

Table.2 Fungal strain isolated from soil and wastewaters from AI-Nasiriya refinery

Fungal species	Numbers of fungal species appear	Frequancy %
Aspergillus niger Tighem	9	94
A.fumigatus Fresenius	6	69
A. flavus Link	8	85
Trichocladium opacum Harz	1	11
Alternaria alternate Keissler	1	11
Fusarium solani Link	1	11
Penicillum sp. Thom.	6	72
Trichoderma lignorum (Tode)Harz	1	11
Rhizoctonia solani Kuhn	1	11

Table.3 Values of total acidity in wastewaters treatment with *A. niger* in different temperature, PH and salt concentration (Nacl)

Week	Temperature	P_H	Salt concentration(gm)	Total acidity (mg/I)
	10	2	0.0	
1				34
2				39
3				43
4				48
	20	4	0.5	
1				38
2				55
3				78
4				110
	30	6	1.0	
1				37
2				52
3				68
4				87
	40	8	2.0	
1				35
2				44
3				55
4				66
	Control	-	-	
1				34
2				34
3				34
4				34

(-):No treatment

Table.4 Values of total acidity in wastewaters treatment with A.flavus in different temperature, PH and Salt concentrations (Nacl)

Week	Temperature	P_H	Salt concentration(gm)	Total acidity (mg/I)
	10	2	0.0	
1				34
2				39
3				47
4				53
	20	4	0.5	
1				37
2				55
3				75
4				90
	30	6	1.0	
1				36
2				41
3				56
4				77
	40	8	2.0	
1				35
2				49
3				63
4				71
	Control	-	-	
1				34
2				34
3				34
4				34

(-):No treatment

Table.5 Values of total acidity in wastewaters treatment with A.fumigatus in different temperature, PH and Salt concentrations (Nacl)

Week	Temperature	P_H	Salt concentration (gm)	Total acidity (mg/I)
	10	2	0.0	
1				34
2				37
3				42
4				47
	20	4	0.5	
1				36
2				47
3				59
4				73
	30	6	1.0	
1				37
2				56
3				79
4				93
	40	8	2.0	
1				35
2				42
3				53
4				65
	Control	-	-	
1				34
2				34
3				34
4				34

(-):No treatment

Table.6 Values of total acidity in wastewaters treatment with Penicillium sp. in different temperature, PH and Salt concentrations (Nacl)

Week	Temperature	P_H	Salt concentration (gm)	Total acidity (mg/I)
	10	2	0.0	
1				34
2				38
3				42
4				51
	20	4	0.5	
1				38
2				52
3				73
4				95
	30	6	1.0	
1				35
2				49
3				62
4				79
	40	8	2.0	
1				35
2				45
3				56
4				68
	Control	-	-	
1				34
2				34
3				34
4				34

(-):No treatment

Table.7 Values of total acidity in wastewaters treatment with mixed fungi in different temperature, PH and Salt concentrations (Nacl)

Week	Temperature	P_H	Salt concentration(gm)	Total acidity (mg/I)
	10	2	0.0	
1				34
2				39
3				45
4				51
	20	4	0.5	
1				38
2				56
3				82
4				140
	30	6	1.0	
1				36
2				54
3				67
4				85
	40	8	2.0	
1				35
2				49
3				62
4				80
	Control	-	-	
1				34
2				34
3				34
4				34

(-):No treatment

Table.8 Values of Biological Oxygen Demande in wastewaters treatment with fungi strains

Fungi	P_H	Salt concentration(gm)	BOD) Value)	Removal %
A.niger	4	0.5	269	46
A.flavus	4	0.5	285	43
A.fumigatus	4	0.5	263	47
Penicillium sp.	4	0.5	275	45
Mixed fungi	4	0.5	356	29
Control	4	0.5	500	-

Fig.1 Values of Chemical Oxygen Demande in wastewaters treatment with fungi : in: A:10co, B: 20co, C: 30co, D: 40co, E: Mixed fungi in different temperature, PH, Nacl

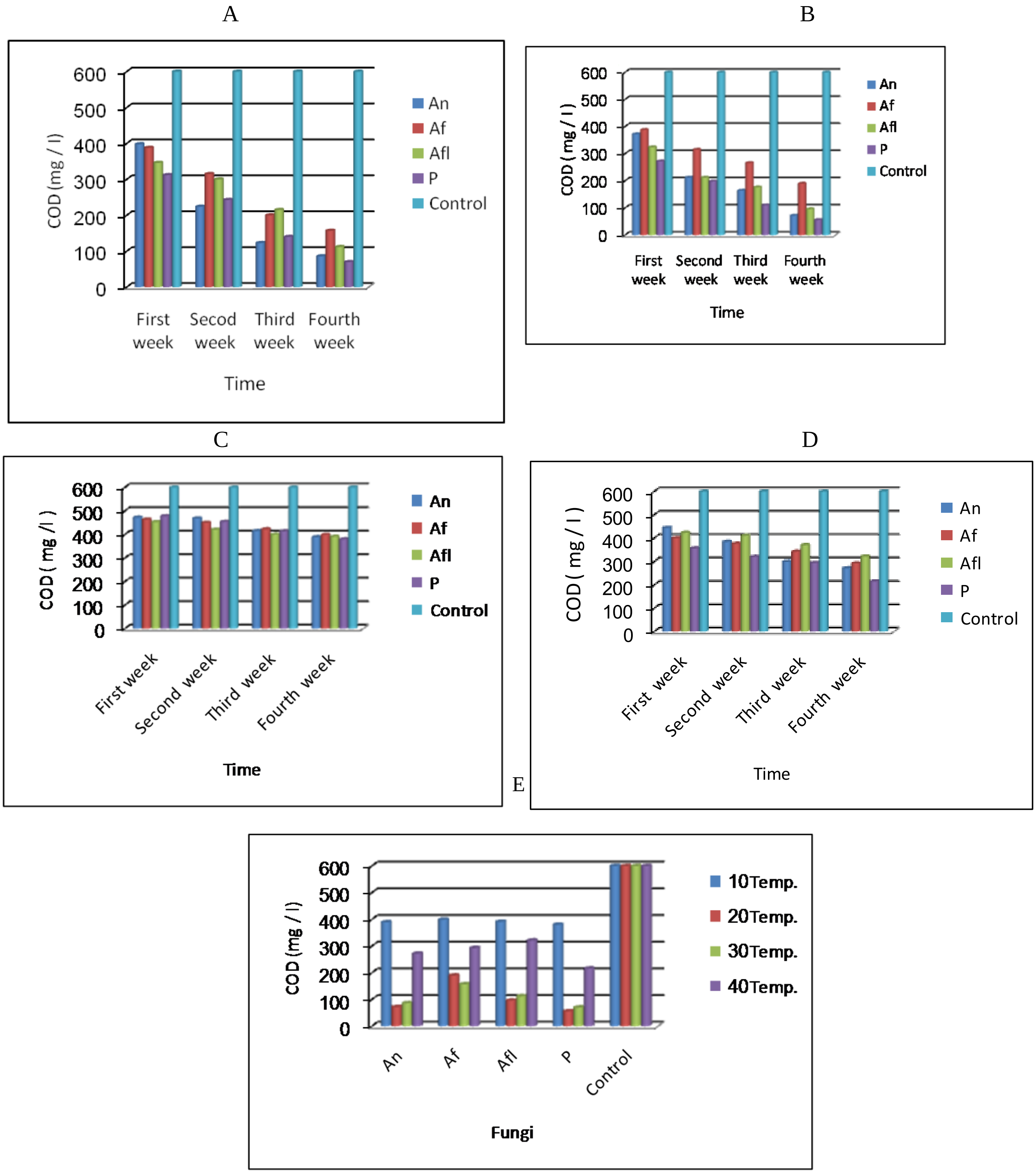
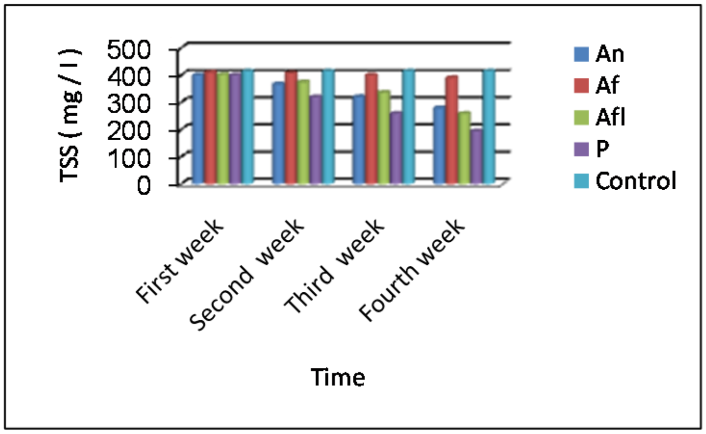


Fig 2. Values of Total Suspended Solids (TSS) in wastewaters treatment with fungi in : A: 10 c^o , B: 20 c^o, C: 30 c^o, D: 40 c^o, E: Mixed fungi in different temperature, P^H, Nacl.

A



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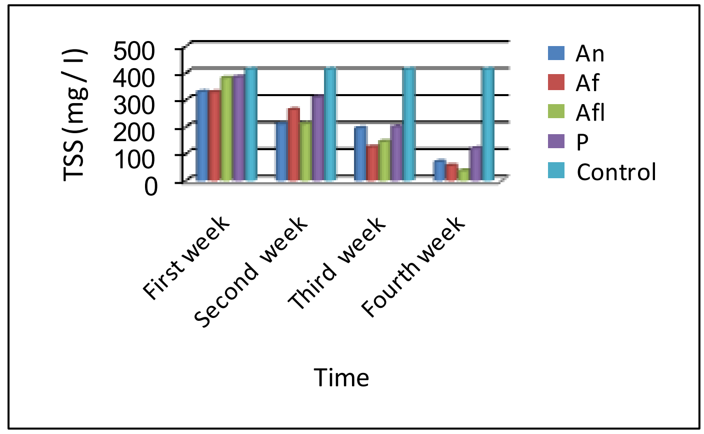

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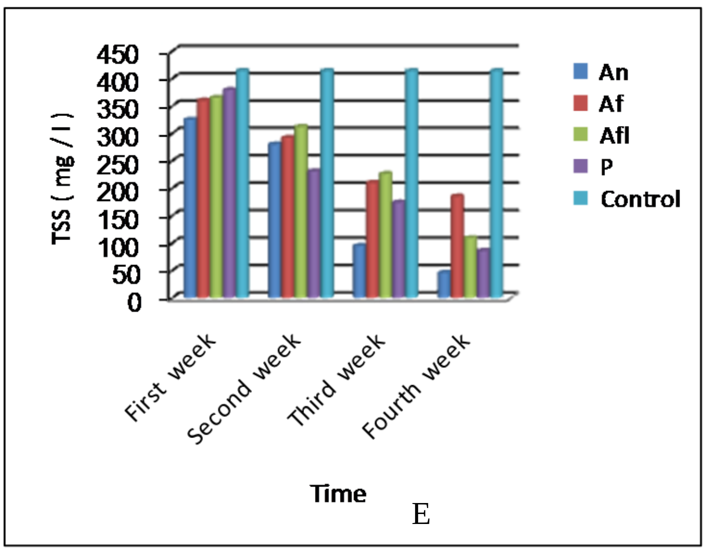

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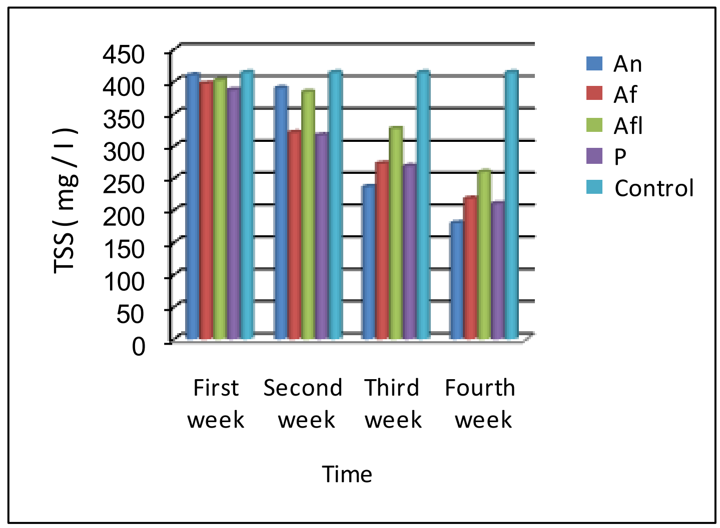


E

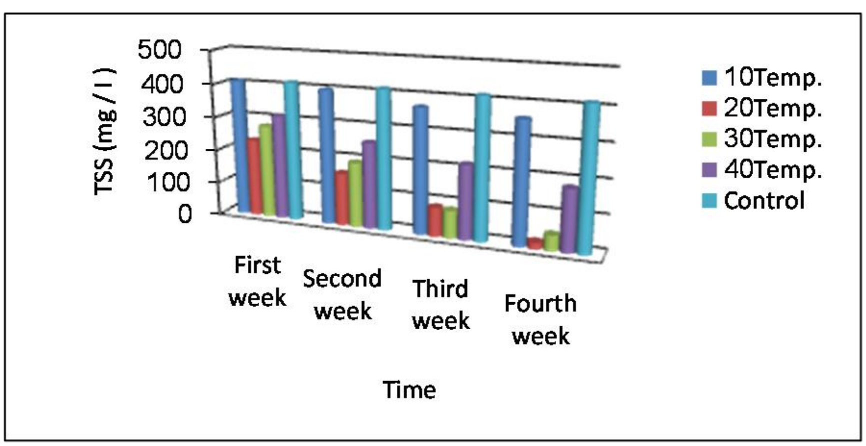
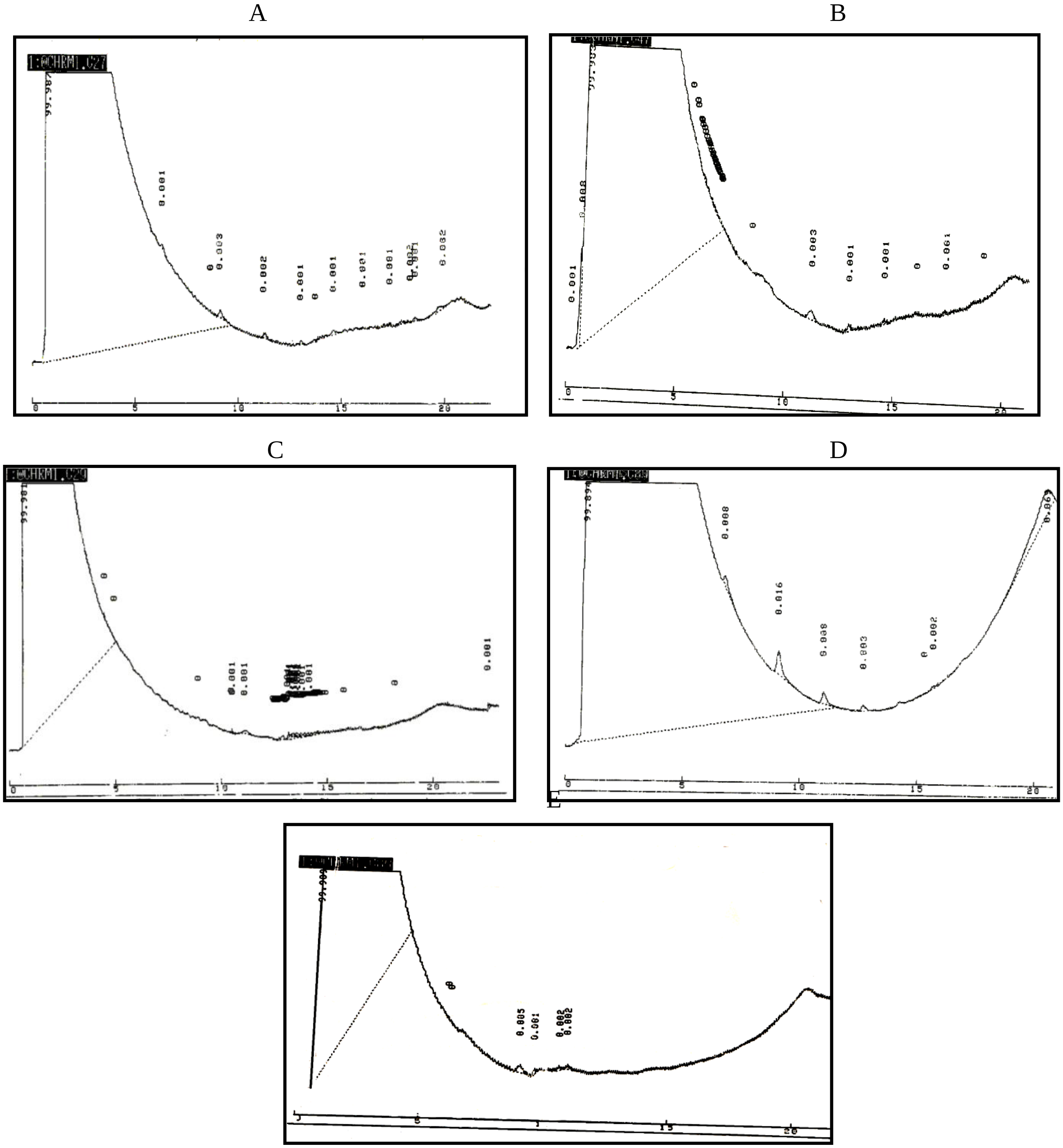


Fig.3 GC- Chromatogram of removal Total Petroleum Hydrocarbon (TPH) in wastewaters in treatment with mixed fungi (A: First week, B:Second week, C:Third week, D:Fourth week, E:control)



This result was similar to findings of (Najafi and Kashi, 2012) which show that the TPH value was reached to 99.98% when compared with initial concentration of 770 mg 1-1.

Davis and Westlake (1979) obtained that the mycelial organisms can penetrate insoluble substances such as crude oil and this increase the surface are available for microbial attack. (Mancera – Lopez et al.,2007) found that *Penicillium funiculosum* and *A.sydowii* were loss TPHS concentration to 86, 81 % respectively, and study of Okoro and Amund (2010) on the biodegradation potential of hydrocarbon, they had been showed that *A.fumigatus* can removed of PHAS with 80 % after 120 days of exposure, and the same result was obtained by (Colombo et al.,1999)in their study reported that *A.terres* and *Fusarium sp.* were the percent degradation to aliphatic compounds reached to 100 %.

Studies of (Abed et al., 2006) and (Diaz et al., 2002) it is clear that very low salt concentrations reduce hydrocarbonoclastic activity and the optimum biodegradation results are reached whitin moderate salinity ranges.

First of all, it is necessary to consider that hydrocarbons are less bioavailable in hypersaline environments that in non saline ones (Luiz and Raquel, 2012).

The oil degrading fungi isolated from the soil and wastewaters in the present study included *A.niger*, *A.flavus*, *A.fumigatus* and *Penicillium sp.*, and the density of these fungi were dominant than other fungi. These fungi were well adapted to degrade and utilize the crude oil. The mixed culture of these fungi were beter than the axenic fungi to removel of COD, TSS, TPH.

The reduction of BOD, COD, TSS and TPH enhanced with increasing reaction's duration time, for given experimental conditions. The P^H was effect on biodegradation of BOD, COD, TSS, and TPH reduction, also the highest reduction obtained at P^H 6.

The 0.5 and 1.0 gm / I salt concentration (Nacl) a chieved the highest efficiency in biodegradation of BOD, COD, TSS and TPH reduction. The temperature 20 c^o, 30 c^o a chieved the highest efficiency in biodegradation of COD, TSS and TPH.

Rehabilitation of oil contaminated soil and wastewaters by the culture of mixed fungi (*A.niger*, *A.flavus*, *A.fumigatus* and *Penicillium sp.)* were promising as it can reduce the oil pollution to acceptable levels for reuse of land and water within a short period.

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