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Research Paper

Efficiency of pseudomonas aeruginosa and Escherichia coli to treatment of effluent petroleum wastewater from AI-Nasiriya refinery – Iraq

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Abstract

Fourbacteria isolated from the soil contaminated with petroleum hydrocarbon and wastewaters from the refinerylocated in the south part of AI-Nasiriya city in Iraq,by using dilution method.The results showed that *Pseudomonas aeruginosa*was more dominant with 100% in soil and wastewaters in inside of refinery,and 93%,95% in soil and wastewaters in outside the refinery respectively.The results showed that the second dominant of bacteria was *Esherchia coil* with 92%,98% in soil and wastewaters in inside the refinery,and 83%,88% in soil and wastewaters in outside of refinery.The remaining two species of bacteria isolated with a very low frequency,these bacteria were *Bacillus subtilis* and *Klebsiella sp*., *B.subtilis* was obtained with 10%,8% in soil and wastewaters in inside the refinery,and 10%,3% in soil and wastewaters in outside of refinery .In the same time that *Klebsiella sp*. Was obtained with 10% in soil and wastewaters in inside the refinery,and 8%,3% in soil and wastewaters in outside the refinery.The results showed that mixed two bacteria (high frequency)were more efficient to decrease Biological Oxygen Demande(BOD)with 59% in pH 4,0.5gm / I salt (NaCI) concentration.Chemical Oxygen Demande (COD) was decreased to 75%.The results showed that the Total Acidity(TA) in wastewater were increased to 82%mg / I in $40c^{o}$,pH 8 and 2.0 gm/ I NaCI , and Total Suspended Solid (TSS) was decreased to 50 mg / I in $40c^{o}$, pH 8,2.0 gm / I NaCI . The results showed that the Total Petroleum Hydrocarbon (TPH) was removal with 99.99%, and in the same time the numbers of mixed bacteria were increased to $5x10^{8}$ after incubation 48h in $40c^{o}$.

Keywords: *Bacteria; Refinery; Soil; Total Acidity; Wastewater.*

1. Introduction

Large amount of water are used for industrial activities and consequently, significant volumes of wastewater are generated. Based on the type of industry, various levels of pollutants are deliberately released 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 [1], [2]. Wastewater released by oil refineries contain large amount 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 [3]. Due to the ineffectiveness of purification systems, wastewater from the refineries may become seriously dangerous, leading to the accumulation of toxic products in the receiving water bodies with potentially serious consequences on the ecosystem [4]. Thus the discharge of these effluents containing persistant chemicals into a receiving water body may result in the long-term effects to aquatic biota [5].

The toxicity of oil refinery effluents to aquatic organisms have being reported in many literatures. Toxicity of petroleum refinery depends on a number of factors, which include quantity, volume and variability of discharge. The different components of the effluents may have varying effects and toxicity [6].[7]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 ability to degrade several pollutants, including crude oil in the aquatic ecosystem and utilize them as a nutrient source [8].They They may also metabolize such as pollutants to substrates with low harmful effect on the environment [9] ,[10] , [11].The efficiency of bacteria isolated from soil and wastewater to treatment of effluent petroleum wastewater from AI-Nasiriya refinery has not been investigated, and the present study investigated the quality of the refinery effluent discharge.

2. Materials and methods

2.1. Characterization of petroleum oil refinery wastewaters

Non-treated Petroleum oil refinery wastewater 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 laboratory within 1h of collection,BOD ,COD ,TSS ,TPH ,Total acidity ,Cations (Cd,Pb,Ni,Zn,Fe and Cu)in wastewater samples were determined according to standard methods [12].

2.2. Bacteria strains and cultures conditions

The bacteria strains used in this study was isolated from soil and wastewater samples collected from effluent discharged to refinery in AI-Nasiriya city.One media was applied to isolated bacteria from soil and wastewater by using Nutrient agar(NA),the media was supplemented with 30 µg /I Nystatin to suppress fungi growth. Plates and media were incubated at 37 c^o in the dark. Stock cultures were maintained on Nutrient agar,subculture periodically and stored at 4 c^o.Bacterial specimens were examined under light microscope after preparations and identified using morphological characters , gram stain, biochemical tests and API20E[13] and taxonomical keys provided in the bacteriological keys [14] , [15].

2.3. Determination of biodegradation potential bacteria

To determine the biodegradation potential of oil degrading isolate bacteria. Add 500 mI of wastewater from AI-Nasiriya refinery after sterilized by autoclave to Erlenmeyer flasks and incubated with 2 mI from axenic and mixed cultures of bacteria colony .All flasks were covered with non-cotton wool absorbent and incubated at (10,20,30,40 c^o) with pH (2,4,6,8) by using HCL or NaOH and add (0.0,0.5,1.0,2.0 gm / I) NaCI. 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 mI of liquid culture was transfer to test tubes containing 9 mI distilled water and 1% pepton and make serial dilution . 1 mI from diluted 10^7 was cultured by using plate count, and determined microbial numbers after incubation 24h [16]. These expermint were duplicated.

A nalysis of the bacteria biodegradation activity was made using a computerized capillary gas chromatography with flame ionized GC- FID (Backard438 A) and flame ionization detector set at 325 c^o . The carrier gas was Helium at flow rate of 30 mI / 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.

2.4. Statistical analysis

The present study conducted on Anova (analysis of variance) which was performed on all the treatments and done using the SPSS (version10.0) package to determine whether or not, a significance difference.

3. Results and discussion

The results of the chemical characterization of the oil refinery wastewater sample are shown in Table 1. The wastewater sample have elevated levels of organic compounds as indicate by the concentrations of BOD,COD,and 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 bacterial strain isolated from soil and wastewater from AI-Nasiriya refinery. P.aeruginosa was more dominant with 100% in soil and wastewater in inside the refinery, and 93%, 95% in soil and wastewater in outside the refinery. The results showed that the second dominance of bacteria was Escherchia coli with 92%, 98% in soil and wastewater in inside the refinery, and 83%, 88% in soil and wastewater in outside of refinery. The remaining two species of bacteria isolated with a very low frequency, these bacteria were Bacillus subtilis and Klebsiellasp. ,B.subtilis was obtained with 10% , 8% in soil and wastewater in inside the refinery , and 10% , 3% in soil and wastewater in

outside of refinery . In the same time that Klebsiella sp. was obtained with 10% in soil and wastewater in inside the refinery, and 8%, 3% in soil and wastewater in outside the refinery.
These results were similar to the findings of [17] which showed that increased in the bacterial population when presence of oil in the soil.

Table 1: Chemical characterization of the oil refinery wastewater.

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: Total numbers of bacteria isolated from soil and wastewater in AI- Nasiriya refinery.

Isolated bacteria	Inside refinery				Outside refinery			
	Soil	%	Water	%	Soil	%	Water	%
P.aeruginosa	$4x10^8$	100	$4x10^8$	100	$4x10^8$	93	$4x10^8$	95
E.coli	$4x10^8$	92	$4x10^8$	98	$4x10^8$	83	$4x10^8$	88
B.subtilis	$1x10^7$	10	$1x10^7$	8.0	$1x10^7$	10	$1x10^7$	3.0
Klebsiella sp.	$1x10^7$	10	$1x10^7$	10	$1x10^7$	8.0	$1x10^7$	3.0

Similar results reports have indicate the increase of microbial population in the presence of oil contaminated soil [18] in their study about the effect of oil spill on the composition of microbes in a soil. 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 [19].
In the present study the total acidity was increased day after day in wastewater treatment with bacterial colony Table 3,4,5. Highly values of total acidity was recorded in wastewater treatment with mixed bacteria , the total acidity reached to 82 mg / I in two day with 40 c^o and 2.0 gm NaCI , and in the same time the statistical methods observed non-significant differences with temperature . The increase of total acidity in wastewater treatment with bacteria refer to chemical changes of the hydrocarbon substrates and must have been precipitated by microbial enzymes [20].These results were similar to the findings of [21] which showed to increased the amount of acidity by using mixed of two strain of bacteria and these bacteria produced acidity to 75 mg / I when compared with 60 mg / I produced by using protozoa. Hydrogen ion concentration is a major variable govering the activity and composition of bacteria. Microbial degradation of hydrocarbon often leads to production of organic acids and other metabolic products [22].

Table 3: Values of Total Acidity in wastewater treatment with E.coli in different temperature, pH.and Salt Concentration (NaCI).

Temperature(c^o)	pH	Salt concentrations (gm)	Total acidity (mg / I)
10	2	0.0	36*
20	4	0.5	42
30	6	1.0	57
40	8	2.0	61
Control	-	-	34

* Non-significant,(-): No record.

Table 4: Values of Total Acidity in wastewater treatment with P.aeruginosa in different temperature, pH.and Salt Concentration (NaCI).

Temperature(c^o)	pH	Salt concentrations (gm.)	Total acidity (mg / I)
10	2	0.0	37*
20	4	0.5	48
30	6	1.0	64
40	8	2.0	78
Control	-	-	34

(-): No record. * Non-significant

Table 5: Values of Total Acidity in wastewater treatment with mixed E.coli and P.aeruginosa in different temperature, pH.and Salt Concentration (NaCI).

Temperature (c^o)	pH	Salt concentrations (gm)	Total acidity (mg / I)
10	2	0.0	38*
20	4	0.5	53
30	6	1.0	69
40	8	2.0	82
Control	-	-	-

* Non-significant,(-): No record.

Table.6 showed decreased of BOD from 500 mg / I in control to 320 mg / I with P.aeruginosa , 390 mg / I with E.coli and 202 mg /I with mixed of two these bacteria , because the bacteria depend on dissolved oxygen in water to consumed organic compounds as asole of carbon and energy [23] , and increases of growth of microorganisms produced reduction of dissolved oxygen during oxidation process of dissolved hydrocarbons , 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 [24].
These results were similar to findings of [21], and the statistical methods obtained no significance differences between the treatments of bacteria.

Table 6: Values of Biological Oxygen Demandein wastewater treatment with different bacteria.

Species of bacteria	pH	Salt concentration (gm)	Values of BOD (mg/I)	Removal %
P.aeruginosa	4	0.5	320*	36
E.coli	4	0.5	390	22
Mixed two bacteria	4	0.5	202	59
Control	4	0.5	500	-

* Non significant, (-): No record.

Figure 1 Showed that the values of Chemical Oxygen Demande (COD) were decreased in wastewater treatment and the highest decreases were recorded with mixed bacteria in 40 c^o, pH8, 2.0 gm / I Nacl and the removal percentage reached to 75% after 48h when compared with control, this result due to the complete of chemical oxidation to all organic chemical compounds and the values of (COD) were higher than (BOD).

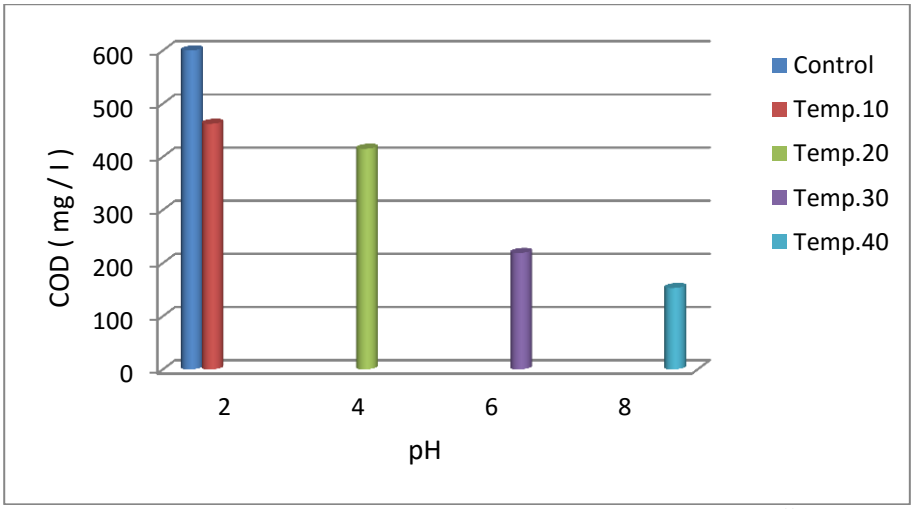


Fig. 1: Valuesof Chemical Oxygen Demandein wastewater treatment with mixed P.aeruginosa and E.coli in different temperature, pH.and 0.0, 0.5, 1.0, 2.0 gm / I Salt Concentration NaCI.

These results were similar to the findings of [21] which showed to efficiency of mixed bacteria to decreased COD values.
These results were similar to the findings of [24] which showed that the pH were the effect on reduction of (COD), PAHS and TPH and the reduction increased with increasing pH value and the optimum amount of pH for reaching to complete mineralization was 7.5.
[24] 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 mainly reduced with decreasing temperature. The highest biodegradation rates that happened 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 [24] 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 2,3,4,5.Show decreased of Total Suspended Solid (TSS) in wastewater treatment with axenic and mixed culture of bacteria, the highest decreased were recorded with mixed bacteria in 40c^o, pH 8, 2.0 gm / I NaCI, and the value reached to 50 mg / I as well as the removal percentage reached to 88%, but the percentage reached to 76%, 56% with alone culture of P.aruginosa and E.coli respectively after incubation 48h when compared with control.

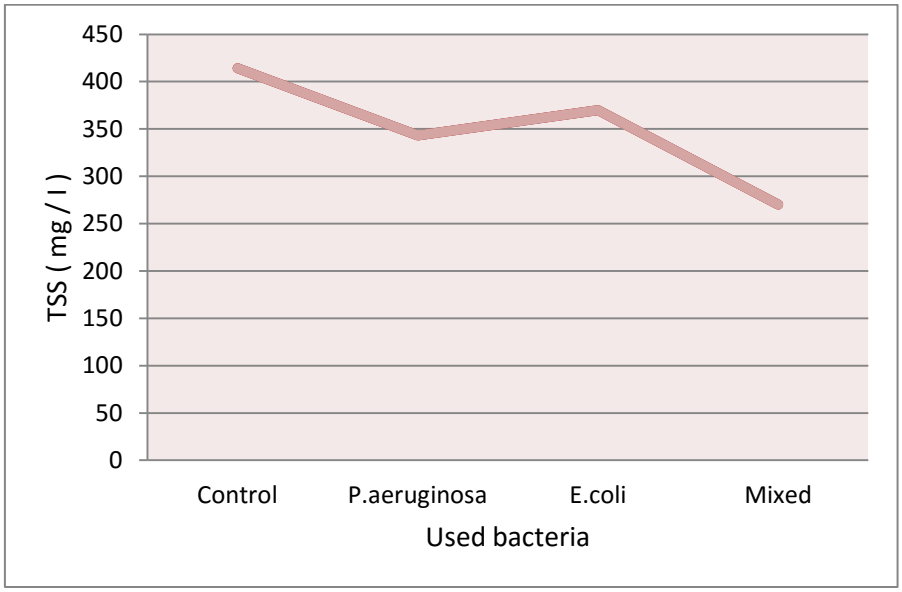


Fig. 2: Values of Total Suspended Solids (TSS) in wastewater treatment with in 10 C°, pH 2 and 0.0 gm / I NaCI.

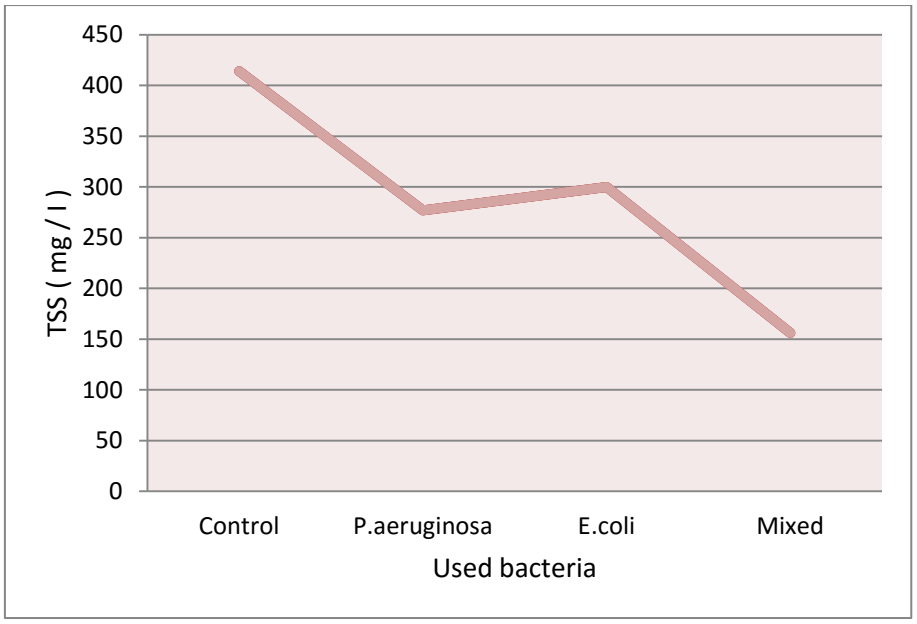


Fig .3: Values of Total Suspended Solids(TSS) in wastewater treatment with in 20 C°, pH 4 and 0.5 gm / I NaCI.

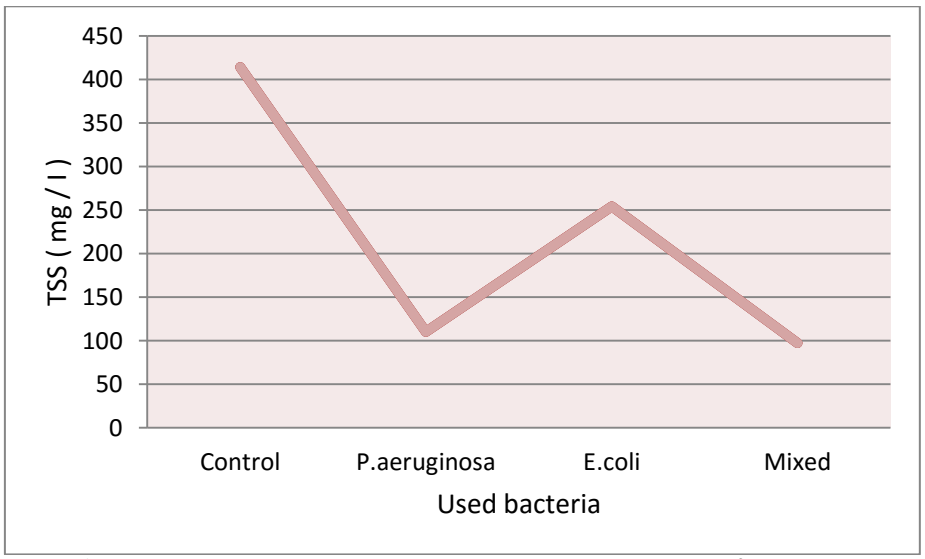


Fig .4: Values of Total Suspended Solid (TSS) in wastewater treatment with bacteria in 30 C°, pH 6 and 1.0 gm / I NaCI.

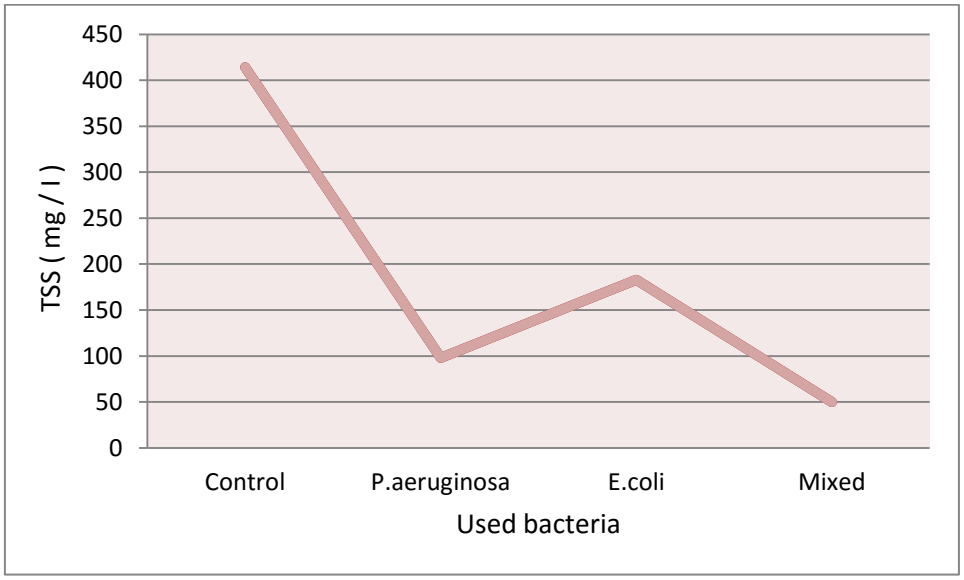


Fig .5: Values of Total Suspended Solids(TSS) in wastewater treatment with in 40 C°, pH 8 and 2.0 gm / I NaCI.

These results refer that bacteria play an important role to degradation TSS , and this point were economic important to reduced the amount of precipitation of chemical substances when added to waters founded in ponds as well as (AI_2SO_4) , and reduced the money and stress . These results were similar to the findings of [21] which showed to decreased of TSS by using bacteria. It means that the bacterial strains were able to degrade crude oil consists of saturated and aromatic hydrocarbons and asphaltic compounds of varying molecular weight , complexity , and degree of susceptibility to microbial oxidation[25].

Figure.6 showed that the reduction of TPH was increased by an increase in the reaction's duration and increased temperature, pH and NaCI concentration in wastewater treatment with mixed bacterial cultures during 48h, and the percentage removal of TPH reached to 99.98% when compared with control. This figure showed decreased concentration and appear many peaks when compared with control , and Fig.7 show containing foam in wastewater treatment with alone and mixed bacteria , these results refer to the ability of these bacteria to consumed hydrocarbons in wastewater .

In the same time the numbers of bacteria were increased to $5x10^8$ after incubation 48h in 40c°Table.7.

Table 7: Total numbers of isolated bacteria after treatment with wastewater.

Isolated bacteria	Temperature (C°)	pH	Salt concentration (gm)	Microbial Numbers
P.aeruginosa	10	2	0.0	2.0×10^8 *
E.coli				1.0×10^8
Mixed				3.0×10^7
Control				0.0
P.aeruginosa	20	4	0.5	2.0×10^7
E.coli				1.0×10^7
Mixed				4.0×10^7
Control				0.0
P.aeruginosa	30	6	1.0	3.0×10^7
E.coli				3.0×10^8
Mixed				4.0×10^8
Control				0.0
P.aeruginosa	40	8	2.0	4.0×10^7
E.coli				4.0×10^8
Mixed				5.0×10^8
Control				0.0

* Non-significant

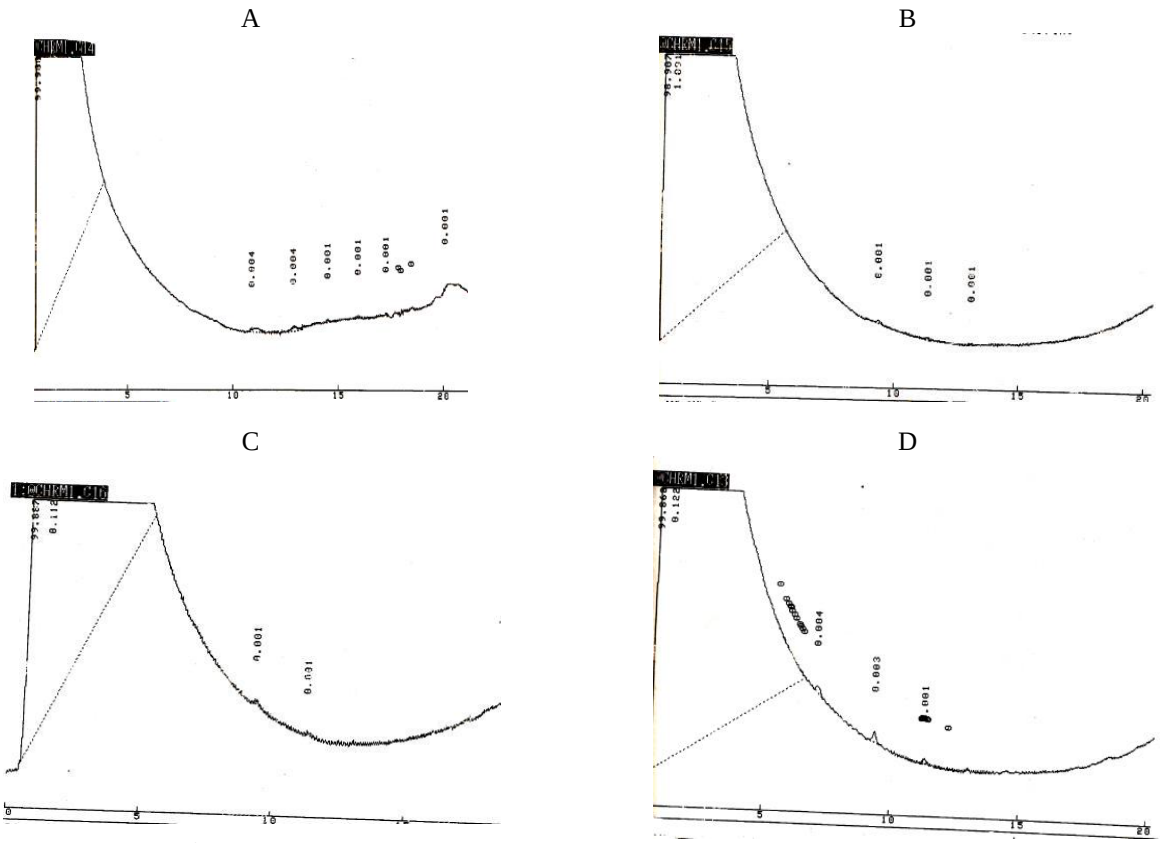


Fig. 6: GC Chromatogram of removal Total Petroleum Hydrocarbon (TPH) in wastewater treatment with (A: P.aeruginosa, B: E.coli, C: Mixed, D: Control) after 48h.

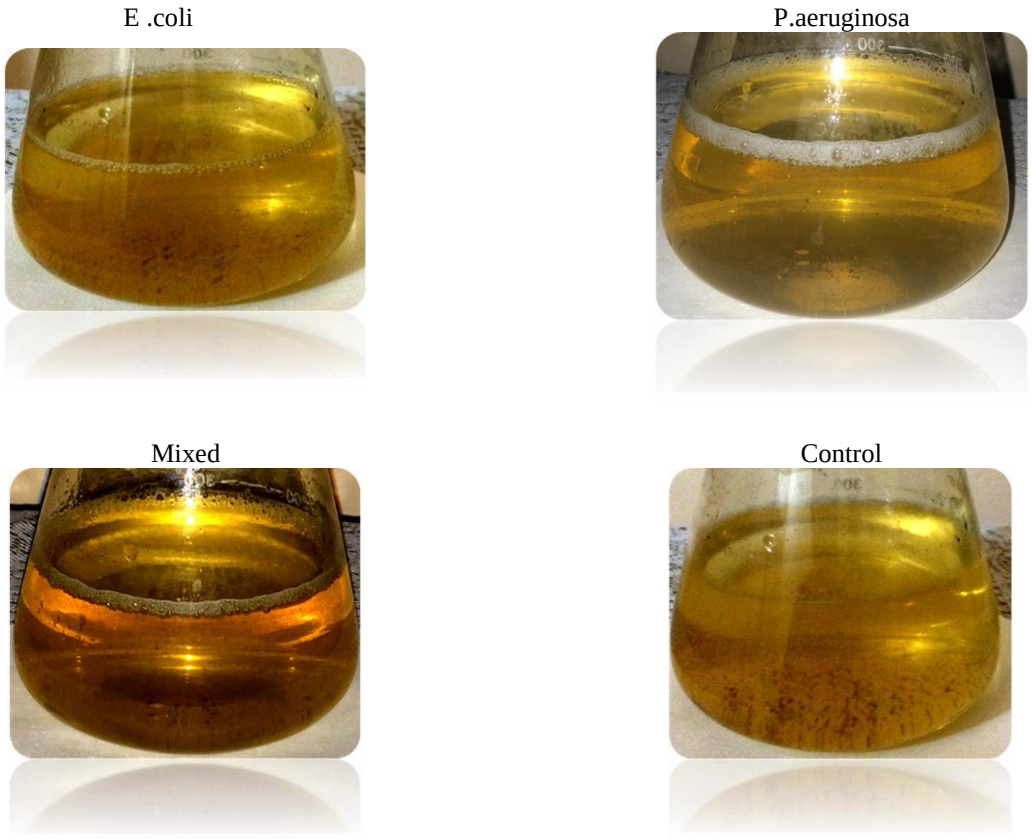


Fig. 7: Ability of isolated bacteria to biodegradation of Total Petroleum Hydrocarbon (TPH) in 40c^{o}, pH 8 and 2.0 gm / I NaCI.

These results were similar to the findings of [24] which show that the TPH value was reached with 99.98% when compared with initial concentration of 770 mg 1-1.
Studies of [26] and [27] 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 [28].

4. Conclusion

The oil degrading bacteria isolated from the soil and wastewater in the present study included P.aeruginosa,E.coli , and the density of these bacteria were dominant than other bacteria . These bacteria were well adapted to degrade and utilize the Petroleum hydrocarbons. The mixed culture of these bacteria were beter than the axenic bacteria to removal of COD,TSS,and TPH.
The reduction of BOD,COD, TSS and TPH enhanced with increasing reaction's duration time, for given experimental conditions. The pH was effect on biodegradation of BOD,COD,TSS, and TPH reduction, also the highest reduction obtained at pH 8.
The 2.0 gm / I salt concentration (NaCI) achieved the highest efficiency in biodegradation of BOD,COD, TSS and TPH reduction. The temperature 40 c^o, achieved the highest efficiency in biodegradation of COD, TSS and TPH.
Rehabilitation of oil contaminated soil and wastewater by the culture of mixed bacteria P.aeruginosa and E.coli 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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